Published in final edited form as:

Cancer J. 2007 ; 13(1): 3–8. doi:10.1097/PPO.0b013e31803c5415.

Cancer as a Manifestation of Aberrant Chromatin Structure

Malcolm V. Brock, MD*, James G. Herman, MD*, and Stephen B. Baylin, MD*

Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD

Abstract

In this article we review many important epigenetic changes in early carcinogenesis and discuss the possibility of these alterations being targeted for therapeutic intervention in the future. Both regional DNA methylation and global chromatin packaging are interrelated partners that function in concert to control gene transcription. We first summarize briefly DNA methylation and its role in gene expression. Then, we focus on how the DNA is packaged into chromatin and the tight relationship between chromatin and DNA methylation. A more complete understanding of these key, regulatory events is vital in approaching a more rational drug therapy to various malignancies.

Keywords

cancer; chromatin modification; DNA methylation; epigenetics

In recent years, there has been an exponential increase in research concerning epigenetic changes that occur during carcinogenesis. Epigenetics refers to alterations in patterns of gene expression that can occur without any modification of the underlying primary DNA sequence. As part of cellular memory, these modifications are heritable and thus are able to be conveyed from one generation to another during replication of somatic cells. In large part, to date, research activity has been devoted mainly to understanding 2 important epigenetic processes that occur during tumorigenesis—regional DNA hypermethylation and alterations in the chromatin components of DNA packaging. What is clearly emerging from the accumulated knowledge is that these 2 processes are not independent but are very much linked to one another. Thus, DNA methylation and chromatin alterations work together to dynamically alter epigenetic regulation of gene transcription from the earliest to the latest steps of tumor progression.^{1–3} In this article, we will consider the impact of both of these epigenetic processes on neoplastic progression with a focus on alterations in chromatin configuration as a key regulatory event. Clear understanding of the chromatin events that occur in carcinogenesis and knowing how they are triggered in neoplastic initiation and progression may be essential to ongoing efforts to construct pharmacologic strategies that target them as cancer prevention and therapy strategies.

DNA METHYLATION CHANGES IN CANCER

DNA methylation refers to the attachment of a methyl moiety, transferred from the donor substrate *S*-adenosyl methionine by DNA methyltransferase (DNMT) enzymes, to the C5 position of cytosines that precede guanines (so-called CpG dinucleotides). There has been,

Copyright © 2007 by Lippincott Williams & Wilkins

Reprints: Stephen B. Baylin, MD, Professor of Oncology, Molecular Determinants of Tumor Progression, Room 544 Cancer Research Building, Johns Hopkins University, School of Medicine, 720 Rutland Avenue, Baltimore, MD 21205–2196. sbaylin@jhmi.edu.

The authors declare no competing financial interests.

over evolution, a genome-wide incidence of deamination with subsequent repair to thymidines of these highly mutagenic methylated cytosines. This has resulted in a global depletion of CpG dinucleotides within the human genome of normal cells.⁴ However, this is not a random loss of CpG sites because, there has been a conservation of small regions of CpG-rich stretches (0.5 to several thousand kb), known as CpG islands, which particularly reside in the 5' end of about 50% of human genes.⁴

This above uneven representation of the CpG dinucleotide is also reflected in the DNA methylation status of the normal human genome. In the majority of our genome, which is transcriptionally inert such as DNA heterochromatic regions in pericentromeric parts of the chromosome and in repeat elements, the majority of the CpG sites are DNA methylated.⁴ However, CpG islands, and especially those associated with gene start sites, are protected from such methylation.⁴ This pattern of DNA methylation is thought to facilitate packaging of our DNA such that unwanted transcription does not occur throughout the genome, that gene expression is ensured in more localized regions, and that chromosomal integrity is maintained during DNA replication.^{4,5}

