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A decade of exploring the cancer epigenome — biological and translational implications

Stephen B. Baylin and
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland 21231, USA

Peter A. Jones
The USC Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California 90089, USA

Stephen B. Baylin: sbaylin@jhmi.edu; Peter A. Jones: pjones@med.usc.edu

Abstract

The past decade has highlighted the central role of epigenetic processes in cancer causation, progression and treatment. Next-generation sequencing is providing a window for visualizing the human epigenome and how it is altered in cancer. This view provides many surprises, including linking epigenetic abnormalities to mutations in genes that control DNA methylation, the packaging and the function of DNA in chromatin, and metabolism. Epigenetic alterations are leading candidates for the development of specific markers for cancer detection, diagnosis and prognosis. The enzymatic processes that control the epigenome present new opportunities for deriving therapeutic strategies designed to reverse transcriptional abnormalities that are inherent to the cancer epigenome.

The past decade has seen a remarkable acceleration in the validation of the concept that cancer is a disease of epigenetic, as well as genetic, abnormalities. Exploration of these connections constitutes one of the most exciting areas in basic cancer biology — with rich potential for clinical translation. Cancer research in epigenetics in the 1990s was dominated by a focus on understanding and extending the discoveries in the 1980s of DNA methylation abnormalities[1]. During the past 10 years, this focus has merged with an explosion of knowledge about the role of chromatin covalent modifications and organization and their relevance to gene expression[2–6], resulting in an emerging view of what may now be called ‘the cancer epigenome’, which harbours myriad abnormalities that are based on somatically heritable alterations that are not due to primary DNA sequence changes[7,8] (TIMELINE). Each year, new surprises arise regarding how interactions between epigenetic and genetic changes help to drive the initiation and progression of cancer. This knowledge fosters new potential cancer biomarker strategies and therapeutic opportunities. We highlight examples of these recent advances and what the future holds for them.

Functional organization of the genome

Few would have predicted how our view of the human epigenome has expanded over the past 10 years. Next-generation sequencing techniques, as applied to mapping chromatin and DNA methylation in normal, cancer and induced pluripotent stem cells (iPSs), have

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revolutionized our knowledge of chromatin states, nucleosome positioning and how alterations in these contribute to disease[3,9–12]. The architecture of gene expression states is being clarified. Nucleosome positions are dynamic and change during cell replication and with gene expression changes[11]. Active gene promoters, particularly those that are CpG-rich and that normally lack DNA methylation, have nucleosome-depleted regions (NDRs) just upstream of their transcription start sites (TSSs). The nucleosomes that flank these NDRs are marked by the histone modification H3 trimethylated on lysine 4 (H3K4me3), have extensive lysine acetylation and harbour the histone variant H2A.Z, which may destabilize nucleosomes to facilitate transcriptional initiation (FIG. 1). The transcribed regions or gene bodies of active genes also show enrichment of specific covalent marks, including H3K36me3, which may facilitate transcriptional elongation[13]. These regions normally have dense cytosine methylation, even in downstream CpG islands[14], which might also promote transcription elongation rather than repress transcription initiation — as methylation does in promoter regions[14]. Importantly, structural features of enhancers are being defined (FIG. 1), including deoxyribonuclease 1 (DNase1) sensitivity, nucleosome depletion, and the presence of H3K4me1 and H3 acetylated on lysine 27 (H3K27ac) in the active state[15]. By contrast, DNA methylation stabilizes epigenetic gene silencing in promoters that lack H2A.Z, that have nucleosomes positioned over the TSS and that harbour repressive histone modifications, such as H3K9me2 or H3K9me3 marks[16]. Long-term silencing of genes with promoter CpG islands by DNA methylation is normally only associated with inactive X-linked genes, imprinted genes and germ cell-specific genes, but it is also common in many abnormally silenced genes in cancer[7,8].

