REVIEW ARTICLE

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[1] or cloned animals[2] 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,"[3] epigenetics was later defined as heritable changes in gene expression that are not due to any alteration in the DNA sequence.[4]

The best-known epigenetic marker is DNA methylation. The initial finding of global hypomethylation of DNA in human tumors[5] was soon followed by the identification of hypermethylated tumor-suppressor genes,[6-11] and then, more recently, the discovery of inactivation of microRNA (miRNA) genes by DNA methylation.[12,13] These and other demonstrations of how epigenetic changes can modify gene expression have led to human epigenome projects[14] and epigenetic therapies.[15] 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.[16-19]

Epigenetic research uses powerful techniques for the study of DNA methylation, such as sodium bisulfite modification associated with polymerase-chain-reaction procedures.[20,21] Terms used in epigenetic research are defined in the Glossary. Comprehensive epigenomic techniques[22] have yielded preliminary descriptions of the epigenomes of human cancer cells.[23-25] This review summarizes new developments concerning hypermethylation of the promoter regions of tumor-suppressor genes[26] 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.[26,27] 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.[26,27] 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,[28] and germ-line genes such as the *MAGE* genes, which are silent in almost all tissues except malignant tumors.[29] Genomic imprinting also requires DNA hypermethylation at one of the two parental alleles of a gene to ensure monoallelic expression,[30] and a similar gene-dosage reduction is involved in X-chromosome inactivation in females.[31] The

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hypermethylation of repetitive genomic sequences probably prevents chromosomal instability, translocations, and gene disruption caused by the reactivation of transposable DNA sequences.[32] Cells that lack the stabilizing effect of DNA methylation because they have spontaneous defects in DNA methyltransferases (DNMTs)[33] or experimentally disrupted DNMTs[34] have prominent nuclear abnormalities.

DNA methylation occurs in the context of chemical modifications of histone proteins.[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."[36] Acetylation of histone lysines, for example, is generally associated with transcriptional activation.[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).[15,16] Methylation of H3 at K4 is closely linked to transcriptional activation,[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.[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.[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.[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[39] (Fig. 1).

Three mechanisms have been proposed to ex-

Glossary

Acetylation: A reaction that introduces a functional acetyl group into an organic compound. Deacetylation is the removal of the acetyl group. Acetylation is a post-translational chemical modification of histones, tubulins, and the tumor suppressor p53.

Bisulfite sequencing: The bisulfite treatment of DNA in order to determine its pattern of methylation. Treatment of DNA with bisulfite converts cytosine residues to uracil but leaves 5-methylcytosine residues unaffected.

Chromatin: The complex of DNA and protein that composes chromosomes. Chromatin packages DNA into a volume that fits into the nucleus, allows mitosis and meiosis, and controls gene expression. Changes in chromatin structure are affected by DNA methylation and histone modifications.

CpG islands: Regions in DNA that contain many adjacent cytosine and guanine nucleotides. The "p" in CpG refers to the phosphodiester bond between the cytosine and the guanine. These islands occur in approximately 40% of the promoters of human genes.

DNA methylation: The addition of a methyl group to DNA at the 5-carbon of the cytosine pyrimidine ring that precedes a guanine.

DNA methyltransferases: Family of enzymes that catalyze the transfer of a methyl group to DNA, using S-adenosylmethionine as the methyl donor.

Epigenome: The overall epigenetic state of a cell.

Genomic imprinting: The epigenetic marking of a locus on the basis of parental origin, which results in monoallelic gene expression.

Histone: The main protein components of chromatin. The core histones — H2A, H2B, H3, and H4 — assemble to form the nucleosome; each nucleosome winds around 146 base pairs of DNA. The linker histone H1 locks the DNA into place and allows the formation of a higher-order structure.

Histone deacetylase: A class of enzymes that remove acetyl groups from an N-acetyl-lysine amino acid on a histone.

Transposons: Sequences of DNA that can move around within the genome of a single cell. In this process, called transposition, the sequences can cause mutations and change the organization of DNA in the genome.

