MOLECULAR ORIGINS OF CANCER

Epigenetics in Cancer

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CLASSIC GENETICS ALONE CANNOT EXPLAIN THE DIVERSITY OF PHENOTYPES WITHIN A POPULATION. NOR DOES CLASSIC GENETICS EXPLAIN HOW, DESPITE THEIR IDENTICAL DNA SEQUENCES, MONOZYGOTIC TWINS OR CLONED ANIMALS CAN HAVE DIFFERENT PHENOTYPES AND DIFFERENT SUSCEPTIBILITIES TO A DISEASE. THE CONCEPT OF EPIGENETICS OFFERS A PARTIAL EXPLANATION OF THESE PHENOMENA. FIRST INTRODUCED BY C.H. WADDINGTON IN 1939 TO NAME “THE CAUSAL INTERACTIONS BETWEEN GENES AND THEIR PRODUCTS, WHICH BRING THE PHENOTYPE INTO BEING,” EPGENETICS WAS LATER DEFINED AS HERITABLE CHANGES IN GENE EXPRESSION THAT ARE NOT DUE TO ANY ALTERATION IN THE DNA SEQUENCE.

The best-known epigenetic marker is DNA methylation. The initial finding of global hypomethylation of DNA in human tumors was soon followed by the identification of hypermethylated tumor-suppressor genes, and then, more recently, the discovery of inactivation of microRNA (miRNA) genes by DNA methylation. These and other demonstrations of how epigenetic changes can modify gene expression have led to human epigenome projects and epigenetic therapies. Moreover, we now know that DNA methylation occurs in a complex chromatin network and is influenced by the modifications in histone structure that are commonly disrupted in cancer cells.

Epigenetic research uses powerful techniques for the study of DNA methylation, such as sodium bisulfite modification associated with polymerase-chain-reaction procedures. Terms used in epigenetic research are defined in the Glossary. Comprehensive epigenomic techniques have yielded preliminary descriptions of the epigenomes of human cancer cells. This review summarizes new developments concerning hypermethylation of the promoter regions of tumor-suppressor genes and describes possible applications of epigenetics to the treatment of patients with cancer.

EPIGENETIC FEATURES OF A NORMAL CELL

DNA methylation has critical roles in the control of gene activity and the architecture of the nucleus of the cell. In humans, DNA methylation occurs in cytosines that precede guanines; these are called dinucleotide CpGs. CpG sites are not randomly distributed in the genome; instead, there are CpG-rich regions known as CpG islands, which span the 5′ end of the regulatory region of many genes. These islands are usually not methylated in normal cells. The methylation of particular subgroups of promoter CpG islands can, however, be detected in normal tissues.

DNA methylation is one of the layers of control of certain tissue-specific genes, such as MASPIN, a member of the serum protease inhibitor family, and germ-line genes such as the MAGE genes, which are silent in almost all tissues except malignant tumors. Genomic imprinting also requires DNA hypermethylation at one of the two parental alleles of a gene to ensure monoallelic expression, and a similar gene-dosage reduction is involved in X-chromosome inactivation in females.
hypermethylation of repetitive genomic sequences probably prevents chromosomal instability, translocations, and gene disruption caused by the reactivation of transposable DNA sequences.\textsuperscript{32} Cells that lack the stabilizing effect of DNA methylation because they have spontaneous defects in DNA methyltransferases (DNMTs)\textsuperscript{33} or experimentally disrupted DNMTs\textsuperscript{34} have prominent nuclear abnormalities.

DNA methylation occurs in the context of chemical modifications of histone proteins.\textsuperscript{35} Histones are not merely DNA-packaging proteins, but molecular structures that participate in the regulation of gene expression. They store epigenetic information through such post-translational modifications as lysine acetylation, arginine and lysine methylation, and serine phosphorylation. These modifications affect gene transcription and DNA repair. It has been proposed that distinct histone modifications form a “histone code.”\textsuperscript{36} Acetylation of histone lysines, for example, is generally associated with transcriptional activation.\textsuperscript{15,16} The functional consequences of the methylation of histones depends on the type of residue — lysine (K) or arginine — and the specific site that the methylation modifies (e.g., K4, K9, or K20).\textsuperscript{15,16} Methylation of H3 at K4 is closely linked to transcriptional activation,\textsuperscript{37} whereas methylation of H3 at K9 or K27 and of H4 at K20 is associated with transcriptional repression. What emerges from these findings is a flexible but precise pattern of DNA methylation and histone modification that is essential for the physiologic activities of cells and tissues.

