

## Epigenetics of Cancer

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### Introduction

The field of epigenetic research and its role in cancer is undergoing a current growth phase because of the great promise it holds for new cancer biomarkers, novel therapeutic opportunities and prevention possibilities. However, the basic biology of epigenetic reprogramming and control of gene regulation still remains an enigma in both normal cellular development and in cancer. Whilst genetics is concerned with the sequence of DNA coding for a gene, epigenetics relates to how that sequence is read or interpreted and it is the 'misinterpretation' of the DNA sequence and masking of critical regulatory DNA regions that commonly underpins oncogenesis. The Human Genome Project, completed in 2003, defined the exact sequence of almost  $10^9$  bases in the genome. As powerful as it is to know the genetic blueprint, it was clear that DNA sequence alone does not predict how the genome is packaged in chromosomes and chromatin to provide for the differential expression of genes, which is essential for development and differentiation.

Recent advances in epigenetic research have provided new high-throughput technologies for the analyses of DNA methylation patterns and histone modification marks that will give structure and function to the human

genome. A Human Epigenome Project is now gaining momentum, with recent funding opportunities announced in 2008 from the NIH RoadMap Initiative, which aims to consolidate epigenetic research and pave the way for unforeseen breakthroughs in understanding normal and disease states. Important epigenetic lesions that trigger a malignant state can only be found by combining gene expression patterns in normal and cancer cells with DNA methylation and histone modification profiles. This article will focus on repression of tumour suppressor genes in cancer cells that commonly occurs by aberrant DNA methylation and the formation of heterochromatin and how this can be used as a biomarker of cancer and as a potential target of epigenetic therapy.

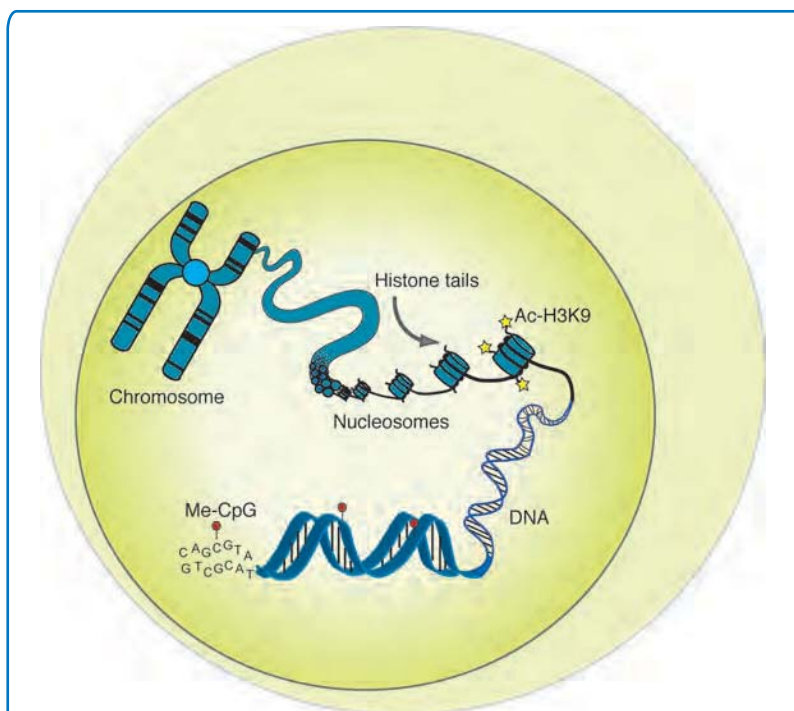
### Cancer is a Disease of the DNA

It has long been known that cancer involves genetic lesions including DNA mutations, deletions and amplification, but it is now also clear that the epigenetic changes in DNA methylation and chromatin modification play a key role in cancer and its development. Two of the most studied 'epigenetic' players that are altered in cancer are **DNA methylation** – the methylation of cytosine residues that are next to guanine residues (CpG sites) –

and **histone modification** – the modification of histones that form part of and regulate the structure of the chromatin (Fig. 1). Both of these epigenetic players are grossly deregulated in cancer, which impacts on gene expression, gene silencing and chromatin accessibility.