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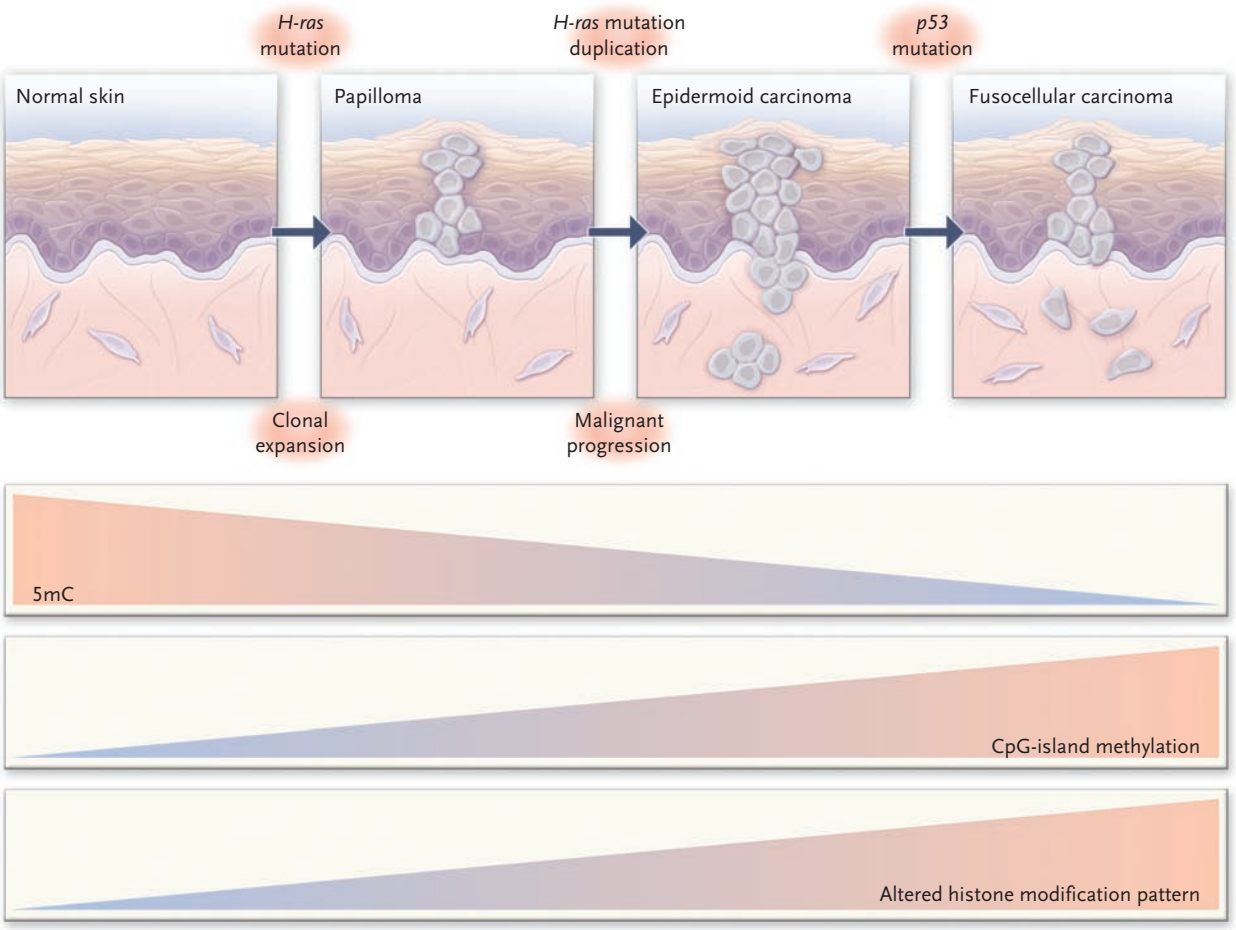

Figure 1. Epigenetic Alterations in Tumor Progression.

A multistage model of carcinogenesis in skin is shown. In conjunction with phenotypic cellular changes and the accumulation of genetic defects, there is a progressive loss of total DNA methylation content, an increased frequency of hypermethylated CpG islands, and an increased histone-modification imbalance in the development of the disease. *H-ras* denotes Harvey–ras oncogene, and 5mC 5-methylcytosine.