### DNA Hypomethylation in Tumors

The low level of DNA methylation in tumors as compared with the level of DNA methylation in their normal-tissue counterparts was one of the first epigenetic alterations to be found in human cancer.\textsuperscript{5} The loss of methylation is mainly due to hypomethylation of repetitive DNA sequences and demethylation of coding regions and introns — regions of DNA that allow alternative versions of the messenger RNA (mRNA) that is transcribed from a gene.\textsuperscript{38} A recent large-scale study of DNA methylation with the use of genomic microarrays has detected extensive hypomethylated genomic regions in gene-poor areas.\textsuperscript{24} During the development of a neoplasm, the degree of hypomethylation of genomic DNA increases as the lesion progresses from a benign proliferation of cells to an invasive cancer\textsuperscript{99} (Fig. 1).

Three mechanisms have been proposed to ex-
plain the contribution of DNA hypomethylation to the development of a cancer cell: generation of chromosomal instability, reactivation of transposable elements, and loss of imprinting. Undermethylation of DNA can favor mitotic recombination, leading to deletions and translocations, and it can also promote chromosomal rearrangements. This mechanism was seen in experiments in which the depletion of DNA methylation by the disruption of DNMTs caused aneuploidy. Hypomethylation of DNA in malignant cells can reactivate intragenomic endoparasitic DNA, such as L1 (long interspersed nuclear elements), and Alu (recombinogenic sequence) repeats. These undermethylated transposons can be transcribed or translocated to other genomic regions, thereby further disrupting the genome.

The loss of methyl groups from DNA can also disrupt genomic imprinting. In the hereditary Beckwith–Wiedemann syndrome (a syndrome characterized by exomphalos, macroglossia, and gigantism), for example, there is loss of imprinting of IGF2 (the insulin-like growth factor gene) and an increased risk of cancer. Loss of imprinting of IGF2 is also a risk factor for colorectal cancer, and disrupted genomic imprinting contributes to the development of Wilms’ tumor. In animal models, mice with a loss of imprinting of
Hypermethylation of the CpG islands in the promoter regions of tumor-suppressor genes is a major event in the origin of many cancers. The initial reports of hypermethylation of the CpG islands in the promoter region of the retinoblastoma tumor-suppressor gene (Rb) were followed by the findings that hypermethylation of the CpG island was a mechanism of inactivation of the tumor-suppressor genes VHL (associated with von Hippel–Lindau disease), p16 (a homologue of MutL homologue 2 [EZH2], a component of the polycomb family of gene-silencing proteins, and BRCA1 (breast-cancer susceptibility gene 1). Hypermethylation of the CpG-island promoter can affect genes involved in the cell cycle, DNA repair, the metabolism of carcinogens, cell-to-cell interaction, apoptosis, and angiogenesis, all of which are involved in the development of cancer. Hypermethylation occurs at different stages in the development of cancer and in different cellular networks, and it interacts with genetic lesions (Table 1). Such interactions can be seen when hypermethylation inactivates the CpG island of the promoter of the DNA-repair genes hMLH1, BRCA1, MGMT (O6-methylguanine–DNA methyltransferase), and the gene associated with Werner’s syndrome (WRN). In each case, silencing of the DNA-repair gene blocks the repair of genetic mistakes, thereby opening the way to neoplastic transformation of the cell.

The profiles of hypermethylation of the CpG islands in tumor-suppressor genes are specific to the cancer type (Fig. 2 and Table 1). Each tumor type can be assigned a specific, defining DNA “hypermethylome.” Such patterns of epigenetic inactivation occur not only in sporadic tumors but also in inherited cancer syndromes, in which hypermethylation can be the second lesion in Knudson’s two-hit model of how cancer develops. Recently devised epigenomic techniques have revealed maps of hypermethylation of the CpG islands that suggest the occurrence of 100 to 400 hypermethylated CpG islands in the promoter regions of a given tumor.

We still do not understand how CpG islands become hypermethylated in some types of cancer but not in others. Inactivation of a particular gene by methylation could give certain tumor types a growth advantage. CpG islands can have a location within a particular nucleotide sequence that allows them to become hypermethylated, or they can be located in a chromosomal region that is subject to large-scale epigenetic dysregulation. In addition, there is a mechanism in which modifications of histones mark a gene for hypermethylation. This marking occurs in the binding of the methyltransferase enhancer of zeste drosophila homologue 2 (EZH2), a component of the polycomb family of gene-silencing proteins, to histones in stem cells with unmethylated gene promoters and in the histone-associated silencing of p16 in colon-cancer cells.

