Cancer Susceptibility: Epigenetic Manifestation of Environmental Exposures

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Abstract: Cancer is a disease that results from both genetic and epigenetic changes. Discordant phenotypes and varying incidences of complex diseases such as cancer in monozygotic twins as well as genetically identical laboratory animals have long been attributed to differences in environmental exposures. Accumulating evidence indicates, however, that disparities in gene expression resulting from variable modifications in DNA methylation and chromatin structure in response to the environment also play a role in differential susceptibility to disease. Despite a growing consensus on the importance of epigenetics in the etiology of chronic human diseases, the genes most prone to epigenetic dysregulation are incompletely defined. Moreover, neither the environmental agents most strongly affecting the epigenome nor the critical windows of vulnerability to environmentally induced epigenetic alterations are adequately characterized. These major deficits in knowledge markedly impair our ability to understand fully the etiology of cancer and the importance of the epigenome in diagnosing and preventing this devastating disease.

Key Words: cancer epigenetics, DNA methylation, imprinting, metastable epiallele

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EPIGENETIC MECHANISMS AFFECTING HEALTH AND DISEASE

It was initially thought that complete sequencing of the human genome would result in the ready identification of the molecular events involved in human disease formation; however, it is now clear that the etiology of most human pathologic conditions will not be fully understood by simply analyzing genomic DNA sequence for genetic variants. This is because mammalian genomes are also subject to DNA methylation and histone modifications that significantly alter gene function. Thus, cell phenotype is not only dependent on its genotype but also on its unique epigenotype, which is shaped by developmental history and environmental exposures.

The role of epigenetic gene regulation in the etiology of cancer is increasingly being recognized. The most commonly described epigenetic change in cancer is an alteration in the methylation pattern of DNA cytosine residues. The covalent addition of a methyl group to the C5 position of cytosine (Fig. 1A) occurs most frequently in CpG islands (cytosine–phosphate–guanine islands), and it results in a conformational change in the major groove of DNA that alters protein binding. CpG islands are regions in the genome with a high GC content and frequent CpG occurrence.[1] The human and mouse genome projects identified approximately 15,500 and 29,000 CpG islands, respectively.[2–4] Hypermethylation of CpG-rich regions of gene promoters inhibits expression by blocking the initiation of transcription. DNA methylation is also involved in the allelic inactivation of imprinted genes, the silencing of genes on the inactive X chromosome, and the reduction of expression of transposable elements.[5,6]

DNA methyltransferase (DNMT) enzymes catalyze the covalent addition of a methyl group to the C5 position of a cytosine residue (Fig. 1A). The activities of the 2 major types of DNMT enzymes result in either DNA methylation maintenance or de novo addition of methyl groups to genomic DNA.[7] After de novo methylation, which is catalyzed by DNMT3L, DNMT3A, and DNMT3B, the DNMT1 maintenance enzyme preserves methylation at CpG sites by recognizing postreplicative, hemimethylated sequences (Fig. 1B). Because these epigenetic modifications are copied after DNA synthesis by DNMT1, they are inherited during somatic cell replication.

Epigenetic modification of cellular phenotype is also driven by alterations in chromatin structure via covalent modification of histone proteins and reorganization of nucleosomes (Fig. 2). Histone acetylation is usually associated with transcriptional activation because the affinity of acetylated histone proteins for DNA is reduced and chromatin packaging is thus relaxed. Histone methylation results in various transcriptional consequences depending on which histone is affected and which lysine residue is modified. For example, histone H3 methylation at lysine 9 is associated with hetero-

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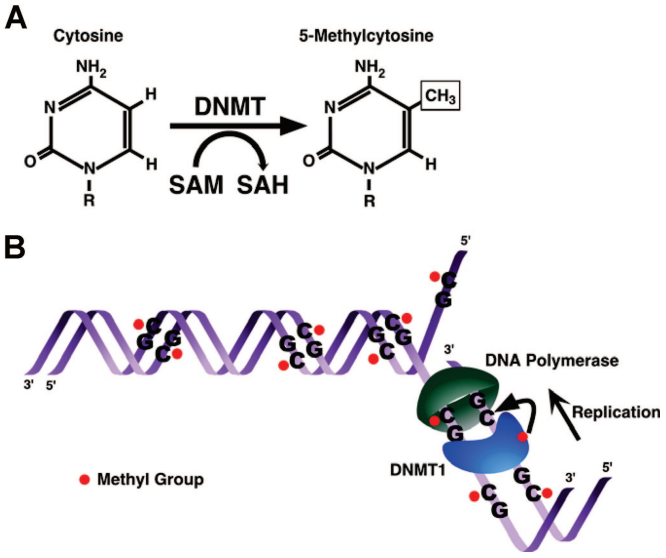

FIGURE 1. Maintenance of DNA methylation. A, Cytosine methylation is the only known biologic DNA modification. It is performed by DNMT enzymes and occurs predominantly at CpG dinucleotide pairs. The DNMT transfers a methyl group from *S*-adenosylmethionine (SAM) to the 5-carbon position of cytosine (boxed CH_3), forming 5-methylcytosine and leaving *S*-adenosylhomocysteine (SAH). B, During DNA replication, the newly synthesized daughter strand is methylated by DNMT within 1 minute of synthesis. DNMT1, the maintenance DNMT, recognizes the hemimethylated state of the parent/daughter strand duplex and copies the methylation pattern of the parent strand CpG dinucleotide onto the daughter strand. (Reprinted with permission of the author and artist, Dr. Susan Murphy.)