One exciting recent advance in our understanding has been that it is the balance between transcriptionally permissive and transcriptionally repressive chromatin modifications that maintains genome-wide gene expression states[17,18]. In contrast to the strong localization of active marks to TSSs, the H3K27me3 inhibitory mark applied by the Polycomb group proteins (PcGs) can extend over many nucleosomes around genes that typically lack DNA methylation but that have a DNase1-insensitive state owing to the presence of nucleosomes at the TSS[19]. The unexpected finding is that PcG occupancy can also coexist around the TSS with the active mark, H3K4me3, to form what has been termed a bivalent state[3,17,18,20,21] for key developmental and lineage-specific genes in embryonic stem cells (ESCs). This bivalency may allow regulatory flexibility by keeping these genes quiescent to maintain ESC pluripotency but allowing for their rapid activation when needed during differentiation[3,17,18]. Intriguingly, as discussed below, these gene promoters are prone to undergoing an epigenetic switch[22,23] and become *de novo* DNA methylated in cancer and pre-cancerous cells[24–26].

There has also been an explosion of knowledge regarding the molecular determinants of global-scale chromatin architecture. Insulator proteins such as CCCTC-binding factor (CTCF) and others[27], together with PcG occupancy[19], organize DNA into loops of transcriptionally repressive heterochromatin or into active euchromatin, which facilitates blocks or which connects distal enhancers and proximal promoters. As addressed below, the accessibility of CTCF to DNA is linked to the DNA methylation status of target regions. A dizzying array of enzymes is now known to not only catalyse the addition of transcriptionally activating and repressing histone marks, but also to remove them[28–30]. The discovery of the demethylases that can remove lysine methylation marks on histones is a particularly exciting advance that is proving to be pivotal for understanding the normal epigenome and several key aspects of cancer biology[30–34]. Similarly, we are refining our view of how DNA methylation patterns are established. For example, an elegant tetrameric complex has been defined that can neatly surround a nucleosome and presumably initiate methylation in this context[35]. This complex comprises two molecules of the *de novo* DNA methyltransferase DNMT3A and two of DNMT3L, a catalytically inactive isomer that is

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expressed in ESCs. Nucleosomes containing the active histone mark H3K4me3 cannot be encompassed by this complex[35], thus targeting DNA methylation to regions such as inactive promoters, intragenic regions, gene bodies and non-control regions.

Cooperation between DNMT1 (a maintenance DNA methyltransferase) and DNMT3A and DNMT3B, is being defined in normal and cancer cells[36]. It has been suggested that DNMT3A and DNMT3B repair the errors made by DNMT1 after DNA synthesis. Unlike DNMT1, DNMT3A and DNMT3B are firmly anchored to nucleosomes, perhaps allowing these enzymes to remain closely associated with the DNA methylation that they produce to facilitate epigenetic inheritance[37]. Excitingly, enzyme systems that remove DNA methylation, such as the TET family proteins, which form 5-hydroxylmethyl cytosine from methylated cytosine, have been discovered to be crucially important during development and tumorigenesis[38–41].

Visualizing the cancer epigenome

The recent findings outlined above now frame observations from the 1980s that multiple types of cancers abnormally gain and lose DNA methylation[1], and these findings are providing a greater understanding of the importance of these abnormalities to tumorigenesis. Juxtaposing these changes with other abnormalities of chromatin organization is rapidly reshaping our view of the cancer epigenome.

Promoter CpG island DNA hypermethylation

Abnormal gains of DNA methylation in normally unmethylated gene promoter CpG islands, and associated stabilization of transcriptional repression and loss of gene function, are the most extensively studied epigenetic alterations in cancer[1,7,8]. Rapid advances that have enabled the precise mapping of DNA methylation across normal and cancer genomes confirms that almost all cancer types harbour hundreds of genes with these abnormal gains in methylation[1,7] (FIG. 2), affecting some 5–10% of the thousands of promoter CpG islands that never normally contain DNA methylation from embryonic development onwards[42]. Some genes do, however, show changes in CpG island DNA methylation with ageing[43,44]. Although most genes that become DNA hypermethylated in cancer are usually affected on an individual basis[45], subchromosomal domains with multiple hypermethylated loci have recently been discovered[46,47].