Mass spectrometry, the most reliable method for detecting changes in histones, is time-consuming and highly specialized. Moreover, histone modifications occur in different histone proteins, histone variants (e.g., H3.3), and histone residues such as lysine, arginine, and serine. These modifications

**Inactivation of Tumor-Suppressor Genes**

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**Histone Modifications of Cancer Cells**

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also involve different chemical groups (e.g., methyl, acetyl, and phosphate) and have different degrees of methylation (e.g., monomethylation, dimethylation, and trimethylation). Acetylation and methylation of histones have direct effects on a variety of nuclear processes, including gene transcription, DNA repair, DNA replication, and the organization of chromosomes. Generally, histone acetylation is associated with transcriptional activation,15,16 but the effect of histone methylation depends on the type of amino acid and its position in the histone tail.15,16 The many permutations and combinations form a complex web of histone modifications.

Hypermethylation of the CpG islands in the promoter regions of tumor-suppressor genes in cancer cells is associated with a particular combination of histone markers: deacetylation of histones H3 and H4, loss of H3K4 trimethylation, and gain of H3K9 methylation and H3K27 trimethylation.23,64 The presence of the hypo-acetylated and hypermethylated histones H3 and H4 silences certain genes with tumor-suppressor–like properties, such as p21WAF1, despite the absence of hypermethylation of the CpG island. In human tumors generally, modifications of histone H4 entail a loss of monoacetylated and trimethylated forms.18 These changes appear early and accumulate dur-

| Table 1. Epigenetic Aberrations among Different Tumor Types. |
|---------------------------------------------------------------|
| **Type of Cancer** | **Epigenetic Disruption** |
| Colon cancer | CpG-island hypermethylation (hMLH1, p16INK4a, p14ARF, RARB2, SFRP1, and WRN), hypermethylation of miRNAs (miR-124a), global genomic hypomethylation, loss of imprinting of IGF2, mutations of histone modifiers (EP300 and HDAC2), diminished monoacetylated and trimethylated forms of histone H4 |
| Breast cancer | CpG-island hypermethylation (BRCA1, E-cadherin, TMS1, and estrogen receptor), global genomic hypomethylation |
| Lung cancer | CpG-island hypermethylation (p16INK4a, DAPK, and RASSF1A), global genomic hypomethylation, genomic deletions of CBP and the chromatin-remodeling factor BRG1 |
| Glioma | CpG-island hypermethylation (DNA-repair enzyme MGMT, EMP3, and THBS1) |
| Leukemia | CpG-island hypermethylation (p15INK4b, EXT1, and ID4), translocations of histone modifiers (CBP, MOZ, MORF, MLL1, MLL3, and NSD1) |
| Lymphoma | CpG-island hypermethylation (p16INK4a, p73, and DNA-repair enzyme MGMT), diminished monoacetylated and trimethylated forms of histone H4 |
| Bladder cancer | CpG-island hypermethylation (p16INK4a and TPEF/HPP1), hypermethylation of miRNAs (miR-127), global genomic hypomethylation |
| Kidney cancer | CpG-island hypermethylation (VHL), loss of imprinting of IGF2, global genomic hypomethylation |
| Prostate cancer | CpG-island hypermethylation (GSTP1), gene amplification of polycomb histone methyltransferase EZH2, aberrant modification pattern of histones H3 and H4 |
| Esophageal cancer | CpG-island hypermethylation (p15INK4b and p14ARF), gene amplification of histone demethylase JMJD2C/GASC1 |
| Stomach cancer | CpG-island hypermethylation (hMLH1 and p14ARF) |
| Liver cancer | CpG-island hypermethylation (SOCS1 and GSTP1), global genomic hypomethylation |
| Ovarian cancer | CpG-island hypermethylation (BRCA1) |