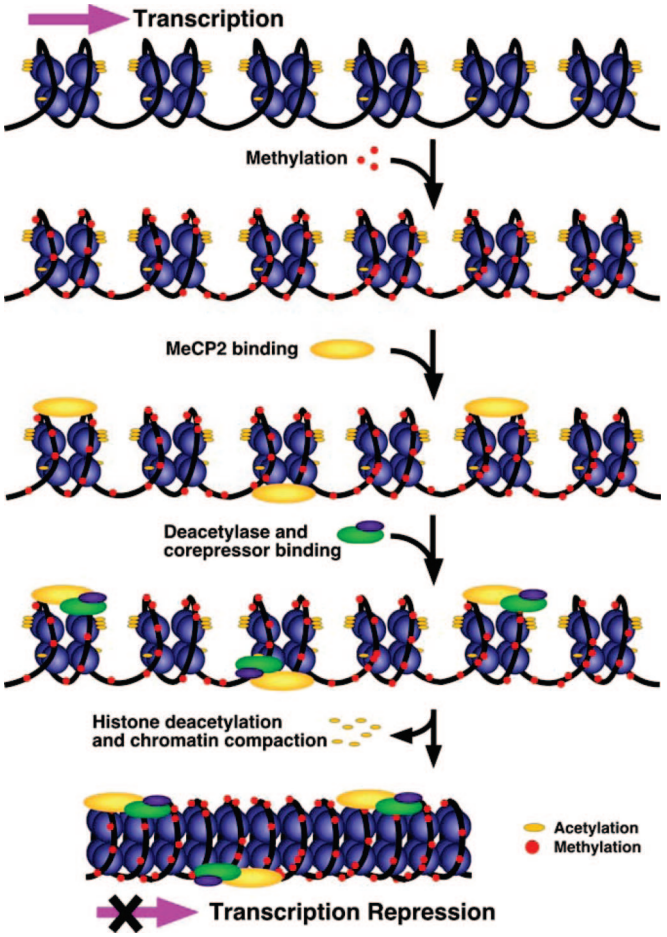

FIGURE 2. Histone acetylation and gene transcription. In transcriptionally inactive chromatin, the histones (blue spheres) lack acetyl groups and are tightly compacted with the DNA. In transcriptionally active chromatin, histones H3 and H4 are acetylated (yellow ovals) on their N-terminal tails. This posttranslational modification neutralizes the inherent positive charge of the histone tails, decreasing the affinity of the histones for the negatively charged DNA phosphate backbone, which contributes to an open chromatin structure that is receptive to interaction with transcription factors. (Adapted from Jones and Laird.[102] (Reprinted with permission of the author and artist, Dr. Susan Murphy.))

chromatin, a more compact formation of chromatin, and subsequent gene silencing. Furthermore, methylated DNA recruits methyl cytosine binding protein 2, which binds histone deacetylases (HDACs) and associated corepressor proteins, resulting in transcriptional repression. In contrast, histone H3 methylation at lysine 4 or lysine 27 is associated with transcriptional activation. Histone lysine residues may be methylated in the form of monomethylation, dimethylation, or trimethylation, adding enormous complexity to the histone code.[8] Moreover, multisubunit chromatin–protein complexes, such as the repressive polycomb group proteins or the activating SWI-SNF proteins, add yet another layer of complexity to epigenetic gene regulation (see reviews[8–10]). These epigenetic modifications therefore provide an intricate and reversible mechanism for modulating gene expression in response to intracellular or extracellular cues.

EPIGENETICS AND NEOPLASIA

Inherited and spontaneous or environmentally induced epigenetic alterations are increasingly being recognized as early molecular events in cancer formation.[11,12] Furthermore, such epigenetic alterations are potentially more adverse than nucleotide mutations because their effects on regional chromatin structure can spread, thereby affecting multiple genetic loci.[13] Transcription of a number of tumor suppressor genes such as *p16^{INK4a}*, *BRCA1*, *p53*, and *hMLH-1* has now be demonstrated to be inhibited by promoter hypermethylation.[5]

Although tumor suppressor gene silencing by DNA methylation occurs frequently in cancer, genome-wide hypomethylation is one of the earliest events to occur in the genesis of cancer.[14–16] Demethylation of the genome can lead to the reactivation of transposable elements, thereby altering the transcription of adjacent genes, the activation of oncogenes such as *H-RAS*, and biallelic expression of imprinted loci (eg, loss of *IGF2* imprinting).[14,15,17] Furthermore, genomic instability associated with the hypermethylation of the DNA mismatch repair enzyme gene *MLH1* may deregulate not only critical genes involved in the initial stages of

carcinogenesis but also those involved in the later invasion and metastasis stages of transformation.[18]

Posttranslational histone modifications such as histone deacetylation and inappropriate remodeling of chromatin structure also lead to a wide range of neoplasias, including rhabdoid tumors (influenced by mutations in the activating SWI-SNF chromatin protein complex), chronic myeloid leukemia, retinoblastoma, and lung, breast, and prostate cancer.[19–21] The human breast cancer susceptibility protein BRCA1 is a stable component of the SWI-SNF chromatin complex.[22] In cells transfected with a dominant-negative version of the SWI-SNF ATPase, BRCA1 is not able to synergize with the tumor suppressor p53, a process that has previously been demonstrated to activate transcription.[23] Additionally, mutated SWI-SNF complexes disrupt c-Myc transactivation.[24] Together, these data strongly indicate that dysregulation of chromatin remodeling activity promotes cancer. Furthermore, treatment with HDAC inhibitors, such as trichostatin-A, stimulates reactivation of the actin-binding protein gelsolin (a candidate suppressor of breast cancer).[25,26]

EPIGENETICALLY LABILE CANCER SUSCEPTIBILITY LOCI

Two distinct sets of genes that potentially link environmental exposures during pregnancy to adult disease susceptibility are imprinted genes and genes with metastable epialleles. Genes with metastable epialleles have highly variable expression because of stochastic allelic changes in the epigenome.[27] In contrast, imprinted genes have monoallelic, parent-of-origin–dependent expression, and they are functionally haploid.[28,29] This is particularly important because imprinting eliminates the protection normally afforded by diploidy against the deleterious effects of recessive mutations (Fig. 3).