Understanding current views of genetic changes in cancers may provide a perspective on how this large number of epigenetic changes contributes to the cancer phenotype. Similar to DNA mutations that frequently occur in specific genes, such as *TP53* or *KRAS*, which act as major drivers for cancer initiation and progression[48–50], there are a few rare, high-frequency epigenetic mutations, or ‘mountains’, being discovered in specific genes. For example, the tumour suppressor genes Von Hippel–Lindau (*VHL*) in renal cancer and *CDKN2A* (encoding the tumour suppressors INK4A and ARF) in several tumour types are DNA hypermethylated, and these have emphasized the importance of epigenetic changes mediating the loss of gene function in cancer[1,7].

What then are the roles, if any, for the hundreds of other DNA hypermethylated cancer genes? Just as the majority of gene mutations are increasingly recognized as low frequency and have been termed ‘hills’, as opposed to mountains[48], their importance may be their aggregation in the same signalling pathways, thus helping in the derivation of the cancer phenotype[48]. Importantly, epigenetic changes can be much more common for many of the same infrequently mutated genes[51–53]. For example, on average, one gene in the extracellular matrix construction pathway is mutated compared with four being DNA hypermethylated in an individual colon or breast tumour[53]. Frequent simultaneous

hypermethylation occurs in both the *GATA4* and *GATA5* transcription factors and their downstream targets in colon cancer: GATA4 and GATA5 function in a pathway that is crucial for proper epithelial differentiation[54]. DNA hypermethylation also affects multiple genes that negatively regulate the WNT pathway from the cell membrane to the nucleus in this same tumour type[55]. This finding shows how epigenetic changes can complement single driver mutations, such as those occurring in adenomatous polyposis coli (APC) or β-catenin that activate the WNT pathway[56,57]. Additionally, DNA hypermethylation of a range of genes can collectively contribute to the disruption of the p53 pathway.

One recently recognized and growing important new role for abnormal, promoter DNA hypermethylation is the fostering of pathway disruption that is associated with the transcriptional repression of multiple microRNAs (miRNAs). This can result in the upregulation of oncogenic targets of the microRNAs, such as BCL-6 (REF. 58), and the constitutive activation of signalling that promotes invasiveness and metatstatic activities[59,60]. Intriguingly, downregulation of the miRNA-29 family has also been linked to overexpression of DNA methyltransferases[61,62], possibly creating a scenario that is permissive for gene promoter DNA hypermethylation. miR-101 is often down-regulated in cancer, leading to overexpression of enhancer of zeste 2 (EZH2)[63,64] — a key component of the PcG system, which, as previously noted, marks genes that are prone to cancer-specific DNA hypermethylation[24–26]. Cancer-specific DNA hypermethylation has also been reported to silence other non-coding RNAs[65]. Intriguingly, non-coding RNAs and anti-sense RNA sequences have also been linked to the development of cancer-specific DNA hypermethylation at gene promoters, and to the recruitment of silencing complexes, such as PcGs, which may subsequently make these regions vulnerable to DNA methylation changes[66–69].

Experimentally, the importance of the above gene changes, many involving epigenetically altered cancer genes not affected by base pair mutations, is illustrated by mouse knockout studies of the DNA binding transcriptional repressor hypermethylated in cancer 1 (*Hic1*). Homozygous knockout mice die from developmental effects[70] but heterozygotes develop a range of tumour types that are common in humans as they age[71]. In these cancers, the wild-type *Hic1* allele almost always acquires promoter DNA methylation[71]. When crossed with mice in which other tumour suppressors have been knocked out, many tumour phenotypes emerge from an initial expansion of progenitor cells and increased transcriptional repression of HIC1 targets[72–75]. Most recently, targeted DNA hypermethylation of the *HIC1* promoter has been found to transform human mesenchymal stem cells[76].