* BRCA1 denotes breast-cancer susceptibility gene 1, BRG1 BRM/SWI2-related gene 1, CBP cyclic AMP response-element–binding protein (CREB)–binding protein, DAPK death-associated protein kinase, EMP3 epithelial membrane protein 3, EP300 E1A binding protein p300, EXT1 exostosin 1, EZH2 enhancer of zeste drosophila homologue 2, GASTP1 glutathione S-transferase 1, HDAC2 histone deacetylase 2, hMLH1 homologue of MutL. *Escherichia coli*, ID4 inhibitor of DNA binding 4, IGF2 insulin-like growth factor 2, JMJD2C/GASC1 Jumonji domain-containing protein 2C, MGMT O6-methylguanine–DNA methyltransferase, MLL1 mixed-lineage leukemia 1, MLL3 mixed-lineage leukemia 3, MORF monocytic leukemia zinc finger protein–related factor, MOZ monocytic leukemia zinc finger, NSD1 nuclear receptor binding SET-domain protein 1, RARB2 retinoic acid receptor β 2, RASSF1A ras association domain family protein 1, SFRP1 secreted frizzled-related protein 1, SOCS1 suppressor of cytokine signaling 1, THBS1 thrombospondin 1, TMS1 target of methylolysis-induced silencing 1, TPEF/HPP1 hyperplastic polyposis gene 1, VHL von Hippel–Lindau disease, and WRN Werner’s syndrome.
ing the development of the tumor (Fig. 1). The losses occur predominantly at the monoacetylated Lys16 and trimethylated Lys20 residues of histone H4 in association with hypomethylated repetitive DNA sequences. They have been found in breast and liver cancer. In prostate cancer, weak immunohistochemical staining of two histone modifications (the dimethylation of lysine 4 and the acetylation of lysine 18 of histone H3) has been proposed as a marker of a high risk of recurrence.

There are also genetic lesions to consider in the aberrant epigenetic landscape of the cancer cell (Fig. 3 and Table 1). Expression patterns of histone-modifying enzymes distinguish cancer tissues from their normal counterparts, and they differ according to tumor type. In leukemias and sarcomas, chromosomal translocations that involve histone-modifier genes, such as histone acetyltransferases (e.g., cyclic AMP response-element-binding protein [CREB]–binding protein–monocytic leukemia zinc finger [CBP-MOZ]) and
Histone methyltransferases (e.g., mixed-lineage leukemia 1 [MLLI], nuclear-receptor binding SET-domain protein 1 [NSD1], and nuclear-receptor binding SET-domain protein 3 [NSD3]), create aberrant fusion proteins. In solid tumors, there is amplification of genes for histone methyltransferases such as EZH2, mixed-lineage leukemia 2 (MLL2), or NSD3 or a demethylase (e.g.,...
Jumonji domain-containing protein 2C (JMJD2C/GASCI).\textsuperscript{70}

**EPIGENETIC FACTORS AND miRNA**

Short, 22-nucleotide, noncoding RNAs that regulate gene expression by sequence-specific base pairing in the 3’ untranslated regions of the target mRNA are called miRNAs. The result is mRNA degradation or inhibition of translation.\textsuperscript{71} Patterns of miRNA expression are tightly regulated and play important roles in cell proliferation, apoptosis, and differentiation.\textsuperscript{71} The number of human genes known to lose activity as a result of the binding of an miRNA to the untranslated regions of the mRNA is growing rapidly.\textsuperscript{72,73}

Recent studies have shown that profiles of miRNA expression differ between normal tissues and tumor tissues and among tumor types.\textsuperscript{72-74} Down-regulation of subgroups of miRNAs, a common finding,\textsuperscript{72-74} implies a tumor-suppressor function for miRNAs\textsuperscript{72,73} as in the examples of down-regulated let-7 and miR-15/miR-16, which target the RAS and BCL2 oncoproteins, respectively.\textsuperscript{75,76}

DNA hypermethylation in the miRNA 5’ regulatory region is a mechanism that can account for the down-regulation of miRNA in tumors.\textsuperscript{12,13} In colon-cancer cells with disrupted DNMTs, hypermethylation of the CpG island does not occur in miRNAs.\textsuperscript{13} The methylation silencing of miR-124a also causes activation of the cyclin D–kinase 6 oncogene (CDK6),\textsuperscript{13} and it is a common epigenetic lesion in tumors.\textsuperscript{13}

**EPIGENETIC FACTORS AND miRNA**

The DNA-methylation and histone-modification patterns associated with the development and progression of cancer have potential clinical use. DNA hypermethylation markers are under study as complementary diagnostic tools, prognostic factors, and predictors of responses to treatment (Fig. 4). For instance, the glutathione S-transferase gene (GSTP1) is hypermethylated in 80 to 90% of patients with prostate cancer,\textsuperscript{77-79} but it is not hypermethylated in benign hyperplastic prostate tissue.\textsuperscript{80} Thus, the detection of GSTP1 methylation could help to distinguish between prostate cancer and a benign process. Hypermethylation of CpG islands can be a marker of cancer cells in all types of biologic fluids and biopsy specimens,\textsuperscript{21,81} making detection of GSTP1 methylation in urine\textsuperscript{79,82} a possible clinical application.