Imprinted Genes

Imprinting can be deregulated in both germ cells and somatic cells. Because imprinted genes are frequently clustered and their expression is coordinately regulated by imprinting control regions, a single genetic or epigenetic change in an imprinting control region can result in the disruption of many genes. Consequently, imprinted genes are associated not only with severe complex developmental disorders such as Angelman, Beckwith-Wiedemann, and Prader-Willi syndromes but also with cancer.[28,29]

Imprinted genes are at a much greater risk of somatic cell inactivation by mutation, loss of heterozygosity, and epigenetic alterations because one allele is already inactivated by genomic imprinting (Fig. 3). The silent, imprinted allele is frequently equated to the first hit in Knudson's 2-hit hypothesis for oncogenesis (Fig. 3).[30] Because imprinted genes normally encode for either positive or negative growth effectors, they are frequently involved in the formation of a wide range of tumors.[28]

Aberrant methylation upstream of the maternally expressed *H19* gene is associated with the development of pediatric Wilms tumors in patients with Beckwith-Wiedemann syndrome because it results in loss of imprinting at the

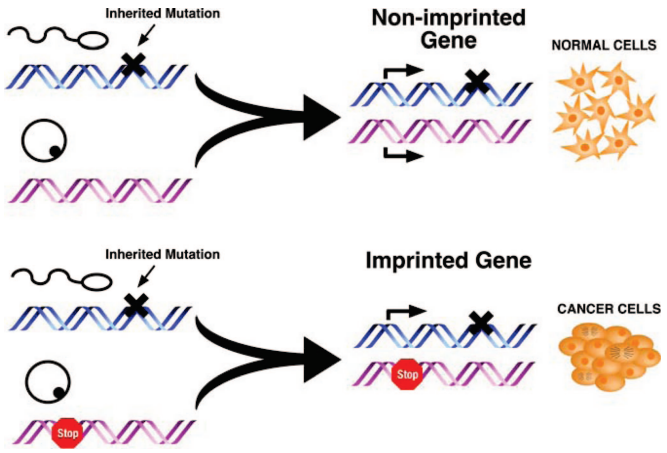

FIGURE 3. Imprinted genes as susceptibility loci in cancer. For most nonimprinted genes, an acquired mutation (X) does not contribute directly to carcinogenesis because of the presence of a second transcriptionally active wild-type allele (paternal DNA, blue; maternal DNA, pink). In contrast, imprinted genes are functionally haploid because one parental allele is epigenetically inactivated (Stop). Thus, the acquisition of a single mutation on the active allele of an imprinted tumor suppressor gene (paternal in this example) can directly result in cancer because only the defective allele is transcribed. (Reprinted with permission of the author and artist, Dr. Susan Murphy.)

IGF2 locus.[31] IGF2 biallelic expression is also involved in a number of adult-onset cancers, including colorectal carcinoma, bladder cancer, osteosarcoma, and breast cancer.[15,29,32,33] *DLK1* is overexpressed in neuroendocrine tumors, suggesting a potential oncogenic role.[34] In contrast, *ARHI*[35] and *ZAC1*[36] are imprinted genes that function as tumor suppressors in human breast and ovarian cancer. *NNAT* may also provide a tumor suppressor function because it is frequently subject to promoter hypermethylation with concomitant decreased expression in myeloid and lymphoid acute leukemia.[37]

There is also genetic evidence that *IGF2R*, which is imprinted in mice but not in humans,[38] functions as a tumor suppressor gene, as demonstrated by frequent loss of heterozygosity and mutations in a number of human[39–45] and rodent[46,47] cancers. The loss of *IGF2R* imprinting in the primate lineage (ie, humans have 2 functional alleles) indicates that humans would be more resistant to cancers involving this tumor suppressor than mice that are still imprinted at this locus.[38,48] Thus, the divergent evolution of *IGF2R* brings into question the relevance of human carcinogen risk assessments based solely on nonprimate animal studies.[28,38]

Metastable Epialleles

Metastable epialleles are genetically identical alleles that are variably expressed because of different epigenetic modifications established during early development.[27] These metastable epialleles can also be environmentally modified later in life.[49] The establishment of an epigenotype at each allele is stochastic and can be associated with widely varying phenotypes (Fig. 4). Furthermore, the epigenetic modifica-

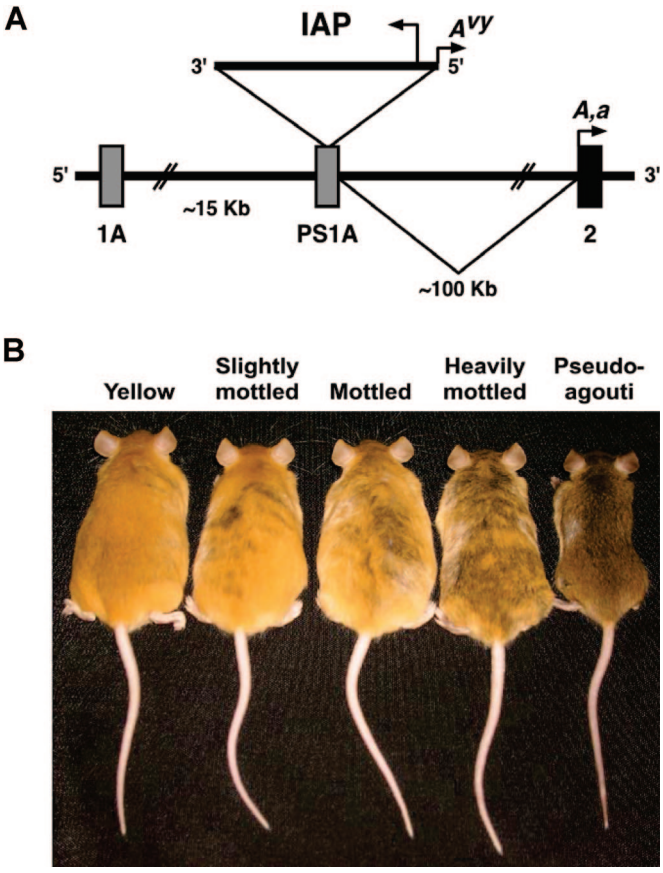

FIGURE 4. *Agouti* viable yellow (A^{vy}) metastable epiallele. A, Transcription of *A* and *a* alleles normally initiates from a hair-cycle–specific promoter in exon 2 (short arrowhead labeled *A,a*). In the A^{vy} mice, a contraoriented IAP insertion within pseudoexon 1A (PS1A) of the murine *Agouti* gene created a cryptic promoter (short arrowhead labeled A^{vy}) that can drive ectopic *Agouti* expression. Hypomethylation of this cryptic promoter causes the formation of yellow mice, whereas hypermethylation results in brown mice. B, Genetically identical 15-week-old A^{vy} mouse littermates representing 5 coat color phenotypes. (Reprinted with permission from Dolinoy et al,[75] and permission of the author and artist, Dr. Susan Murphy.)

tions regulating metastable epiallele expression are not always completely reset during genome-wide epigenetic reprogramming in gametogenesis.[50,51] This incomplete erasure can lead to transgenerational epigenetic inheritance.