The genome of cancer cells harbors what appears to be a gross imbalance in the above DNA methylation patterns, and, thus, not surprisingly, in functional packaging of the DNA. Many tumor types are characterized by global losses of DNA methylation where it should normally be present.^{2,6} This has the potential to result in unwanted areas of transcription and the losses, particularly in pericentromeric regions, may contribute to chromosomal instability.^{7,8} However, the best studied change involves more localized gains of DNA methylation, in the same tumors, which occur in the CpG islands at the start sites of a growing list of genes involved in normal growth control, DNA repair, cell cycle regulation, and cell differentiation.¹⁻³ This hypermethylation is associated with an aberrant loss of transcriptional capacity of involved genes. In fact, in cancer, this promoter DNA hypermethylation now rivals the impact of genetic mutations in that it occurs at least as frequently as point mutations and deletions as a cause of altered gene expression.¹⁻³ Moreover, it is becoming increasingly apparent that these DNA methylation changes are occurring during the earliest stages of malignant transformation of the cell.^{1,9} Thus, many epigenetically silenced genes appear at stages before frank invasion and malignancy.⁹ For example, in a premalignant state for leukemia, myelodysplastic syndrome, hypermethylation of the tumor suppressor genes *p15^{INK4B}* is common, and the incidence increases with onset of leukemic transformation.^{10,11} Furthermore, early gene hypermethylation changes are now well recognized in preinvasive states of common tumors such as breast, colon, and lung, as well as other neoplasms, and may play a critical role in the actual genesis of such malignancies by facilitating early abnormal clonal cell expansion.^{1,9}

A key example of these above-mentioned early alterations in various tumor types include the hypermethylation of either both copies of the cell cycle regulatory gene, *p16^{INK4}*, or the loss of heterozygosity and methylation of the remaining copy on chromosome 9p21.^{12,13} Interestingly, the rate of point mutations in p16 is low in most primary tumors with allelic loss of 9p21, but p16 is a frequent target for early methylation in these same tumors, such as breast cancer and non-small cell lung cancer.^{12,13} In fact, in histologically normal mammary epithelium from healthy women without malignancy undergoing reduction mammoplasty, evidence of p16 promoter hypermethylation was found focally in almost half of the tissues.¹⁴ Similarly, in the resected surgical specimens of many patients with primary malignancies, a large field of early methylation changes occurs in histologically normal tissue adjacent to these cancers, probably reflecting a clonal expansion of cells with abnormal hypermethylation.¹⁵

Experimentally, the loss of p16 in human epithelial cells, such as those from mammary tissues, appears to facilitate early tumorigenesis and may predispose the cell to subsequent genomic instability.¹⁶⁻¹⁸ In cultured human breast epithelial cells, subpopulations of methylated p16 clones accumulate multiple chromosomal changes displaying premalignant and eventually malignant phenotypes, suggesting that the methylation of p16 is of critical importance in the formation of aberrant clonal expansion in breast cancer.¹⁴ Furthermore, recent data from knockout mice suggest that germline loss of this gene can increase stem cell life span.¹⁹⁻²¹ Thus, epigenetic silencing may predispose stem/progenitor cells to abnormal clonal expansion. Another salient example of early epigenetic gene silencing occurs in aberrant crypt foci of the colonic intestinal wall. In these foci, the normal colonic epithelial villi have given way to premalignant hyperplastic, preadenomatous cells that are at high risk for progressing to colon cancer.^{22,23} The neoplastic progression in these cells usually involves the abnormal constitutive activation of the Wnt developmental pathway. In early carcinogenesis, however, these aberrant crypt foci, do not always contain genetic mutations of the Wnt pathway but instead harbor abnormal promoter hypermethylation of the *SFRP* gene family members, which encode for membrane localized factors that antagonize Wnt interaction with its receptors.^{24,25} In addition, the hypermethylation of the *SFRPs* is present in all aberrant crypt foci examined and persist throughout malignant transformation, whereas further downstream mutations, such as APC, which is thought to be the earliest genetic alteration in colon tumorigenesis, may be acquired later.²⁵ So, the epigenetic changes involved in *SFRP* inactivation drive an oncogenic pathway that results in an abnormal expansion of stem or progenitor cells that depend on Wnt signaling for proliferation rather than differentiation.²⁵ Furthermore, there is a compelling line of evidence that the early epigenetic alterations are required for this malignant transformation of colonic cells and that later mutational events are insufficient, alone, to produce the transformed phenotype.⁹