Analysis of hypermethylation of the CpG island has potential diagnostic applicability for carriers of high-penetrance mutations in tumor-suppressor genes. For example, identification of DNA hypermethylation in a breast-biopsy specimen from a carrier of a BRCA1 mutation could be useful when the pathological diagnosis is uncertain, because hypermethylation of the CpG island is an early event in the development of cancer.\textsuperscript{56} Analysis of several hypermethylated genes detects twice as many tumor cells in breast ductal fluids as conventional cytologic analysis,\textsuperscript{83} and hypermethylated genes can be found in exfoliated cells at different stages in the development of cervical cancer.\textsuperscript{84} The application of DNA-hypermethylation markers as tumor markers in routine clinical practice will require rapid, quantitative, accurate, and cost-effective techniques and objective criteria for selection of the genes that are applicable to different tumor types.

Hypermethylation of a tumor-suppressor gene and DNA hypermethylome profiles can be indicators of the prognosis in patients with cancer. Hypermethylation of the death-associated protein kinase (DAPK), p16\textsuperscript{INK4a}, and epithelial membrane protein 3 (EMP3) has been linked to poor outcomes in lung, colorectal, and brain cancer, respectively.\textsuperscript{22} Prognostic dendrograms similar to those in gene-expression microarray analyses, with the use of a combination of hypermethylated markers and CpG-island microarrays, have been developed.\textsuperscript{22} These epigenomic profiles are complementary to profiles of gene-expression patterns and can be developed with DNA extracted from archived material.\textsuperscript{21,22}

The hypermethylation of particular genes is potentially a predictor of the response to treatment. The methylation-associated silencing of the gene for the DNA-repair protein MGMT in gliomas is an example.\textsuperscript{85} MGMT reverses the addition of alkyl groups to the guanine base of DNA and is thus a point of attack for alkylating agents.\textsuperscript{52} Two studies have shown that the hypermethylation of MGMT is an independent predictor of a favorable response of gliomas to carmustine (BCNU)\textsuperscript{86} or temozolomide.\textsuperscript{87} These findings have been confirmed by others.\textsuperscript{88} Moreover, the hypermethylation of MGMT in untreated patents with low-grade astrocytoma and other tumor types is a marker of a poor prognosis,\textsuperscript{89,90} and it is probably related to
the accumulation of mutations in these tumors. The potential of the methylation status of MGMT and other DNA-repair genes to predict the response to chemotherapy has also been seen with cyclophosphamide (with the MGMT gene), cisplatin (with the hMLH1 gene), methotrexate (with the reduced folate carrier [RFC] gene), and irinotecan (with the WRN gene).

**Epigenetic Therapy of Cancer**

Unlike mutations, DNA methylation and histone modifications are reversible. Epigenetic alterations allow the cancer cell to adapt to changes in its microenvironment, but dormant, hypermethylated tumor-suppressor genes can be awakened with drugs (Fig. 3). It is possible to re-express DNA-demethylated genes in cancer cell lines by using demethylating agents and to rescue their functionality. DNA demethylating drugs in low doses have clinical activity against some tumors. Two such agents, 5-azacytidine (Vidaza) and 5-aza-2′-deoxycytidine (decitabine), have been approved as treatments for the myelodysplastic syndrome and leukemia. However, these demethylating agents have not yet been shown to have clinical activity against solid tumors. Histone deacetylase (HDAC) inhibitors can induce differentiation, cell-cycle arrest, and apoptosis in vitro, although it has not been possible to pinpoint a specific mechanism that explains these effects. In clinical trials, HDAC inhibitors are associated with a low incidence of adverse events. The first drug of this type, suberoylanilide hydroxamic acid (vorinostat), has been approved by the Food and Drug Administration for the treatment of cutaneous T-cell lymphoma. The efficacy of HDAC inhibitors in the treatment of other tumors is limited.