### DNA Methylation

In normal cells, DNA methylation is largely confined to repeated parts of the genome, in what used to be referred to as 'junk' DNA, but it is now clear that the so-called junk DNA also contains regulatory sequences, including non-coding RNA. In contrast to the extensively methylated bulk of the genome, there are also CpG sites that remain unmethylated in normal cells. These commonly occur in clusters and are known as CpG islands. CpG islands, which range from 200 bp to several kb in length, are up to five times richer in CpG density than the rest of the genome, and typically span the promoter region and first exon of approximately 60% of all genes (1,2). Unmethylated CpG islands also commonly span the promoter regions of tumour suppressor genes (genes controlling cell growth) and the lack of methylation is critical to ensure active gene expression and an open chromatin structure (Fig. 2).



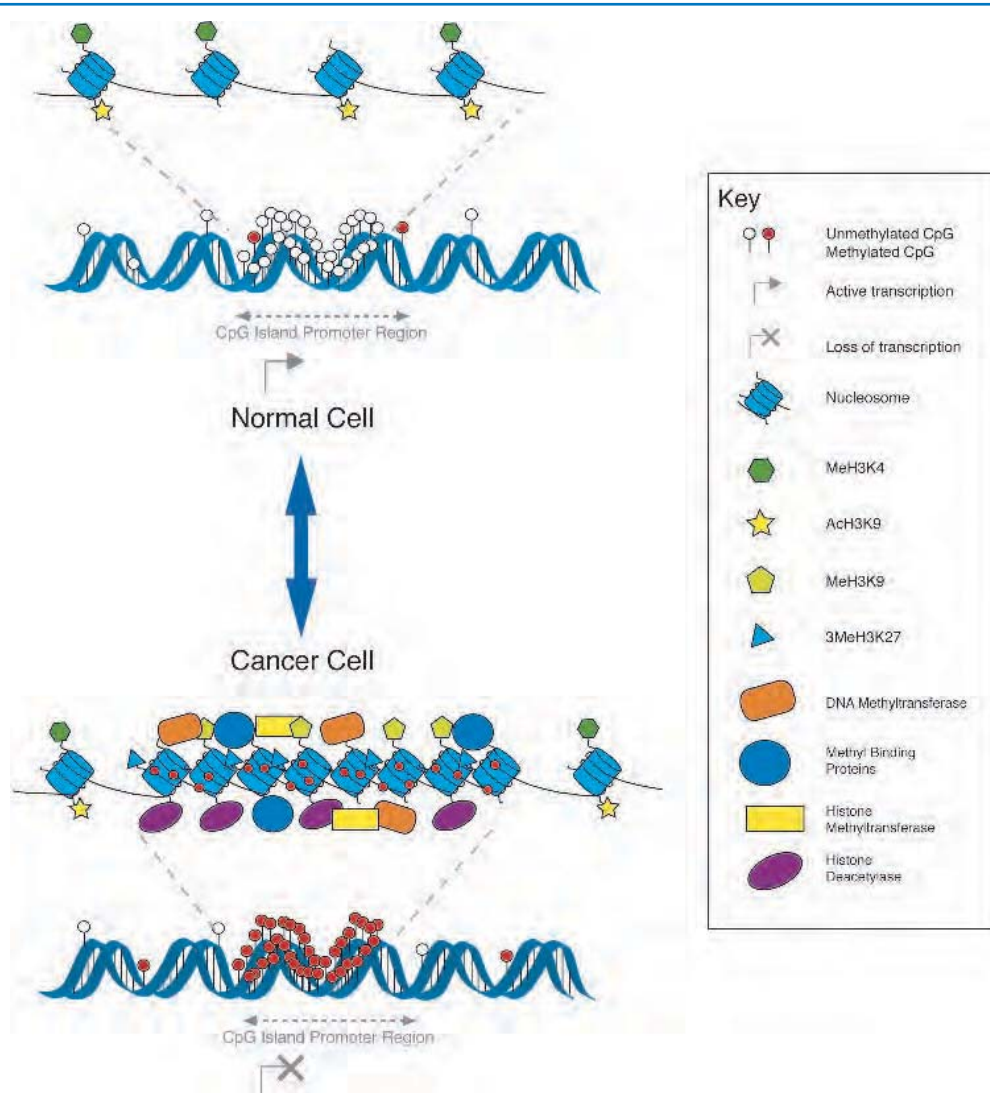
**Fig. 1. Zooming in on epigenetic targets.**

This model depicts the many levels of chromatin packaging that give rise to the highly condensed chromosome. The two main epigenetic modifications that occur in gene silencing are modifications of the histone tails (chromatin remodelling) and methylation of CpG residues (DNA methylation).