Metastable epialleles are most often associated with retroelements and transgenesis. Three of the identified murine metastable epialleles (A^{vy}, $Axin^{Fu}$, and $Cabp^{IAP}$) are associated with contraoriented intracisternal A particle (IAP) retrotransposon insertions. IAP proviral elements are prevalent in the mouse genome and consist of the full-length 7-kilobase elements as well as shorter elements with deletions of varying lengths.[52] Approximately 1000 copies of IAP retrotransposons are present in the mouse genome,[52] and about 9% of the human genome contains retrotransposons.[53] IAP transcripts have been identified in most murine tissues,[52] and they are increased dramatically in DNMT knockout mice.[54] The long terminal repeats of IAPs carry promoters that initiate transcription of the IAP and in some cases adjacent host sequences,[55] contain multiple protein binding sites,[56] and are influenced by methylation status.[57] In fact, IAP hypomethylation at the 5′ long terminal repeat has been attributed to constitutive expression of IAP transcripts in many mouse tumors.[52,57]

The murine *Agouti* gene encodes a paracrine signaling molecule that promotes follicular melanocytes to produce yellow pheomelanin pigment instead of black eumelanin pigment. Transcription is normally initiated from a hair-cycle–specific promoter in exon 2 of the *Agouti* (*A*) allele (Fig. 4A). Transcription of the *Agouti* gene normally occurs only transiently in hair follicles during a specific stage of hair growth. It results in a subapical yellow band on each black hair shaft, causing the brown (agouti) coat color of wild-type mice.[58]

The A^{vy} allele resulted from the insertion of an IAP murine retrotransposon upstream of the transcription start site of the *Agouti* gene (Fig. 4A).[58,59] A cryptic promoter in the proximal end of the A^{vy} IAP promotes constitutive ectopic *Agouti* transcription, leading to yellow fur.[51,60] Moreover, CpG methylation in the A^{vy} IAP correlates inversely with ectopic *Agouti* expression. The degree of methylation varies dramatically among individual isogenic A^{vy}/a mice, causing a wide variation in coat color ranging from yellow (unmethylated IAP) to pseudoagouti (methylated IAP) (Fig. 4B).[51] Yellow animals with hypomethylation at the cryptic promoter also experience increased incidence of obesity, diabetes, and tumorigenesis, whereas genetically identical brown pseudoagouti animals with hypermethylation in the same region are protected from these pathologic conditions.[51,60,61] The identification and categorization of additional metastable epialleles in the mouse, as well as the human genome, will significantly enhance our understanding of how epigenetic lability contributes to human health and disease, including cancer.

CANCER SUSCEPTIBILITY AND THE ENVIRONMENT

The epigenome is particularly susceptible to dysregulation by environmental factors during gestation, neonatal development, puberty, and old age. In fact, age-correlated increases in DNA promoter methylation occur in a number of genes involved in cancer, including *IGF2*, *Versican*, and *PAX6*, among others.[13] Alterations in epigenotype have also been observed after adult exposure to environmental xenobiotic chemicals. Exposure of adult mice to sodium arsenite in vivo revealed decreased genomic methylation, whereas coexposure to sodium arsenite and a methyl-deficient diet showed gene-specific hypomethylation in the promoter region of the oncogenic gene, *Ha-ras*.[62] Other metals, including cadmium,[63] lead,[64] and nickel,[65] have also been shown to interact with the epigenome. In addition, decreased histone acetylation, increased histone methylation, and subsequent decreased gene expression occur after in vitro nickel exposure.[66,67] Furthermore, in vitro chromium exposure is linked to epigenetically controlled gene expression alterations via interactions with histone acetyltransferase and HDAC enzymes.[68]

Although alterations in the epigenome can occur during adulthood, it is most vulnerable to environmental factors during embryogenesis because the DNA synthetic rate is high and the elaborate DNA methylation patterning required for normal tissue development is established during early development. Endocrine active chemicals have been associated with epigenetic alterations after in utero exposures. Methylation studies conducted with the estrogenic pharmaceutical agent diethylstilbestrol (DES) demonstrated hypomethylation in the estrogen-responsive lactoferrin promoter and in the promoter and intronic regions of the c-*fos* oncogene in mice exposed in utero or in the perinatal period, respectively[69,70]; however, significant methylation changes after neonatal DES exposure were not observed in the murine abdominal B-like *Hoxa* genes, important regulators of reproductive tract development.[71]

Human epidemiologic evidence reveals that individuals exposed to DES in utero during the first 3 months of pregnancy exhibited increased incidences of reproductive disorders and the rare cancer, clear cell adenocarcinoma of the vagina.[72] Increased incidences of uncommon disorders were also seen in the granddaughters and grandsons of DES-exposed women, suggesting epigenetic transgenerational inheritance.[72] Rodent studies likewise demonstrated that the effects of maternal DES exposure are transmitted through the maternal germline to offspring via both genetic and epigenetic mechanisms.[73] Recently, the chaperone protein, Hsp90, was associated with DES-induced epigenetic gene alterations via interactions with histone proteins important for active chromatin conformations.[74]

The effects of dietary exposure to naturally occurring plant-based phytoestrogens, including the isoflavinoid genistein, are also of growing interest. We recently demonstrated that maternal genistein supplementation of mice during gestation, at levels comparable to those of humans consuming high soy diets, shifted the coat color of viable yellow Agouti (A^{vy}/a) offspring toward pseudoagouti by increasing methylation of 6 CpG sites in a retrotransposon upstream of the metastable *Agouti* gene (Fig. 4A).[75] The genistein-induced hypermethylation additionally protected A^{vy}/a animals from obesity and tumorigenesis in adulthood.