Some 20 years ago DNA hypermethylation was implicated in the expansion of progenitor cells that can initiate tumour development in the kidney through a process termed loss of imprinting (LOI)[77,78]. In this process, a gain of CpG methylation (hypermethylation not hypomethylation) in the insulator region immediately upstream of the imprinted *H19* gene on chromosome 11 is associated with loss of CTCF binding to the region[79,80]. This, in turn, results in the activation of the imprinted insulin-like growth factor 2 (*IGF2*) promoter on the same chromosome by providing access of this region to a downstream enhancer[78,81]. Mosaicism for the epigenetic lesion has been shown in pre-neoplastic kidneys ahead of the development of Wilms' tumour, and in some instances Wilms' tumour can arise in this setting through DNA microdeletions that are upstream of *H19* (REF. 82). This scenario may well illustrate that epigenetic changes can be the earliest initiating factor in a human cancer[83]. More recently, this change in *IGF2* has been linked to the expansion of abnormal stem and progenitor cells in the progression of colon cancer[84].

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Other DNA methylation changes

Key roles for DNA methylation are emerging for regions other than the most proximal promoter areas. DNA methylation patterns for conserved gene sequences several kilobases upstream or downstream of promoter CpG islands, and termed shores, are tightly linked with the tissue of origin[85,86]. Alterations of this methylation pattern in cancer cells have been reported to be more frequent than those in nearby CpG islands[86], but the importance of these changes is not yet known. Most recent studies indicate that, in cancer, such shores are isolated targets for DNA hypermethylation much less frequently than the CpG islands[87,88].

Although regional gains in DNA methylation in cancer are important, simultaneous losses of DNA methylation have long been recognized and are even more widespread[1,7,89]. Recent genome-wide sequencing of CpG sites in small numbers of colon cancer versus normal colon samples is beginning to reveal intriguing insights into the relationships between these abnormal losses of DNA methylation and the previously discussed gains in many CpG islands. The large regions of DNA methylation losses are not randomly distributed but rather span megabase regions on multiple chromosomes. In turn, these losses, in predominantly CpG-poor regions, are punctuated with narrow gains of DNA methylation, which are those predominantly involving the regional gains in promoter, and other non-annotated, CpG islands[87,88,90]. Importantly, these domains of losses and gains are associated with late-replicating, lamin-associated nuclear regions and this is profoundly important. It is these regions in ESCs that contain the majority of the genes with bivalent, chromatin promoter domains, which, as discussed earlier, are those that are highly vulnerable to abnormal CpG island DNA hypermethylation in cancer[87,88,90]. Also, this change and the methylation losses that occur within the bodies of many genes could be functionally related to reduced expression in cancer[14]. These regional changes in cancer DNA methylation will undoubtedly be the subject of intense investigation over the next few years.