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,[40] 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[37] 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.[32] 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.[41] Loss of imprinting of *IGF2* is also a risk factor for colorectal cancer,[42,43] and disrupted genomic imprinting contributes to the development of Wilms' tumor.[41] In animal models, mice with a loss of imprinting of

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IGF2[44] or overall defects in imprinting[45] have an increased risk of cancer. Normally, certain testis-specific genes, genes that encode melanoma antigens, or specific proliferation-linked genes[38] are silent in somatic cells because promoter-region CpG islands are methylated. In some cancer cells, by contrast, these promoter regions undergo demethylation, and the usually repressed genes become expressed. Two notable examples of the hypomethylation mechanism are the activation of *PAX2* (a gene that encodes a transcription factor involved in proliferation and other important activities of cells) and the activation of the *let-7a-3* miRNA gene, which has been implicated in endometrial and colon cancer.[46,47]

The hypomethylation of DNA can have unpredictable effects. The progeny of a mouse deficient in DNA methylation and a Min mouse, which has a genetic defect in the adenomatous polyposis coli (*APC*) gene and is prone to colon adenoma, have fewer tumors than one would expect[48]; by contrast, another DNMT-defective mouse strain has an increased risk of lymphoma.[49] Moreover, hypomethylation suppresses the later stages of intestinal tumorigenesis[48] but promotes early precancerous lesions in the colon and liver through genomic deletions.[50]

INACTIVATION OF TUMOR-SUPPRESSOR GENES

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*)[6,7] 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), $p16^{INK4a}$,[8-11] *hMLH1* (a homologue of MutL *Escherichia coli*),[26] and *BRCA1* (breast-cancer susceptibility gene 1).[26,51]

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.[22,26] 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* (O^6-methylguanine–DNA methyltransferase), and the gene associated with Werner's syndrome (*WRN*).[26,51-53] 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[54,55] (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,[56] in which hypermethylation can be the second lesion in Knudson's two-hit model of how cancer develops.[56,57] 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.[22]

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,[24] or they can be located in a chromosomal region that is subject to large-scale epigenetic dysregulation.[22] 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,[58,59] to histones in stem cells with unmethylated gene promoters[60-62] and in the histone-associated silencing of $p16^{INK4a}$ in colon-cancer cells.[63]

HISTONE MODIFICATIONS OF CANCER CELLS

Mass spectrometry, the most reliable method for detecting changes in histones, is time-consuming and highly specialized.[22] 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

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Table 1. Epigenetic Aberrations among Different Tumor Types.*

Type of Cancer	Epigenetic Disruption
Colon cancer	CpG-island hypermethylation (*hMLH1, p16^{INK4a}, p14^{ARF}, 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 (*p16^{INK4a}*, *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 (*p15^{INK4b}, EXT1,* and *ID4*), translocations of histone modifiers (*CBP, MOZ, MORF, MLL1, MLL3,* and *NSD1*)
Lymphoma	CpG-island hypermethylation (*p16^{INK4a}, p73,* and DNA-repair enzyme *MGMT*), diminished monoacetylated and trimethylated forms of histone H4
Bladder cancer	CpG-island hypermethylation (*p16^{INK4a}* 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 (*p16^{INK4b}* and *p14^{ARF}*), gene amplification of histone demethylase *JMJD2C/GASC1*
Stomach cancer	CpG-island hypermethylation (*hMLH1* and *p14^{ARF}*)
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, *GSTP1* 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* O^6-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, *RARβ2* 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 methylation-induced silencing 1, *TPEF/HPP1* hyperplastic polyposis gene 1, *VHL* von Hippel–Lindau disease, and *WRN* Werner's syndrome.