Increasingly, cancer stem/progenitor cells are thought to play a critical role, rather than progeny daughter cells, in both the early abnormal clonal expansion and in the continued maintenance of neoplastic progression. Furthermore, overexpression of stem cell genes have been shown to drive tumorigenesis in intestinal epithelium.²⁶ Loss of a key regulatory gene that would otherwise normally suppress stem/precursor cell function may then be an essential element in the expansion of cells at risk for future formation of invasive cancer.^{1,9,27}

CHROMATIN STRUCTURAL CHANGES IN CANCER

Although methylation abnormalities are indeed early, recent evidence in numerous experimental systems suggests that connected chromatin events, such as altered posttranslational histone modifications, chromatin remodeling, and nuclear positioning of genes, may even precede the DNA methylation process. It has been known for some time that although DNA methylation can suppress gene activity through promoter transcriptional repression, this change alone is not sufficient to initiate such a block.²⁸ For RNA polymerase, transcription factors, and other coactivators to be denied open access to DNA, there also must be a concomitant change in the way the DNA is organized and maintained as chromatin in the nucleus. The fundamental unit of chromatin is the nucleosome, which consists of 146 base pairs of DNA wrapped around an octamer of core histone proteins, termed H2A, H2B, H3, and H4.²⁹ In DNA, in which the CpG islands are unmethylated and there is actively transcribed chromatin, the nucleosome is less tightly wrapped, and the nucleosomes are arranged by remodeling factors into a linear array providing for an open configuration which more readily allows unfettered access to the transcriptional machinery.^{4,28} Conversely, the chromatin structure is reversed in DNA associated with densely methylated CpG islands in gene promoter regions.^{4,28} In this transcriptionally

inhibited, inactive chromatin, the DNA is tightly wrapped in the nucleosome, and multiple nucleosomes are compacted into a higher order structure, which results in a closed, repressive local chromatin configuration.^{4,28} This nucleosome structure is, in turn, very dependent on the states of chromatin balance involving differing ratios of active and repressive histone modifications.^{30–32} These histone modifications or marks are being described in ever-increasing numbers and include lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, glutamic acid ADP-ribosylation, and lysine ubiquitination and sumoylation.^{30–32} Together they form a complex histone code that assists in maintaining chromatin in either open or closed configurations, and, thus, actively participate in modulating gene expression as well as normal chromosome structure and function.^{30–32}

The best described of these histone marks are the methylation and acetylation state of conserved amino acid residues on the NH₂-terminal tails of the core histone proteins. Each amino acid residue can be a marker, therefore, for a different signal.

Histone methylation is dynamically regulated by histone methyltransferases and histone demethylases.^{30–32} For example, methylation of lysine 4 of histone 3 is associated with the chromatin that assumes an open chromatin state, characteristic of active transcription, whereas methylation of lysine 9 or lysine 27, is associated with the chromatin configuration characteristic of repressed gene expression.^{30–32} Furthermore, lysine methylation may be monomeric, dimeric, or trimeric, which allows even more variability to the histone code.^{30–32} In the acetylation of histones, a balance exists between the enzymatic activity of histone acetyltransferases, on the one hand, and histone deacetylases (HDACs), on the other.^{4,28,30–32} In general, acetylation of histone amino acids is characteristic of open chromatin and transcriptional activation whereas deacetylation is associated with nucleosomal condensation and transcriptional inhibition.