In the cancer cell, dramatic and opposing changes to the epigenetic landscape often occur and these changes consist of global hypomethylation of the junk DNA and localised hypermethylation of CpG island-associated promoters (3,4) (Fig. 2). DNA methylation of the repeated fraction of DNA in a normal cell results in transcriptional silencing and is thought to be one of the mechanisms involved in suppression of foreign DNA and potentially non-coding RNA. Genome-wide demethylation in cancer lifts transcriptional restriction, prompting ectopic gene activation (5,6), for example, activation of oncogene or viral gene expression. Inappropriate demethylation of intergenic regions is also linked with an increased susceptibility to genetic instability, which is a common feature of cancer cells. Conversely, when unmethylated

CpG island promoters associated with the tumour suppressor genes become hypermethylated in the cancer cell, gene expression is restricted and the associated chromatin remodelled into a repressive state.

Exactly what the initiating signal is that promotes epigenetic silencing is currently unknown, but there are two main opposing 'chicken and egg' hypotheses. In the first hypothesis, DNA hypermethylation triggers aberrant tumour suppressor gene silencing in cancer (7,8). In the second hypothesis, aberrant tumour suppressor gene silencing occurs early in tumourigenesis and it is this gene silencing that promotes the recruitment of the DNA methylation machinery and subsequent DNA hypermethylation (9-12). It is possible that both mechanisms occur and are gene and cell type dependent.



**Fig. 2. Epigenetic modifications occurring in a tumour suppressor gene during the transition from a normal cell to a cancer cell.**

In the normal cell, actively expressing tumour suppressor genes are associated with open chromatin, typically marked by active histone marks such as H3K4 methylation and H3K9 acetylation. The CpG island associated promoter region is characteristically unmethylated (open circles). However, in the cancer cell, tumour suppressor gene silencing is associated with compacted chromatin, typically bound by repressive proteins, such as histone deacetylases (HDAC) and histone methyltransferases (HMT), which deacetylate H3K9, and methylate H3K9 and H3K27 residues, respectively. These are recruited in conjunction with methyl binding proteins (MBD), which cooperatively work to enforce the silenced, compacted state. The CpG island associated promoter region is associated with extensive DNA hypermethylation (red circles).

Table 1. Genes that are epigenetically regulated by DNA hypomethylation or hypermethylation in breast carcinogenesis.