Exposure to endocrine active compounds has recently been linked with epigenetic alterations that are inherited transgenerationally even in the absence of continued exposure. Anway et al[76] reported that maternal transient exposure to the antiandrogenic fungicide vinclozolin or the estrogenic pesticide methoxychlor during reprogramming of the germline resulted in increased spermatogonial germ cell apoptosis and decreased epididymal sperm count and motility in not only the first generation but also in generations 2 through 4. The high frequency of these phenotypic changes in subsequent generations and altered methylation patterns in the sperm that persisted transgenerationally excluded genetic mutations as the cause of these pathologic conditions.

EPIGENETIC BASIS FOR CANCER TREATMENT, DIAGNOSIS, AND PREVENTION

Our enhanced understanding of the importance of epigenetics in the etiology of cancer has ushered in a new era in which treatment, diagnosis, and prevention are focused not solely on the genome but also on the epigenome. Typical cancer treatment involves either broad therapies such as surgery, radiation, and chemotherapy or more specific individualized therapy targeting specific genetic mutations and more recently epimutations. Broad epigenetic therapeutic approaches, some of which are currently approved for human use, include DNA methylation inhibition[77] and HDAC inhibition. Epigenetic therapies are also undergoing development to target directly specific sites in the genome, including the directed silencing of oncogenes via DNA methylation.

DNA methylation inhibition therapies involve both nucleoside and nonnucleoside analogs. The archetypal DNMT inhibitor 5-azacytidine is a nucleoside inhibitor that is incorporated into DNA. Upon incorporation, both cytosine and 5-azacytidine residues are methylated by DNMTs; cellular DNMT is rapidly depleted because of its inactivation by bonding in covalent protein–DNA adducts.[78] The side effects of accumulated nucleoside inhibitor enzymes, however, are often toxic, as manifested by myelosuppression with neutropenic fever.[79] Nonnucleoside DNMT inhibitors (hydralazine,[80] procainamide,[81] and epigallocatechin-3-gallate[82]) interfere with DNMT activity by binding directly to the enzymes instead of incorporating into the DNA sequence itself. Because these compounds directly block DNMT activity as opposed to covalently binding DNA, nonnucleoside DNMT inhibitors appear to be less toxic.

HDAC inhibitors are a structurally diverse group of compounds, including cyclic and noncyclic hydroamates, short-chain fatty acids, cyclic peptides, and benzamides.[83] A number of HDAC inhibitors have now been demonstrated to inhibit histone deacetylation in human tumor cells,[84–86] although the development of HDAC inhibitors is still in early preclinical stages. Treatment with nucleoside or nonnucleoside methylation inhibitors followed by HDAC inhibitors often yields additive or synergistic effects in reexpression of silenced tumor suppressor genes.[78,87] In addition, cotreatment with HDAC inhibitors and other chemotherapeutic agents results in additive and sometimes synergistic therapeutic responses.[83] Finally, HDAC inhibitor treatment may protect normal cells from the toxic side effects of ionizing radiation.[83]

Specific epimutations have been documented for a variety of cancers, and, as a consequence, novel therapeutic measures are being developed to target directly specific sites in the genome. Inhibition of inappropriate transcriptional initiation of growth factors (*IGF2*)[88] and various oncogenes, such as *BCL-2*,[89], by directed DNA methylation is a precise and heritable therapeutic alteration of the epigenome. Although not yet approved for therapeutic human use, directed epigenetic gene therapy holds tremendous promise for enhancing cancer prevention and therapy.

Epigenetic alterations not only are ideal targets for cancer therapeutics but can also serve as biomarkers for cancer diagnosis and targets for prevention. The phenomenon of interindividual variability in methylation and expression may represent a common characteristic of epigenetically labile genes in the mouse and human genomes whose expression is controlled by DNA methylation or other epigenetic

mechanisms established early in development.[49] It has been demonstrated that epigenetic discordance, including differences in DNA methylation and histone modification patterns, is present between monozygotic twins and that this discordance increases with age and is associated with differences in disease incidence[90,91]; therefore, identifying the epigenetic molecular changes associated with aging and cancer will have important implications for future diagnostic and prevention strategies.

Additionally, gestation represents a "developmental window of vulnerability" to epigenetic changes that are modifiable by nutritional and environmental factors.[59,75,92,93] In principle, epigenetic changes established during gestation and neonatal development are also reversible in adulthood, as demonstrated by studies showing that epigenetic marks influenced by neonatal maternal behavior are reversed in adulthood after methionine or HDAC inhibitor infusion[94–96]; therefore, understanding specific epigenetic mechanisms that affect clinical outcomes may ultimately enable early-life nutritional interventions to prevent adult-onset cancers in humans.[49]

PROFILING THE HUMAN EPIGENOME

As discussed here, epigenome dysregulation is implicated in a broad range of cancers.[14–16,97–99] Currently, primary cancer therapies focus on the genetic basis of how individuals respond to drug treatment. Small epigenetic variations, however, have the capability to influence, for example, the way an individual metabolizes drugs, and identifying such variations has potential applications in drug development research and in medical treatment. An individual's epigenetic profile might help predict whether a particular drug is likely to be effective or likely to cause an adverse reaction. The identification of environmentally responsive, epigenetically labile genes in both the mouse and human genomes should ultimately allow for improved diagnosis, treatment, and prevention of chronic human diseases such as cancer. One robust method for accomplishing this objective makes use of machine-learning algorithms trained to identify genomic motifs that are predictive of imprinted genes and metastable epialleles.