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[65] silences certain genes with tumor-suppressor–like properties, such as *p21^{WAF1}*, 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-

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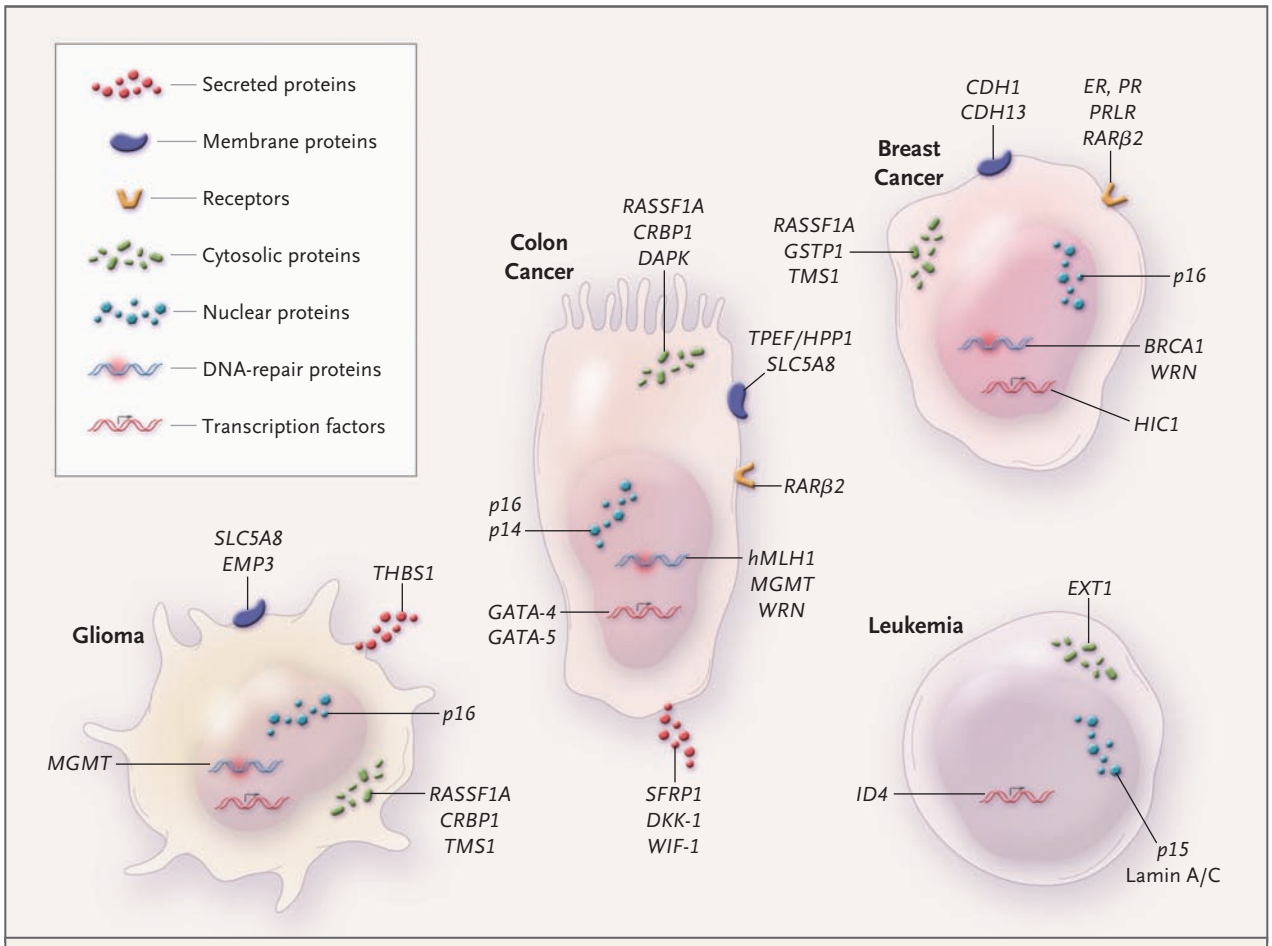