The nonspecific effects of DNA demethylating agents and HDAC inhibitors could have unintended consequences with regard to gene expression, and as a paradoxical result, they could have growth-promoting effects on a tumor. However, there are prospects for directed epigenetic-specific therapy with the use of transcription factors that target particular gene promoters.

For instance, the engineered zinc finger proteins target unique sequences in the MASPIN promoter; these proteins not only reactivate the epigenetically silenced gene but also inhibit tumor growth in vitro. Until now, therapy with DNA demethylating agents and HDAC inhibitors has been based on classic protein-coding tumor-suppressor genes, but the possibility of rescuing the growth-inhibitory effects.
of miRNAs by means of DNA-demethylation treatment\textsuperscript{12,13} suggests new epigenetic treatment strategies that are worthy of further exploration.

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\textbf{REFERENCES}

1. Fraga MF, Ballestar E, Paz MF, et al. Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A 2005;102:10066-9.
2. Humphreys D, Eggan K, Akutsu H, et al. Epigenetic instability in ES cells and cloned mice. Science 2001;293:95-7.
3. Waddington CH. Preliminary notes on the development of the wings in normal and mutant strains of drosophila. Proc Natl Acad Sci U S A 1939;25:299-307.
4. Holliday R. The inheritance of epigenetic defects. Science 1987;238:163-70.
5. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 1989;331:847-9.
6. Greger V, Passarge E, Hopping W, Messmer E, Hoesthemke B. Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. Hum Genet 1989;83:155-8.
7. Sakai T, Toguchida J, Ohtani N, Yandell DW, Rapaport JM, Dryja TP. Allele-specific hypermethylation of the retinoblastoma tumor-suppressor gene. Am J Hum Genet 1991;48:880-8.
8. Herman JG, Latif F, Weng Y, et al. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci U S A 1994;91:9700-4.
9. Merlo A, Herman JG, Mao L, et al. 5′ CpG island methylation is associated with transcriptional silencing of the tumour suppressor gene p16/CDKN2/MTS1 in human cancers. Nat Med 1995;1:686-92.
10. Herman JG, Merlo A, Mao L, et al. Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. Cancer Res 1995;55:4525-30.
11. Gonzalez-Zulueta M, Bender CM, Yang AS, et al. Methylation of the 5′ CpG island of the p16/CDKN2 tumor suppressor gene in normal and transformed human tissues correlates with gene silencing. Cancer Res 1995;55:4531-5.
12. Saito Y, Liang G, Egger G, et al. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell 2006;9:435-43.
13. Lujambio A, Rapoport S, Ballestar E, et al. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res 2007;67:1424-9. [Erratum, Cancer Res 2007;67:3492.]
14. Jones PA, Martiussen R. A blueprint for a Human Epigenome Project: the ACR Human Epigenome Workshop. Cancer Res 2005;65:11241-5.
15. Mack GS. Epigenetic cancer therapy makes headway. J Natl Cancer Inst 2006;98:1443-4.
16. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell 2007;128:693-705.
17. Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693-705.
18. Fraga MF, Ballestar E, Villar-Garea A, et al. Loss of acetylation at Lys16 and tri-methylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet 2005;37:391-400.
19. Seligson DB, Horvath S, Shi T, et al. Global histone modification patterns predict risk of prostate cancer recurrence. Nature 2005;435:426-2.
20. Herman JG, Graff JR, Myöhänen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A 1996;93:9821-6.
21. Laird PW. The power and the promise of DNA methylation markers. Nat Rev Cancer 2003;3:253-66.
22. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet 2007;8:286-98.
23. Ballestar E, Paz MF, Valle L, et al. Methyl-CpG binding proteins identify novel sites of epigenetic inactivation in human cancer. EMBO J 2003;22:6335-45.
24. Weber M, Davies JJ, Wittig D, et al. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nat Genet 2005;37:853-62.
25. Keshet I, Schlesinger Y, Farkash S, et al. Evidence for an instructive mechanism of de novo methylation in cancer cells. EMBO J 2003;22:6335-45.
26. Weber M, Davies JJ, Wittig D, et al. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nat Genet 2005;37:853-62.
27. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003;349:2042-54.
28. Weber M, Hellmann I, Stadler MB, et al. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat Genet 2006;38:149-53.
29. Herman JG, Baylin SB. Gene silencing in cancer association with promoter hypermethylation. N Engl J Med 2003;349:2042-54.
30. Weber M, Hellmann I, Stadler MB, et al. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat Genet 2006;38:149-53.
31. Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. Science 2003;300:455.
32. Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer 2004;4:143-53.
33. Fraga MF, Herranz M, Espada J, et al. A mouse skin multistage carcinogenesis model reflects the aberrant DNA methylation patterns of human tumors. Cancer Res 2004;64:5527-34.
34. Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. Science 2003;300:455.
35. Feinberg AP. Imprinting of a genomic domain of 11p15 and loss of imprinting in cancer: an introduction. Cancer Res 1999;59:Suppl:1744s-1746s.
36. Cui H, Cruz-Correa M, Giardello FM, et al. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 2003;299:1735-3.
37. Kaneda A, Feinberg AP. Loss of im-
printing of IGFl2: a common epigenetic modifier of intestinal tumor risk. Cancer Res 2005;65:11236-40.