THE DNA AND CHROMATIN PARTNERSHIP

The addition of DNA methylation to the above-described repressive chromatin changes provides an additional layering of epigenetic events that can tightly, heritably, repress transcription. Histone methyltransferases in *Neurospora*, *Arabidopsis*, and mammalian X-chromosome inactivation are known to recruit DNMTs to gene promoters.^{33–37} It is postulated that during the early stages of malignant transformation, histone methylation of residues on the tails of critical histone proteins occurs followed closely by DNA methylation at the gene promoter.²⁷ In an emerging model of DNA promoter hypermethylation changes during tumorigenesis, increased density of promoter DNA methylation may function as a lock to ensure permanent silencing of transcriptional repression initiated by chromatin changes.²⁷ There is a precedent for this concept in the events that initiate and maintain silencing of one X chromosome in mammalian female cells.³⁸

This partnership between DNA methylation and chromatin, once established, does not appear, by any means, to be an equal one, especially with respect to histone deacetylation changes. The dominant lock by DNA methylation must be diminished with a demethylating agent before the type I and II HDACs are able to reactive transcription effectively.³⁹ This dominance has important clinical implications. Treatment of cancer cells with select type I and II inhibitors of HDACs, alone, usually fails to result in gene reexpression of tumor suppressor genes in tumor cells with densely methylated promoter regions.³⁹ Pharmacologic induction of DNA demethylation with inhibitors of DNA methylation alone, such as azacytidine (5-azacytidine; Vidaza; Pharmion Corp., Boulder, CO) and decitabine (Dacogen; SuperGen Inc., Dublin, CA, and MGI Pharma, Minneapolis, MN), however, does result in derepression of silenced genes and restoration of normal gene expression. These

DNA demethylation agents also cause the loss of certain transcriptional-silencing marks, such as H3K9me₂, and the restoration of transcriptional-activation marks, such as acetylation of K9 and K14 of H3 and H3K4me.^{27,40} Interestingly, when HDAC inhibitors are administered after low doses of DNA demethylation agents, the 2 drugs synergize to promote gene reexpression.^{39,41} It is known that HDACs are associated with the DNMTs and with methyl cytosine-binding proteins (MBPs), which also mediate the transcriptional repressive activities of DNA methylation.^{4,28,42–45} Presumably, the initial dose of demethylation agents can cause release of the MBPs and also the HDACs from the promoters.⁴⁶ As a result of elucidating this biologic paradigm, clinical trials have been undertaken to exploit the therapeutic potential of sequential treatment of low-dose demethylation agents followed closely by HDAC inhibitors, especially in hematopoietic malignancies.^{47,48}

There are, however, chromatin events that may lie “downstream” of DNA methylation in terms of maintaining transcriptional repression. For example, removal of MBPs, which can complex with type I and II HDACs to facilitate DNA methylation-mediated transcriptional repression, can relieve the associated gene silencing even while DNA methylation remains intact.⁴⁹ Similarly, inhibition of SIRT1, a so-called class III HDAC, with a completely different mechanism of histone deacetylation, which associates with the promoters of DNA hypermethylated tumor genes, has been shown to result in transcriptional activation even in the presence of dense DNA promoter hypermethylation.⁵⁰ Although there is, then, much more to learn about how various histone modifications are initiated during carcinogenesis, these chromatin-mediated changes and the DNA methylation that can accompany them provide clues to events initiating the very earliest stages of neoplasia and, thus, represent targets for establishing new means of cancer prevention and therapy.

POLYCOMB REPRESSIVE COMPLEXES (PRCs)