Figure 2. Profile of Hypermethylation of the CpG Island in the Promoter Region of Tumor-Suppressor Genes in Human Cancer. Four tumor cells are shown undergoing transcriptional silencing by DNA hypermethylation of the regulatory regions of tumor-suppressor genes. In colon cancer, entrance into the cell cycle occurs by means of *p16*^{INK4a} methylation. In leukemia cells, *p15*^{INK4b} methylation initiates proliferation. In breast-cancer cells, defects in DNA repair are related to methylation of *BRCA1*, and in glioma cells, methylation of O^6-methylguanine–DNA methyltransferase (*MGMT*) initiates defects in DNA repair. Other depicted hypermethylated tumor-suppressor genes are *CDH1* (cadherin 1), *CDH13* (cadherin 11), *CRBP1* (cellular retinol binding protein 1), *DKK-1* (dickkopf homologue 1), *ER* (estrogen receptor), *GATA-4* (GATA-binding protein 4), *GATA-5* (GATA-binding protein 5), *HIC1* (hypermethylated in cancer 1), *PR* (progesterone receptor), *PRLR* (prolactin receptor), *RARβ2* (retinoic acid receptor β 2), *SLC5A8* (solute carrier family 5 iodide transporter member 8), *WIF-1* (WNT inhibitory factor 1), and lamin A/C.

ing the development of the tumor[18] (Fig. 1). The losses occur predominantly at the monoacetylated Lys16 and trimethylated Lys20 residues of histone H4 in association with hypomethylated repetitive DNA sequences.[18] They have been found in breast and liver cancer.[66,67] 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.[19]

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.[68] 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

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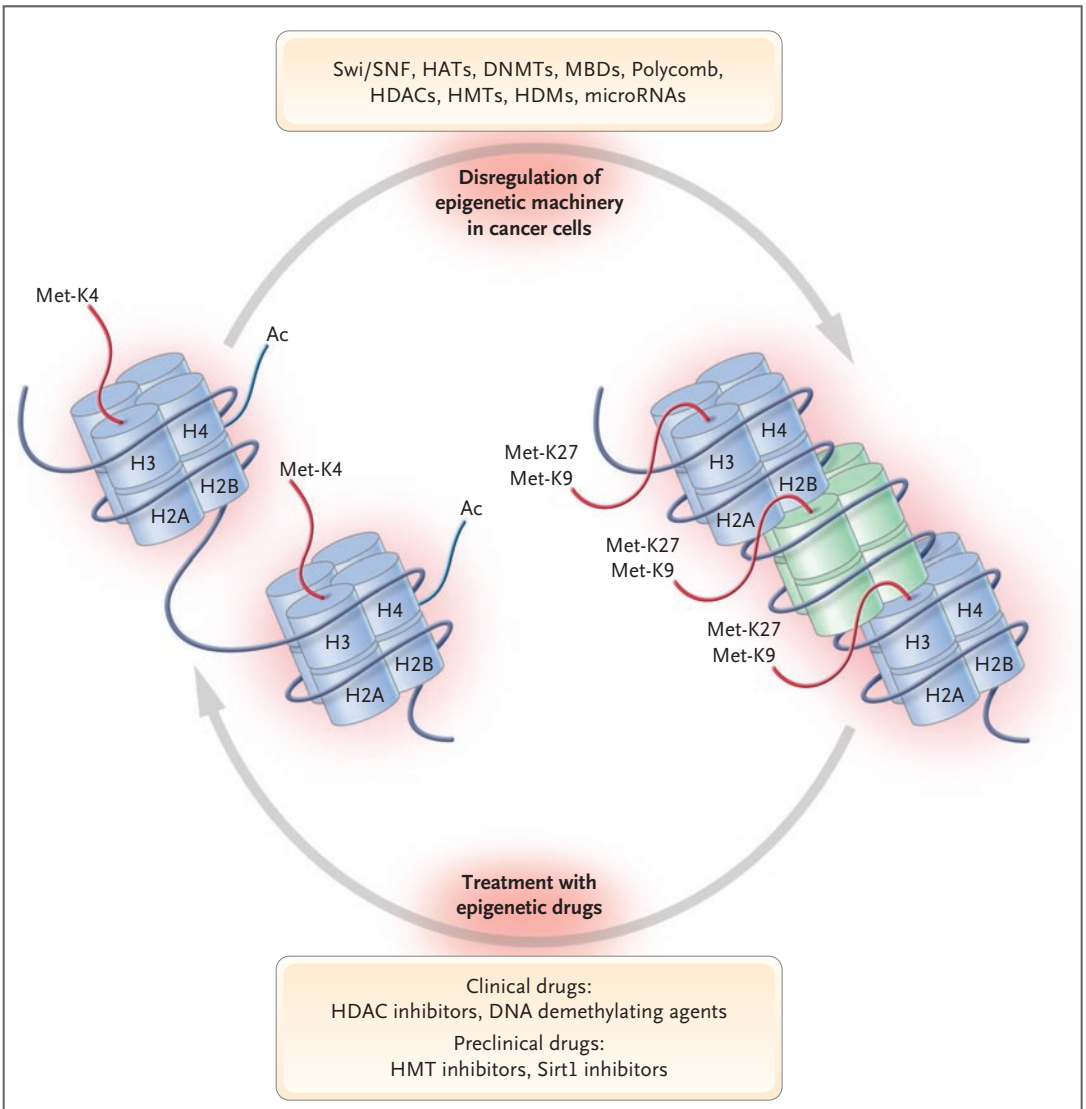