We recently developed such a bioinformatic approach for interrogating the entire mouse genome to identify genes with high probabilities of being imprinted.[100] A number of genes identified in this study have human homologs that show linkage to diseases. Furthermore, many of these orthologs share CpG islands or transposable elements in syntenic regions within 10 kilobases of their promoters, arguably 2 of the most straightforward target elements for epigenetic control of adjacent gene expression. Because genomic imprinting is epigenetically regulated,[28,101] these putative imprinted genes may be especially vulnerable to environmental influences. The detection of a comparable genome-wide set of epigenetically labile metastable epialleles in mice and humans will allow us to establish their role in human health and to also determine better the value of the mouse as a model for assessing human risk from agents that elicit their biologic effect primarily by altering the epigenome. Although we do predict some overlap between mouse and human candidate imprinted genes identified through our machine-learning approach, it is likely that the most significant criterion in species-specific identification will differ. This difference underscores the importance for increased caution when assessing human risk from environmental agents that alter the epigenome using rodent models; the molecular pathways targeted may be independent.

To this end, the Human Epigenome Project (http://www.epigenome.org/index.php) seeks to identify, catalog, and interpret genome-wide DNA methylation patterns of all human genes in all major tissues. Although providing a solid platform for analysis, this approach would be incomplete without further identification of environmentally responsive epigenetically labile loci in the human genome.

REFERENCES

1. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. *J Mol Biol.* 1987;196:261–282.
2. Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. *Science.* 2001;291:1304–1351.
3. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature.* 2001;409:860–921.
4. Waterston RH, Lindblad-Toh K, Birney E, et al. Initial sequencing and comparative analysis of the mouse genome. *Nature.* 2002;420:520–562.
5. Jones P, Baylin S. The fundamental role of epigenetic events in cancer. *Nat Rev Genet.* 2002;3:415–428.
6. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev.* 2002;16:6–21.
7. Goll MG, Bestor TH. Eukaryotic methyltransferases. *Annu Rev Biochem.* 2005;74:481–514.
8. Jenuwein T, Allis CD. Translating the histone code. *Science.* 2001; 293:1074–1080.
9. Cheung P, Lau P. Epigenetic regulation by histone methylation and histone variants. *Mol Endocrinol.* 2005;19:563–573.
10. Saha A, Wittmeyer J, Cairns BR. Chromatin remodelling: the industrial revolution of DNA around histones. *Nat Rev Mol Cell Biol.* 2006;7: 437–447.
11. Costa M. Model for the epigenetic mechanism of action of nongenotoxic carcinogens. *Am J Clin Nutr.* 1995;61:666S–6669S.
12. Silva Lima B, Van der Laan J. Mechanisms of nongenotoxic carcinogenesis and assessment of the human hazard. *Regul Toxicol Pharmacol.* 2000;32:135–143.
13. Issa JP. CpG-island methylation in aging and cancer. *Curr Top Microbiol Immunol.* 2000;249:101–118.
14. Esteller M, Herman JG. Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. *J Pathol.* 2002; 196:1–7.
15. Feinberg AP, Tycko B. The history of cancer epigenetics. *Nat Rev Cancer.* 2004;4:143–153.
16. Yamamoto S, Sobue T, Sasaki S, et al. Validity and reproducibility of a self-administered food-frequency questionnaire to assess isoflavone intake in a Japanese population in comparison with dietary records and blood and urine isoflavones. *J Nutr.* 2001;131:2741–2747.
17. Cruz-Correa M, Cui H, Giardiello FM, et al. Loss of imprinting of insulin growth factor II gene: a potential heritable biomarker for colon neoplasia predisposition. *Gastroenterology.* 2004;126:964–970.
18. Coleman WB, Rivenbark AG. Quantitative DNA methylation analysis: the promise of high-throughput epigenomic diagnostic testing in human neoplastic disease. *J Mol Diagn.* 2006;8:152–156.
19. Davis PK, Brackmann RK. Chromatin remodeling and cancer. *Cancer Biol Ther.* 2003;2:22–29.
20. Hendricks KB, Shanahan F, Lees E. Role for BRG1 in cell cycle control and tumor suppression. *Mol Cell Biol.* 2004;24:362–376.
21. Oruetxebarria I, Venturini F, Kekarainen T, et al. P16INK4a is required for hSNF5 chromatin remodeler-induced cellular senescence in malignant rhabdoid tumor cells. *J Biol Chem.* 2004;279:3807–3816.