In addition to the local chromatin changes, studies from the field of embryonic development have elucidated a complex series of events that mark genes for active versus repressed transcription as stem and progenitor cells commit themselves to specific maturation pathways. These events result in more global alterations in the levels of proteins that participate in chromatin modifications. The polycomb group protein complexes (PRCs) are well conserved from *Drosophila* to man and function to mediate long-term transcriptional silencing.^{51–55} There are at least 4 PRC protein complexes, PRC2, 3, and 4, which serve to initiate the silencing, and PRC1 complexes, which function to maintain the silencing.⁵⁵ In early carcinogenesis, levels of PRC1 and PRC2–4 constituents, which contain the histone methyltransferase activity catalyzing the placement of the H3K27me mark, are noted to be increased.^{56–59} There are critical potential targets of these increases. For example, the often methylated tumor suppressor gene, p16^{CDKN2A}, for example, is a silencing target of BMI1, a constituent of the PRC1 complex.⁵² Furthermore, p16 methylation changes have been shown in cultured mammary epithelial cells to constitute an early loss of cell cycle control that may directly allow for additional epigenetic silencing via PRCs.¹⁸ Another polycomb constituent, EZH2 of the PRC2–4 complexes, contributes to the methylation of both H3K27 and H1K26. H3K27me has been found at the promoters of all DNA hypermethylated and silenced cancer genes examined.⁴⁰ Up-regulation of EZH2 and another PRC2 complex constituent, SUZ12, has been implicated in several cancer types.^{57,59} The implication, again, is that there may be a progressive layering of PRC epigenetic regulation that culminates in DNA methylation and the eventual effect of prohibiting access of transcription factors and other components of the transcriptional machinery to modify gene expression.

The complete elucidation and understanding of these basic biologic concepts can be exploited therapeutically in the clinical application of demethylation agents (Fig. 1). After

induction of demethylation, for example, although gene expression is restored and certain histone modifications are reversed, the chromatin configuration is not fully active and retains certain repressive marks such as K27methylation-H3, other PRC-related marks,⁴⁰ and H3K9me3. Retention of PRC proteins and H3K9 methylation may then, in turn, make the gene promoter vulnerable to the DNA methylation machinery, leading to only partial or incomplete demethylation, or even, remethylation.²⁷ Critical proteins involved in DNA methylation and chromatin changes are potential targets for pharmacologic intervention. As chromatin aberrations become increasingly targeted as part of cancer therapy, many unanswered questions remain. The long-term effect of inducing hypomethylation in humans, for example, is largely unknown. Hypomethylation is known to occur predominantly in repetitive DNA sequences and may be linked to chromosomal instability.^{8,60,61} Therefore, the consequences of nonspecifically activating genes or transposable elements in normal cells is not fully understood and must be closely monitored. Secondly, hematopoietic malignancies currently seem to have the most profound response to epigenetic therapy whereas solid neoplasms have been recalcitrant to therapy. Will similar pharmacologic strategies be effective for hematologic and solid tumors, or would more refined targeted therapies be needed? Finally, as noted, many of these epigenetic abnormalities are very early in onset. Will epigenetic therapy be more effective as a chemopreventive strategy, and what is the appropriate timing of such an intervention? The molecular barriers to demethylation agents are numerous. Many cancer cell lines after treatment with demethylation agents, for example, reaccumulate their dense promoter methylation and become transcriptionally silent again⁶² (Fig. 1). This, of course, suggests a paradigm of continued administration of drug for effect. The complex tumor biology involved may suggest that one of our more immediate therapeutic goals in epigenetic therapy may be to turn treatment of cancer into a maintenance therapy such as is currently the case with hypertension or diabetes. Even with this incremental step, the utilization of therapies directed at reversing or controlling abnormal epigenetic events in cancer is ever nearer.