Figure 3. Epigenetic Inactivation of Tumor-Suppressor Genes.

In a normal cell, expression of the mRNA of a tumor-suppressor gene occurs in the context of an unmethylated promoter CpG island and histone modification, such as hyperacetylation and methylation of lysine 4 of histone H3. Gray cylinders indicate octamers of histones, consisting of histones H2A, H2B, H3, and H4. They form the nucleosomes, and the double strand of DNA is wrapped around them. A combination of selection and targeted disruption of the DNA methylation and histone-modifier proteins disrupts the epigenetic circumstances in the cancer cell. Epigenetic inactivation of tumor-suppressor genes is associated with dense CpG-island promoter hypermethylation and the appearance of repressive histone markers such as methylation of lysines 9 and 27 of histone H3. Epigenetic drugs can partially restore the distorted epigenetic picture by removing inactivation markers (e.g., DNA methylation) and inducing the presence of active markers (e.g., histone acetylation). AC denotes acetylation, DNMTs DNA methyltransferases, HATs histone acetyltransferases, HDAC histone deacetylase, HDMs histone demethylases, HMTs histone methyltransferases, MBDs methyl-CpG–binding domain proteins, Met-K4 methylation of lysine 4, Met-K9 methylation of lysine 9, Met-K27 methylation of lysine 27, Sirt1 sirtuin 1, and Swi/SNF switching/sucrose nonfermenting chromatin-remodeling complex.

histone methyltransferases (e.g., mixed-lineage leukemia 1 [*MLL1*], nuclear-receptor binding SET-domain protein 1 [*NSD1*], and nuclear-receptor binding SET-domain protein 3 [*NSD3*]), create aberrant fusion proteins.[69] In solid tumors, there is amplification of genes for histone methyltransferases such as *EZH2*, mixed-lineage leukemia 2 (*MLL2*), or *NSD3*[58,69] or a demethylase (e.g.,

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Jumonji domain-containing protein 2C [*JMJD2C/GASC1*]).[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.[71] Patterns of miRNA expression are tightly regulated and play important roles in cell proliferation, apoptosis, and differentiation.[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.[72,73]

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

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

EPIGENETICS IN CANCER MANAGEMENT

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,[77-79] but it is not hypermethylated in benign hyperplastic prostate tissue.[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,[21,81] making detection of *GSTP1* methylation in urine[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.[56] Analysis of several hypermethylated genes detects twice as many tumor cells in breast ductal fluids as conventional cytologic analysis,[83] and hypermethylated genes can be found in exfoliated cells at different stages in the development of cervical cancer.[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*), $p16^{INK4a}$, and epithelial membrane protein 3 (*EMP3*) has been linked to poor outcomes in lung, colorectal, and brain cancer, respectively.[22] Prognostic dendrograms similar to those used in gene-expression microarray analyses, with the use of a combination of hypermethylated markers and CpG-island microarrays, have been developed.[22] These epigenomic profiles are complementary to profiles of gene-expression patterns and can be developed with DNA extracted from archived material.[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.[85] MGMT reverses the addition of alkyl groups to the guanine base of DNA and is thus a point of attack for alkylating agents.[52] Two studies have shown that the hypermethylation of *MGMT* is an independent predictor of a favorable response of gliomas to carmustine (BCNU)[86] or temozolomide.[87] These findings have been confirmed by others.[88] Moreover, the hypermethylation of *MGMT* in untreated patents with low-grade astrocytoma and other tumor types is a marker of a poor prognosis,[89,90] and it is probably related to