44. Sakatani T, Kaneda A, Iacobuzio-Donahue CA, et al. Loss of imprinting of Igfl2 alters intestinal maturation and tumorigenesis in mice. Science 2005;307:976-8.

45. Holm TM, Jackson-Grusby L, Brambrink T, Yamada Y, Rideout WM III, Jænisch R. Global loss of imprinting leads to widespread tumorigenesis in adult mice. Cancer Cell 2005;8:275-85. [Errata, Cancer Cell 2005;8:433; 2006;9:69.]

46. Wu H, Chen Y, Liang J, et al. Hypomethylation-linked activation of PAx2 mediates tamoxifen-stimulated endometrial carcinogenesis. Nature 2005;438:981-5.

47. Brueckner B, Stresmann C, Kuner R, et al. The human let-7a-3 locus contains an epigenetically regulated microRNA gene with oncogenic function. Cancer Res 2007; 67:1419-23.

48. Laird PW, Jackson-Grusby L, Fazeli A, et al. Suppression of intestinal neoplasia by DNA hypomethylation. Cell 1995;81: 197-205.

49. Gaudet F, Hodgson JG, Eden A, et al. Induction of tumors in mice by genomic hypomethylation. Science 2003;300:489-92.

50. Yamada Y, Jackson-Grusby L, Linhart H, et al. Opposing effects of DNA hypomethylation on intestinal and liver carcinogenesis. Proc Natl Acad Sci U S A 2005;102:13580-5.

51. Esteller M, Silva JG, Dominguez G, et al. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 2000;92:564-9.

52. Esteller M, Herman JG. Generating mutations but preserving chromosensitivity: the role of O6-methylguanine DNA methyltransferase in human cancer. Oncogene 2004;23:1-8.

53. Agrelo R, Cheng WH, Setien F, et al. Epigenetic inactivation of the premature aging Werner syndrome gene in human cancer. Proc Natl Acad Sci U S A 2006;103:8823-7.

54. Costello JF, Frühwald MC, Smiraglia DJ, et al. Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. Nat Genet 2000;24:132-8.

55. Esteller M, Corn PG, Baylin SB, Herman JG. A gene hypermethylation profile of human cancer. Cancer Res 2001;61:3225-31.

56. Esteller M, Fraga MF, Guo M, et al. DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis. Hum Mol Genet 2001;10:3001-7.

57. Grady WM, Willis J, Guilford IJ, et al. Methylation of the CDH1 promoter as the causal event in gastric carcinomas. Nat Genet 2000;26:167-70.

58. Bracken AP, Pasini D, Capra M, Prosperi E, Colli E, Helin K, EZH2 is downregulated in prostate cancer. Nat Genet 2000;26:16-7. [Erratum, Nat Genet 2000;26:16-7.]

59. Biré E, Brenner C, Deplus R, et al. The Polycomb group protein EZH2 directly controls DNA methylation. Nature 2006; 439:871-4. [Erratum, Nature 2007;446:824.]

60. Schlesinger Y, Straussman R, Keshet I, et al. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. Nat Genet 2007;39:232-6.

61. Widschwendter M, Fiegl H, Egle D, et al. Epigenetic stem cell signature in cancer. Nat Genet 2007;39:157-8.

62. Ohm JE, McGarvey KM, Yu X, et al. A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. Nat Genet 2007;39:237-42.

63. Bachman KE, Park BH, Rhee I, et al. Histone modifications and silencing prior to DNA methylation of a tumor suppressor gene. Cancer Cell 2003;3:89-95.

64. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128:683-92.

65. Richon VM, Sudhoff TW, Rifkind RA, Marks PD. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. Proc Natl Acad Sci U S A 2000;97:10014-9.

66. Tryndyak VP, Kovalchuk O, Pogribny IP. Loss of DNA methylation and histone H4 lysine 20 trimethylation in human breast cancer cells is associated with aberrant expression of DNA methyltransferase 1, SUV4-20h2 histone methyltransferase and methyl-binding proteins. Cancer Biol Ther 2006;5:65-70.

67. Pogribny IP, Ross SA, Tryndyak VP, Pogribna M, Poier UA, Karpinets TV. Histone H3 lysine 9 and H4 lysine 20 trimethylation and the expression of SUV-4-20h2 and SUV-39h1 histone methyltransferases in hepatocarcinogenesis induced by methyl deficiency in rats. Carcinogenesis 2006;27:1180-6.

68. Ozdağ H, Teschendorff AE, Ahmed AA, et al. Differential expression of selected histone modifier genes in human solid cancers. BMC Genomics 2006;7:90.

69. Hoque MO, Topaloglu O, Begum S, et al. Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects. J Clin Oncol 2005;23:6569-75.

70. Fackler MJ, Malone K, Zhang Z, et al. Quantitative multiplex methylation-specific PCR analysis doubles detection of tumor cells in breast ductal fluid. Clin Cancer Res 2006;12:3306-10.

71. Feng Q, Balasubramanian A, Hawes SE, et al. Detection of hypermethylated genes in women with and without cervical neoplasia. J Natl Cancer Inst 2005; 97:273-82.

72. Esteller M, Hamilton SB, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer Res 1999;59:793-7.

73. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med 2000;344:1350-4. [Erratum, N Engl J Med 2000;344:13470.]

74. Hagi ME, Diserens A-C, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352:997-1003.

75. Haas P, Stupp R, Hugi ME, MGMT methylation status: the advent of stratified therapy in glioblastoma? Dis Markers 2007; 23:97-104.

76. Komin C, Watabane T, Katayama Y, Yoshino A, Yokoyama T, Fukushima T.
Promoter hypermethylation of the DNA repair gene O6-methylguanine-DNA methyltransferase is an independent predictor of shortened progression free survival in patients with low-grade diffuse astrocytomas. Brain Pathol 2003;13:176-84.

90. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.

91. Esteller M, Gaidano G, Goodman SN, et al. Hypermethylation of the DNA repair gene O6-methylguanine DNA methyltransferase and survival of patients with diffuse large B-cell lymphoma. J Natl Cancer Inst 2002;94:26-32.

92. Strathdee G, MacKean MJ, Illand M, Brown R. A role for methylation of the hMLH1 promoter in loss of hMLH1 expression and drug resistance in ovarian cancer. Oncogene 1999;18:2335-41.

93. Ferreri AJ, Dell’Oro S, Capello D, et al. Aberrant methylation in the promoter region of the reduced folate carrier gene is a potential mechanism of resistance to methotrexate in primary central nervous system lymphomas. Br J Haematol 2004;126:657-64.

94. Yoo CB, Jones PA. Epigenetic therapy of cancer: past, present and future. Nat Rev Drug Discov 2006;5:37-50. [Erratum, Nat Rev Drug Discov 2006;5:121.]

95. Müller CI, Rüter B, Koeffler HP, Lübbert M. DNA hypermethylation of myeloid cells, a novel therapeutic target in MDS and AML. Curr Pharm Biotechnol 2006;7:315-21.

96. Oki Y, Anki E, Issa JP. Decitabine — bedside to bench. Crit Rev Oncol Hematol 2007;61:140-52.

97. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov 2006;5:769-84.

98. Ropero S, Fraga MF, Ballestar E, et al. A truncating mutation of HDAC2 in human cancers confers resistance to histone deacetylase inhibition. Nat Genet 2006;38:566-9.

99. Marks PA, Breslow R. Dimethyl sulfoside to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. Nat Biotechnol 2007;25:84-90.

100. Moore M, Ullman C. Recent developments in the engineering of zinc finger proteins. Brief Funct Genomic Proteomic 2003;1:342-55.

101. Beltran A, Parikh S, Liu Y, et al. Reactivation of a dormant tumor suppressor gene maspin by designed transcription factors. Oncogene 2007;26:2791-8.

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