REFERENCES

1. Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. *Nat Rev Genet.* 2006; 7:21–33. [PubMed: 16369569]
2. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med.* 2003; 349:2042–2054. [PubMed: 14627790]
3. Jones PA, Laird PW. Cancer epigenetics comes of age. *Nat Genet.* 1999; 21:163–167. [PubMed: 9988266]
4. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev.* 2002; 16:6–21. [PubMed: 11782440]
5. Robertson KD. DNA methylation and human disease. *Nat Rev Genet.* 2005; 6:597–610. [PubMed: 16136652]
6. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature.* 1983; 301:89–92. [PubMed: 6185846]
7. Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell.* 1992; 69:915–926. [PubMed: 1606615]
8. Eden A, Gaudet F, Waghmare A, et al. Chromosomal instability and tumors promoted by DNA hypomethylation. *Science.* 2003; 300:455. [PubMed: 12702868]
9. Baylin SB, Ohm JE. Epigenetic gene silencing in cancer—a mechanism for early oncogenic pathway addiction? *Nat Rev Cancer.* 2006; 6:107–116. [PubMed: 16491070]
10. Leone G, Teofili L, Voso MT, Lubbert M. DNA methylation and demethylating drugs in myelodysplastic syndromes and secondary leukemias. *Haematologica.* 2002; 87:1324–1341. [PubMed: 12495905]

11. Daskalakis M, Nguyen TT, Nguyen C, et al. Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2'-deoxycytidine (decitabine) treatment. *Blood*. 2002; 100:2957–2964. [PubMed: 12351408]
12. Belinsky SA, Nikula KJ, Palmisano WA, et al. Aberrant methylation of *p16^{INK4a}* is an early event in lung cancer and a potential biomarker for early diagnosis. *Proc Natl Acad Sci U S A*. 1998; 95:11891–11896. [PubMed: 9751761]
13. 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; 55:4525–4530. [PubMed: 7553621]
14. Holst CR, Nuovo GJ, Esteller M, et al. Methylation of *p16^{INK4a}* promoters occurs *in vivo* in histologically normal human mammary epithelia. *Cancer Res*. 2003; 63:1596–1601. [PubMed: 12670910]
15. Guo M, House MG, Hooker C, et al. Promoter hypermethylation of resected bronchial margins: a field defect of changes? *Clin Cancer Res*. 2004; 10:5131–5136. [PubMed: 15297416]
16. Foster SA, Wong DJ, Barrett MT, Galloway DA. Inactivation of p16 in human mammary epithelial cells by CpG island methylation. *Mol Cell Biol*. 1998; 18:1793–1801. [PubMed: 9528751]
17. Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA, Klingelutz AJ. Both Rb/p16^{INK4a} inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature*. 1998; 396:84–88. [PubMed: 9817205]
18. Reynolds PA, Sigaroudinia M, Zardo G, et al. Tumor suppressor p16^{INK4A} regulates polycomb-mediated DNA hypermethylation in human mammary epithelial cells. *J Biol Chem*. 2006; 281:24790–24802. [PubMed: 16766534]
19. Janzen V, Forkert R, Fleming HE, et al. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16^{INK4a}. *Nature*. 2006; 443:421–426. [PubMed: 16957735]
20. Krishnamurthy J, Ramsey MR, Ligon KL, et al. p16^{INK4a} induces an age-dependent decline in islet regenerative potential. *Nature*. 2006; 443:453–457. [PubMed: 16957737]
21. Molofsky AV, Slutsky SG, Joseph NM, et al. Increasing p16^{INK4a} expression decreases forebrain progenitors and neurogenesis during ageing. *Nature*. 2006; 443:448–452. [PubMed: 16957738]
22. Jen J, Powell SM, Papadopoulos N, et al. Molecular determinants of dysplasia in colorectal lesions. *Cancer Res*. 1994; 54:5523–5526. [PubMed: 7923189]
23. Siu IM, Robinson DR, Schwartz S, et al. The identification of monoclonality in human aberrant crypt foci. *Cancer Res*. 1999; 59:63–66. [PubMed: 9892186]
24. Rattner A, Hsieh JC, Smallwood PM, et al. A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *Proc Natl Acad Sci U S A*. 1997; 94:2859–2863. [PubMed: 9096311]
25. Suzuki H, Watkins DN, Jair KW, et al. Epigenetic inactivation of *SFRP* genes allows constitutive WNT signaling in colorectal cancer. *Nat Genet*. 2004; 36:417–422. [PubMed: 15034581]
26. Hochedlinger K, Yamada Y, Beard C, Jaenisch R. Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. *Cell*. 2005; 121:465–477. [PubMed: 15882627]
27. Ting AH, McGarvey KM, Baylin SB. The cancer epigenome—components and functional correlates. *Genes Dev*. 2006; 20:3215–3231. [PubMed: 17158741]
28. Bird AP, Wolffe AP. Methylation-induced repression—belts, braces, and chromatin. *Cell*. 1999; 99:451–454. [PubMed: 10589672]
29. Kornberg RD, Lorch Y. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell*. 1999; 98:285–294. [PubMed: 10458604]
30. Briggs SD, Xiao T, Sun ZW, et al. Gene silencing: trans-histone regulatory pathway in chromatin. *Nature*. 2002; 418:498. [PubMed: 12152067]
31. Fischle W, Wang Y, Allis CD. Histone and chromatin cross-talk. *Curr Opin Cell Biol*. 2003; 15:172–183. [PubMed: 12648673]
32. Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001; 293:1074–1080. [PubMed: 11498575]