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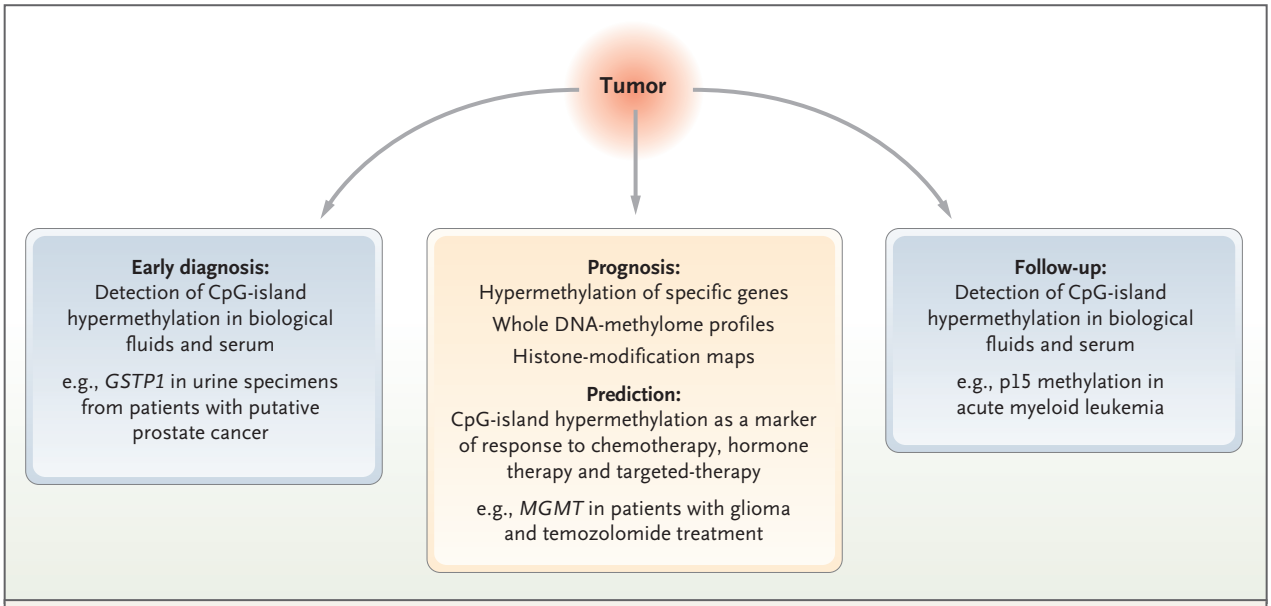

Figure 4. Epigenetics in Cancer Management.

DNA methylation techniques permit the sensitive and quantitative detection of hypermethylated tumor-suppressor genes in all types of biologic fluids and biopsy specimens. The establishment of DNA methylation and histone-modification profiles of the primary tumor specimen itself might be a valuable tool in determining the prognosis and predicting the patient's response to therapies.

the accumulation of mutations in these tumors.[90] 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),[91] cisplatin (with the *hMLH1* gene),[92] methotrexate (with the reduced folate carrier [*RFC*] gene),[93] and irinotecan (with the *WRN* gene).[53]

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-methylated genes in cancer cell lines by using demethylating agents[94] and to rescue their functionality.[22,26] 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.[15,95,96] However, these demethylating agents have not yet been shown to have clinical activity against solid tumors.[15] Histone deacetylase (HDAC) inhibitors[97] can induce differentiation, cell-cycle arrest, and apoptosis in vitro,[97] although it has not been possible to pinpoint a specific mechanism that explains these effects.[97,98] In clinical trials, HDAC inhibitors are associated with a low incidence of adverse events.[15] 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.[99] 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.[100] 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.[101] 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

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of miRNAs by means of DNA-demethylation treatment[12,13] suggests new epigenetic treatment strategies that are worthy of further exploration.

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