Gene	Official Gene Name	Hypo/hyper	Gene	Official Gene Name	Hypo/hyper
1. ARH1	LDLRAP1 (low density lipoprotein receptor adaptor protein 1)	Hypo	54. LATS1/LATS2	Large tumour suppressor, homolog 1/2	Hyper
2. BCSG1	Synuclein (breast cancer-specific protein 1)	Hypo	55. MCJ		Hyper
3. CAV1	Caveolin 1	Hypo	56. MGMT	O-6-methylguanine-DNA methyltransferase	Hyper
4. CDH3	Cadherin 3, P-cadherin	Hyper	57. MLH1	MutL homolog 1, colon cancer, nonpolyposis type 2	Hyper
5. NAT1	N-acetyltransferase 1	Hypo	58. MYOD1	Myogenic differentiation	Hyper
6. SAT2	Spermidine/spermine N1-acetyltransferase family member 2	Hypo	59. PAX5	Paired box 5	Hyper
7. UPA	Plasminogen activator, urokinase	Hypo	60. PCDH10	Protocadherin 10	Hyper
8. 14-3-3sigma	Stratifin (SFN)	Hyper	61. PGR	Progesterone receptor	Hyper
9. ABCB1	ATP-binding cassette, sub-family B, member 1	Hyper	62. PLAGL1	Pleiomorphic adenoma gene-like 1	Hyper
10. AK5	Amnionless homolog	Hyper	63. PTEN	Phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	Hyper
11. AMN		Hyper	64. PTGS2	Prostaglandin-endoperoxide synthase2 (Cox-2)	Hyper
12. APC	Adenomatous Polyposis Coli	Hyper	65. RAD9	RAD9 homolog A	Hyper
13. BCL2		Hyper	66. RAR-β	Retinoic acid receptor, beta	Hyper
14. BRCA1	Breast Cancer 1, early onset	Hyper	67. RARRES1	Retinoic acid receptor responder (tazarotene induced) 1	Hyper
15. CALCA	Calcitonon-related polypeptide alpha	Hyper	68. RASSF1A	Ras associated domain family 1	Hyper
16. CCND2	Cyclin D2	Hyper	69. RBP1	Retinol binding protein 1	Hyper
17. CDCP1	CUB domain containing protein 1	Hyper	70. RIZ1	PR domain containing 2, with ZNF domain (PRDM2)	Hyper
18. CDH1	Cadherin 1, E-cadherin	Hyper	71. RNR1	Mitochondrially encoded 12S RNA	Hyper
19. CDH13	Cadherin 13, H-cadherin	Hyper	72. ROBO1	Roundabout, axon guidance receptor, homolog 1	Hyper
20. CDKN1C	P57	Hyper	73. RUNX3	Runt-related transcription factor 3	Hyper
21. CDKN2A	Cyclin-dependent kinase inhibitor 2A (p16, p14ARF)	Hyper	74. SCGB3A1	Secretoglogin, family 3A, member 1 (HIN-1)	Hyper
22. CEBPD	CCAAT?enhancer binding protein	Hyper	75. SERPINB5	Serpin peptidase inhibitor, clade B, member 5	Hyper
23. CLCA2	Chloride channel, calcium activated, family member 2	Hyper	76. SFRP1	Secreted frizzled-related protein 1	Hyper
24. CST6	Cystatin E/M	Hyper	77. SIM1	Single-minded homolog 1	Hyper
25. Cx26	Connexin 26	Hyper	78. SLIT2	Slit homolog 2	Hyper
26. CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	Hyper	79. SOCS1	Suppressor of cytokine signalling 1	Hyper
27. DAB2	Disabled homolog 2, mitogen-responsive phosphoprotein	Hyper	80. SRBC	Protein kinase C, delta binding protein (PRKCDBP)	Hyper
28. DAL1	Erythrocyte membrane protein band 4.1-like 3	Hyper	81. SULT1A1	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	Hyper
29. DAPK	Death Associated Protein Kinase 1	Hyper	82. SYK	Spleen tyrosine kinase	Hyper
30. DCC	Deleted in colorectal carcinoma	Hyper	83. TDH	L-threonine dehydrogenase	Hyper
31. DLC1	Deleted in liver cancer 1	Hyper	84. TFF1	Trefoil factor 1	Hyper
32. DSC3	Desmocollin 3	Hyper	85. TGF-β R2	Transforming growth factor β receptor 2	Hyper
33. ESR1	Estrogen Receptor 1	Hyper	86. THBS1	Thrombospondin 1	Hyper
34. ESR2	Estrogen Receptor 2	Hyper	87. TIMP3	TIMP metalloproteinase inhibitor 3	Hyper
35. FHIT	Fragile histidine triad gene	Hyper	88. TMEFF2	Transmembrane protein with EGF-like and two follistatin-like domains 2	Hyper
36. FOXA2	Forkhead box A2	Hyper	89. TMS1	PYD and CARD domain containing (PYCARD)	Hyper
37. GPC3	Glypican 3	Hyper	90. TNFRSF12	Tumour necrosis factor receptor superfamily, member 25	Hyper
38. GREM1	Gremlin 1	Hyper	91. TPM1	Tropomyosin 1	Hyper
39. GSTP1	Glutathione S-transferase pi	Hyper	92. TSC1	Tuberous sclerosis 1	Hyper
40. HIC-1	Hypermethylated in cancer 1	Hyper	93. TSC2	Tuberous sclerosis 2	Hyper
41. HOXA5	Homeobox A5	Hyper	94. TSLC1		Hyper
42. HOXD11	Homeobox D11	Hyper	95. TSPAN-2	Tetraspanin 2	Hyper
43. HRAS	Harvey rat sarcoma viral oncogene homolog	Hyper	96. TWIST1	Twist homolog 1	Hyper
44. HS3ST2	Heparan sulfate 3-O-sulfotransferase 2	Hyper	97. TYMS	Thymidylate synthetase	Hyper
45. HSD17B4	Hydroxysteroid (17-beta) dehydrogenase 4	Hyper	98. WIF1	WNT inhibitory factor 1	Hyper
46. hTERT	Telomerase reverse transcriptase	Hyper	99. WRN	Werner syndrome	Hyper
47. ID4	Inhibitor of DNA binding 4	Hyper	100. WT-1	Wilms tumour 1	Hyper
48. IGFBP3	Insulin-like growth factor binding protein 3	Hyper	101. XT3	Solute carrier family 6, member 20 (SLC6A20)	Hyper
49. KLK10	Kallikrein-related peptidase 10 (NES1)	Hyper			
50. KLK6	Kallikrein-related peptidase 6	Hyper			
51. LAMA3	Laminin, alpha 3	Hyper			
52. LAMB3	Laminin, beta 3	Hyper			
53. LAMC2	Laminin, gamma 2	Hyper			