33. Selker EU, Jensen BC, Richardson GA. A portable signal causing faithful DNA methylation de novo in *Neurospora crassa*. *Science*. 1987; 238:48–53. [PubMed: 2958937]
34. Tamaru H, Selker EU. A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature*. 2001; 414:277–283. [PubMed: 11713521]
35. Tamaru H, Selker EU. Synthesis of signals for de novo DNA methylation in *Neurospora crassa*. *Mol Cell Biol*. 2003; 23:2379–2394. [PubMed: 12640122]
36. Jackson JP, Lindroth AM, Cao X, et al. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature*. 2002; 416:556–560. [PubMed: 11898023]
37. Johnson L, Cao X, Jacobsen S. Interplay between two epigenetic marks: DNA methylation and histone H3 lysine 9 methylation. *Curr Biol*. 2002; 12:1360–1367. [PubMed: 12194816]
38. Plath K, Fang J, Mlynarczyk-Evans SK, et al. Role of histone H3 lysine 27 methylation in X inactivation. *Science*. 2003; 300:131–135. [PubMed: 12649488]
39. Cameron EE, Bachman KE, 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. [PubMed: 9916800]
40. McGarvey KM, Fahrner JA, Greene E, et al. Silenced tumor suppressor genes reactivated by DNA demethylation do not return to a fully euchromatic chromatin state. *Cancer Res*. 2006; 66:3541–3549. [PubMed: 16585178]
41. 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. [PubMed: 11992124]
42. Bachman KE, Rountree MR, Baylin SB. Dnmt3a and Dnmt3b are transcriptional repressors that exhibit unique localization properties to heterochromatin. *J Biol Chem*. 2001; 276:32282–32287. [PubMed: 11427539]
43. Rountree MR, Bachman KE, Baylin SB. DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat Genet*. 2000; 25:269–277. [PubMed: 10888872]
44. Fuks F, Burgers WA, Brehm A, et al. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat Genet*. 2000; 24:88–91. [PubMed: 10615135]
45. Robertson KD, Ait-Si-Ali S, Yokochi T, et al. DNMT1 forms a complex with rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nat Genet*. 2000; 25:338–342. [PubMed: 10888886]
46. El-Osta A, Kantharidis P, Zalcberg JR, et al. Precipitous release of methyl-CpG binding protein 2 and histone deacetylase 1 from the methylated human multidrug resistance gene (MDR1) on activation. *Mol Cell Biol*. 2002; 22:1844–1857. [PubMed: 11865062]
47. Gore SD, Baylin S, Sugar E, et al. Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. *Cancer Res*. 2006; 66:6361–6369. [PubMed: 16778214]
48. Egger G, Liang G, Aparicio A, et al. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*. 2004; 429:457–463. [PubMed: 15164071]
49. Bakker J, Lin X, Nelson WG. Methyl-CpG binding domain protein 2 represses transcription from hypermethylated pi-class glutathione *S*-transferase gene promoters in hepatocellular carcinoma cells. *J Biol Chem*. 2002; 277:22573–22580. [PubMed: 11960994]
50. Pruitt K, Zinn RL, Ohm JE, et al. Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation. *PLoS Genet*. 2006; 2:344–352.
51. Orlando V. Polycomb, epigenomes, and control of cell identity. *Cell*. 2003; 112:599–606. [PubMed: 12628181]
52. Lund AH, van Lohuizen M. Polycomb complexes and silencing mechanisms. *Curr Opin Cell Biol*. 2004; 16:239–246. [PubMed: 15145347]
53. Ringrose L, Paro R. Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu Rev Genet*. 2004; 38:413–443. [PubMed: 15568982]
54. Schwartz YB, Kahn TG, Dellino GI, et al. Polycomb silencing mechanisms in *Drosophila*. *Cold Spring Harb Symp Quant Biol*. 2004; 69:301–308. [PubMed: 16117662]