22. Bochar DA, Wang L, Beniya H, et al. BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell.* 2000;102:257–265.
23. Zheng L, Li S, Boyer TG, et al. Lessons learned from BRCA1 and BRCA2. *Oncogene.* 2000;19:6159–6175.
24. Cheng SW, Davies KP, Yung E, et al. c-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function. *Nat Genet.* 1999;22:102–105.
25. Asch HL, Head K, Dong Y, et al. Widespread loss of gelsolin in breast cancers of humans, mice, and rats. *Cancer Res.* 1996;56:4841–4845.
26. Hoshikawa Y, Kwon HJ, Yoshida M, et al. Trichostatin A induces morphological changes and gelsolin expression by inhibiting histone deacetylase in human carcinoma cell lines. *Exp Cell Res.* 1994;214:189–197.
27. Rakyan VK, Blewitt ME, Druker R, et al. Metastable epialleles in mammals. *Trends Genet.* 2002;18:348–351.
28. Murphy SK, Jirtle RL. Imprinting evolution and the price of silence. *Bioessays.* 2003;25:577–588.
29. Falls J, Pulford D, Wylie A, et al. Genomic imprinting: implications for human disease. *Am J Pathol.* 1999;154:635–647.
30. Knudson AG Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A.* 1971;68:820–823.
31. Bliek J, Maas SM, Ruijter JM, et al. Increased tumour risk for BWS patients correlates with aberrant H19 and not KCNQ1OT1 methylation: occurrence of KCNQ1OT1 hypomethylation in familial cases of BWS. *Hum Mol Genet.* 2001;10:467–476.
32. Feinberg A. DNA methylation, genomic imprinting and cancer. *Curr Top Microbiol Immunol.* 2000;249:87–99.
33. Feinberg A. Cancer epigenetics takes center stage. *Proc Natl Acad Sci U S A.* 2001;98:392–394.
34. van Limpt V, Chan A, Caron H, et al. SAGE analysis of neuroblastoma reveals a high expression of the human homologue of the *Drosophila* Delta gene. *Med Pediatr Oncol.* 2000;35:554–558.
35. Yu Y, Fujii S, Yuan J, et al. Epigenetic regulation of ARHI in breast and ovarian cancer cells. *Ann N Y Acad Sci.* 2003;983:268–277.
36. Piras G, El Kharroubi A, Kozlov S, et al. Zac1 (Lot1), a potential tumor suppressor gene, and the gene for ε-sarcoglycan are maternally imprinted genes: identification by a subtractive screen of novel uniparental fibroblast lines. *Mol Cell Biol.* 2000;20:3308–3315.
37. Kuerbitz SJ, Pahys J, Wilson A, et al. Hypermethylation of the imprinted *NNAT* locus occurs frequently in pediatric acute leukemia. *Carcinogenesis.* 2002;23:559–564.
38. Killian JK, Nolan CM, Wylie AA, et al. Divergent evolution in M6P/IGF2R imprinting from the Jurassic to the Quaternary. *Hum Mol Genet.* 2001;10:1721–1728.
39. Huang Z, Wen Y, Shandilya R, et al. High throughput detection of M6P/IGF2R intronic hypermethylation and LOH in ovarian cancer. *Nucleic Acids Res.* 2006;34:555–563.
40. Hankins GR, De Souza AT, Bentley RC, et al. M6P/IGF2 receptor: a candidate breast tumor suppressor gene. *Oncogene.* 1996;12:2003–2009.
41. Calafat A, Kuklenyik Z, Reidy J, et al. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environ Health Perspect.* 2005;113:391–395.
42. De Souza AT, Hankins GR, Washington MK, et al. *M6P/IGF2R* gene is mutated in human hepatocellular carcinomas with loss of heterozygosity. *Nat Genet.* 1995;11:447–449.
43. Yamada T, De Souza AT, Finkelstein S, et al. Loss of the gene encoding mannose 6-phosphate/insulin-like growth factor II receptor is an early event in liver carcinogenesis. *Proc Natl Acad Sci USA.* 1997;94:10351–10355.
44. Souza RF, Appel R, Yin J, et al. The insulin-like growth factor II receptor gene is a target of microsatellite instability in human gastrointestinal tumours. *Nat Genet.* 1996;14:255–257.
45. Jamieson TA, Brizel DM, Killian JK, et al. M6P/IGF2R loss of heterozygosity in head and neck cancer associated with poor patient prognosis. *BMC Cancer.* 2003;3:4.
46. Tsujiuchi T, Sasaki Y, Tsutsumi M, et al. Alterations of the *M6p/Igf2* receptor gene in lung adenocarcinomas induced by *N*-nitrosobis(2-hydroxypropyl)amine in rats. *Mol Carcinog.* 2003;36:32–37.
47. Holmes A, le Guisquet A, Vogel E, et al. Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. *Neurosci Biobehav Rev.* 2005;29:1335–1346.
48. Barlow DP, Stoger R, Herrmann BG, et al. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the *Tme* locus. *Nature.* 1991;349:84–87.
49. Waterland R, Jirtle R. Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition.* 2004;20:63–68.
50. Rakyan VK, Chong S, Champ ME, et al. Transgenerational inheritance of epigenetic states at the murine $Axin^{Fu}$ allele occurs after maternal and paternal transmission. *Proc Natl Acad Sci U S A.* 2003;100:2538–2543.
51. Morgan H, Sutherland H, Martin D, et al. Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet.* 1999;23:314–318.
52. Kuff E, Lueders K. The intracisternal A-particle gene family: structure and functional aspects. *Adv Cancer Res.* 1988;51:183–276.
53. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature.* 2001;209:860–921.
54. Walsh CP, Chaillet JR, Bestor TH. Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. *Nat Genet.* 1998;20:116–117.
55. Lewin B. *Genes*. New York: Oxford University Press; 2000.
56. Falzon M, Kuff E. Multiple protein-binding sites in an intracisternal A particle long terminal repeat. *J Virol.* 1988;62:4070–4077.
57. Mietz J, Kuff E. Tissue and strain-specific patterns of endogenous proviral hypomethylation analyzed by two-dimensional gel electrophoresis. *Proc Natl Acad Sci U S A.* 1990;87:2269–2273.
58. Duhl D, Vrieling H, Miller K, et al. Neomorphic agouti mutations in obese yellow mice. *Nat Genet.* 1994;8:59–65.
59. Waterland R, Jirtle R. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol.* 2003;23:5293–5300.
60. Miltenberger R, Mynatt R, Wilkinson J, et al. The role of the *agouti* gene in the yellow obese syndrome. *J Nutr.* 1997;127:1902S–1907S.
61. Yen T, Gill A, Frigeri L, et al. Obesity, diabetes, and neoplasia in yellow A(vy)/– mice: ectopic expression of the agouti gene. *FASEB J.* 1994;8:479–488.
62. Okoji RS, Yu RC, Maronpot RR, et al. Sodium arsenite administration via drinking water increases genome-wide and Ha-ras DNA hypomethylation in methyl-deficient C57BL/6J mice. *Carcinogenesis.* 2002;23:777–785.
63. Poirier L, Vlasova T. The prospective role of abnormal methyl metabolism in cadmium toxicity. *Environ Health Perspect.* 2002;110:793–795.
64. Silbergeld E, Waalkes M, Rice J. Lead as a carcinogen: experimental evidence and mechanisms of action. *Am J Ind Med.* 2000;38:316–323.
65. Salnikow K, Costa M. Epigenetic mechanisms of nickel carcinogenesis. *J Environ Pathol Toxicol Oncol.* 2000;19:307–318.
66. Chen H, Ke Q, Kluz T, et al. Nickel ions increase histone H3 lysine 9 dimethylation and induce transgene silencing. *Mol Cell Biol.* 2006;26:3728–3737.
67. Yan Y, Kluz T, Zhang P, et al. Analysis of specific lysine histone H3 and H4 acetylation and methylation status in clones of cells with a gene silenced by nickel exposure. *Toxicol Appl Pharmacol.* 2003;190:272–277.
68. Wei Y, Tepperman K, Huang M, et al. Chromium inhibits transcription from polycyclic aromatic hydrocarbon-inducible promoters by blocking the release of histone deacetylase and preventing the binding of p300 to chromatin. *J Biol Chem.* 2004;279:4110–4119.
69. Li S, Hansman R, Newbold R, et al. Neonatal diethylstilbestrol exposure induces persistent elevation of c-*fos* expression and hypomethylation in its exon-4 in mouse uterus. *Mol Carcinog.* 2003;38:78–84.
70. Li S, Washburn K, Moore R, et al. Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. *Cancer Res.* 1997;57:4356–4359.
71. Li S, Ma L, Chiang T, et al. Promoter CpG methylation of Hox-a10 and Hox-a11 in mouse uterus not altered upon neonatal diethylstilbestrol exposure. *Mol Carcinog.* 2001;32:213–219.
72. Newbold R. Lessons learned from perinatal exposure to diethylstilbestrol. *Toxicol Appl Pharmacol.* 2004;199:142–150.
73. Newbold RR, Padilla-Banks E, Jefferson WN. Adverse effects of the