transforming growth factor beta (TGF- $\beta$ ) signalling pathway in breast cancer. This suppression was found to be associated with repressive histone modifications, independent of DNA methylation, and resulted in functional disruption of the signalling pathway. Although there had been isolated reports of TGF- $\beta$  associated genes being epigenetically regulated in cancer, this study was the first to describe concordant epigenetic suppression of multiple genes belonging to the same pathway. In clinical terms, these multigene approaches provide great promise for future cancer management, as it provides a broader target for diagnosis and cancer therapy.

### Cancer Detection and Therapeutic Opportunities

Tumour suppressor genes silenced by DNA methylation provide an attractive target in cancer diagnosis. For example, the CpG island promoter region spanning the *GSTP1* gene is silenced and aberrantly methylated in prostate tumour cells, but is expressed and unmethylated in normal prostate (28). The *GSTP1* methylation signature has been developed into a highly sensitive assay that distinguishes between normal cells and prostate tumour cells that are circulating in the blood of at risk patients (28,29). Methylation assays can be highly sensitive, in this case detecting as low as 10 prostate tumour cells in 1mL of blood, thus representing a powerful way to potentially detect prostate cancer. The *GSTP1* methylation assay is also undergoing trials as a biomarker to monitor patients undergoing therapy. Early data indicate that *GSTP1* methylation in circulating prostate cells and free DNA can detect cancer relapse prior to prostate specific antigen elevation and is proving encouraging as an early indicator of relapse (Clark, unpublished).

Unlike genetic changes, epigenetic changes associated with cancer initiation and progression are potentially reversible with drugs that are designed to target the numerous enzymes involved in gene suppression. These agents could potentially remove the epigenetic changes to the cell, causing the cancer cell to revert back to a normal cell. This provides significant promise for cancer therapy, and there are currently a number of compounds that show promise as epigenetic therapeutics. DNA demethylating agents, such as Vidaza® and decitabine, do not target cells for immediate death like chemotherapeutic drugs. Rather, they are incorporated into the DNA of proliferating cells and reactivate methylated silenced genes (30). There are many DNA methylation and HDAC inhibitors currently in pre-clinical and clinical trials, with the most effective analogues being directed at haematological malignancies. Promise for solid tumour epigenetic therapy may come from combining DNA demethylation drugs with HDAC inhibitors (30,31). Epigenetic drugs may also prove beneficial when combined with conventional chemotherapeutics and immunotherapy, but it is only with a complete understanding of the molecular events underpinning cancer initiation and progression that we will be able to effectively combat this 'disease of the DNA'.

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