55. Sparmann A, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. *Nat Rev Cancer*. 2006; 6:846–856. [PubMed: 17060944]
56. Kuzmichev A, Margueron R, Vaquero A, et al. Composition and histone substrates of polycomb repressive group complexes change during cellular differentiation. *Proc Natl Acad Sci U S A*. 2005; 102:1859–1864. [PubMed: 15684044]
57. Varambally S, Dhanasekaran SM, Zhou M, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature*. 2002; 419:624–629. [PubMed: 12374981]
58. Kirmizis A, Bartley SM, Farnham PJ. Identification of the polycomb group protein SU(Z)12 as a potential molecular target for human cancer therapy. *Mol Cancer Ther*. 2003; 2:113–121. [PubMed: 12533679]
59. Kleer CG, Cao Q, Varambally S, et al. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci U S A*. 2003; 100:11606–11611. [PubMed: 14500907]
60. Feinberg AP, Tycko B. The history of cancer epigenetics. *Nat Rev Cancer*. 2004; 4:143–153. [PubMed: 14732866]
61. Ehrlich M. DNA hypomethylation, cancer, the immunodeficiency, centromeric region instability, facial anomalies syndrome and chromosomal rearrangements. *J Nutr*. 2002; 132:2424S–2429S. [PubMed: 12163705]
62. Liang G, Chan MF, Tomigahara Y, et al. Cooperativity between DNA methyltransferases in the maintenance methylation of repetitive elements. *Mol Cell Biol*. 2002; 22:480–491. [PubMed: 11756544]

THERAPEUTIC TARGETS

FIGURE 1.

Model of chromatin state during demethylation of cancer cells. Heavy black arrows denote targets for drug intervention. Small gray arrow: G9a (a histone methyltransferase) methylates H3-K9 in euchromatin and forms a binding site for HP1 (heterochromatin protein 1), a key interpreter of the silencing mark H3-K9me3. me1, monomethylation of lysine residue; me2, dimethylation of lysine residue; me3, trimethylation of lysine residue; PRC4, polycomb repressive complex 4, which exists in embryonic stem cells and cancer cells with stem cell properties. It is composed of the histone methyltransferase, EZH2, and the stress-sensing protein, SIRT1. In cancer cells (bottom), the heritable epigenetic state is one of deep silencing with layers of repressive marks including mono, di-, and tri-H3K9me and di- and tri-H3K27me as well as a reduction in active marks, such as H3K9 acetylation. The DNA is densely methylated (black circles) and the histones (solid ovals) are compactly spaced, giving a closed chromatin configuration and no gene expression (black arrow with red X). On addition of a demethylation agent (top), the gene expression is induced (black arrow) but the completely active, normal euchromatin state is not fully restored. The histones (ovals are semi-solid not fully clear) are still compactly spaced, repressive histone modifications are still present, but there is a marked reduction of DNA methylation. The promoter in this “intermediate state” is primed to return to the DNA hypermethylated and deep silencing state if drug is not continued.