model environmental estrogen diethylstilbestrol are transmitted to subsequent generations. *Endocrinology.* 2006;147:s11–s17.
74. Ruden DM, Xiao L, Garfinkel MD, et al. Hsp90 and environmental impacts on epigenetic states: a model for the trans-generational effects of diethylstilbestrol on uterine development and cancer. *Hum Mol Genet.* 2005;14:R149–R155.
75. Dolinoy DC, Wiedman J, Waterland R, et al. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ Health Perspect.* 2006;114:567–572.
76. Anway MD, Cupp AS, Uzumcu M, et al. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science.* 2005;308: 1466–1469.
77. Lyko F, Brown R. DNA methyltransferase inhibitors and the development of epigenetic cancer therapies. *J Natl Cancer Inst.* 2005;97:1498–1506.
78. Cameron E, Bachman K, Myohanen S, et al. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat Genet.* 1999;21:103–107.
79. Issa J-PJ, Gharibyan V, Cortes J, et al. Phase II study of low-dose decitabine in patients with chronic myelogenous leukemia resistant to imatinib mesylate. *J Clin Oncol.* 2005;23:3948–3956.
80. Zambrano P, Segura-Pacheco B, Perez-Cardenas E, et al. A phase I study of hydralazine to demethylate and reactivate the expression of tumor suppressor genes. *BMC Cancer.* 2005;5:44.
81. Cornacchia E, Golbus J, Maybaum J, et al. Hydralazine and procainamide inhibit T cell DNA methylation and induce autoreactivity. *J Immunol.* 1988;140:2197–2200.
82. Fang MZ, Wang Y, Ai N, et al. Tea polyphenol (−)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res.* 2003;63:7563–7570.
83. Karagiannis T, El-Osta A. Clinical potential of histone deacetylase inhibitors as stand alone therapeutics and in combination with other chemotherapeutics or radiotherapy for cancer. *Epigenetics.* 2006;1: 121–126.
84. Marks PA, Richon VM, Rifkind RA. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. *J Natl Cancer Inst.* 2000;92:1210–1216.
85. Mei S, Ho A, Mahlknecht U. Role of histone deacetylase inhibitors in the treatment of cancer. *Int J Oncol.* 2004;25:1509–1519.
86. Drummond DC, Noble CO, Kirpotin DB, et al. Clinical development of histone deacetylase inhibitors as anticancer agents. *Annu Rev Pharmacol Toxicol.* 2005;45:495–528.
87. Suzuki H, Gabrielson E, Chen W, et al. A genomic screen for genes upregulated by demethylation and histone deacetylase inhibition in human colorectal cancer. *Nat Genet.* 2002;31:141–149.
88. Yao X, Hu J-F, Daniels M, et al. A novel orthotopic tumor model to study growth factors and oncogenes in hepatocarcinogenesis. *Clin Cancer Res.* 2003;9:2719–2726.
89. Hoffman A, Hu J. Directing DNA methylation to inhibit gene expression. *Cell Mol Neurobiol.* 2006;26:423–436.
90. Petronis A. Epigenetics and twins: three variations on the theme. *Trends Genet.* 2006;22:347–350.
91. 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;102:10604–10609.
92. Waterland R, Dolinoy DC, Lin J-R, et al. Maternal methyl supplements increase offspring DNA methylation at *Axin fused*. *Genesis.* 2006;44: 401–406.
93. Waterland RA, Lin J-R, Smith CA, et al. Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (*Igf2*) locus. *Hum Mol Genet.* 2006;15:705–716.
94. Weaver I, Cervoni N, Champagne FA, et al. Epigenetic programming by maternal behavior. *Nat Neurosci.* 2004;7 847–854.
95. Weaver I, Champagne FA, Brown S, et al. Reversal of maternal programming of stress responses in adult offspring through methyl supplementation. *J Neurosci.* 2005;25:11045–11054.
96. Weaver ICG, Meaney MJ, Szyf M. Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc Natl Acad Sci U S A.* 2006;103:3480–3485.
97. Petronis A. Epigenetics and bipolar disorder: new opportunities and challenges. *Am J Med Genet.* 2003;123:65–75.
98. Kato C, Petronis A, Okazaki Y, et al. Molecular genetic studies of schizophrenia: challenges and insights. *Neurosci Res.* 2002;43:295–304.
99. Jiang Y-H, Bressler J, Beaudet AL. Epigenetics and human disease. *Annu Rev Genom Hum Genet.* 2004;5:479–510.
100. Luedi PP, Hartemink AJ, Jirtle RL. Genome-wide prediction of imprinted murine genes. *Genome Res.* 2005;15:875–884.
101. Reik W, Walter J. Genomic imprinting: parental influence on the genome. *Nat Rev Genet.* 2001;2:21–32.
102. Jones PA, Laird PW. Cancer epigenetics comes of age. *Nat Genet.* 1999;21:163–167.