Origins of the cancer epigenome

Clues from developmental biology

Increasingly, the understanding of the cancer epigenome has been linked to the epigenetic dynamics of development. Abnormal DNA hypermethylation in cancer involves many genes that are important to embryonic development. Three studies of colon cancer in 2007 (REFS 24–26,91) revealed that about 50% of genes with cancer-specific promoter CpG island hypermethylation were among the approximately 10% of genes that are controlled by the PcGs in ESCs. As noted earlier, PcG-mediated transcriptional repression, often in the setting of bivalent chromatin, seems to mediate a low, poised transcription state of CpG island promoters for genes in ESCs that are important for regulating lineage determination[3,17,18]. Importantly, these regulatory events during embryonic development occur in the absence of the abnormal promoter DNA methylation seen in multiple types of cancer[92]. A working model[93] hypothesizes a molecular progression during tumorigenesis that starts with abnormally expanding adult stem or progenitor cell compartments in which vulnerable genes with promoter CpG islands undergo quantitative replacement of flexible, PcG-mediated gene silencing with the more stable silencing that is associated with DNA methylation[91,93]. The mechanisms, however, remain to be fully characterized. Abnormal retention or imposition of PcGs may occur to the exclusion of DNA methylation for some genes[22]. In others, once DNA methylation evolves, the PcG complex and the H3K27me3 histone mark may be quantitatively and/or qualitatively replaced[91,93] (FIG. 3). These possibilities are consistent with recent data that methylated DNA in a nucleosomal context actually repels components of PcGs[94]. Indeed, a specific gene marked by bivalent chromatin in the embryonic setting has reduced levels of H3K27me3 when the gene undergoes DNA methylation in cancer[95].

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What might these changes and switches from PcG marking to DNA methylation mean for tumorigenesis? In stem and/or progenitor cell renewal systems in an adult, such adoption of tight transcriptional repression might prevent their subsequent activation, which is needed for signal transduction events and cell differentiation. In the setting of increased cancer risk states, such as chronic inflammation, where there is an abnormal stress for tissue renewal and a challenge to cell survival, such tight transcriptional repression may favour the abnormal expansion of stem and progenitor cell populations[96,97]. In turn, this expansion may further select for cell addiction to oncogenic gene mutations, which can help to foster abnormal growth and survival and progression to invasive states and malignancy[98]. The exploration of these possibilities provides fertile ground for future research.

Genetics meets epigenetics

We have previously reviewed how CpG methylation directly causes genetic changes in cancer by generating mutational hotspots in somatic tissues[99] or abnormally silencing DNA repair genes such as *MLH1* and *O*-6-methylguanine-DNA methyltransferase (*MGMT*) leading to microsatellite instability and a failure to repair DNA lesions, respectively[1]. However, recent whole-exome sequencing has unexpectedly revealed a high frequency of cancer-specific mutations in genes that are known to directly participate in epigenome organization in multiple tumour types. Thus, many abnormal epigenetic events may lie downstream of genetic abnormalities (FIG. 2). The precise phenotypic consequences of these mutations remain to be determined but important clues are emerging. For example, mutations in the *TET2*, isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* genes occur in gliomas and leukaemia[100,101]. In gliomas[101], *IDH1* mutations correlate with a DNA CpG island hypermethylator phenotype (CIMP)[102], an important concept that is continually being advanced and recognized for multiple tumour types. Strikingly, mutations in TET2 and IDH1 (or IDH2) are mutually exclusive in leukaemias[100], although each may correlate with increased numbers of DNA hypermethylated genes. This suggests a possible mechanism for the DNA methylation changes[100]. The TET proteins are hydroxylating enzymes that generate 5-hydroxymethylcytosine from methylated cytosines and may thus help to protect against unwanted DNA methylation in normal cells[38,39,103]. These enzymes require α-ketoglutarate as a cofactor, and mutant IDH1 and IDH2, through interactions with their wild-type proteins, use α-ketoglutarate to generate a profound build up of the metabolite 2-hydroxyglutarate, which inhibits TET2 (REF. 100). Therefore, focal excesses of 5-methyl-cytosine in the tumours could result from the failure to reduce its accumulation through the TET hydroxylation pathway. The localization of the TET enzymes, and increased levels of 5-hydroxymethylcytosine, at CpG island promoters of the genes that are most vulnerable to cancer-specific DNA hypermethylation (those with bivalent chromatin[39,40,104]) make this an attractive hypothesis. Furthermore, TET proteins may help to direct transcriptional repression[39,40,104,105] that could precede abnormal DNA methylation.

Although the above recent findings for TET enzymes provide insightful leads explaining how regions of the genome, such as promoter CpG islands, could acquire abnormal DNA methylation in cancer, much remains to be resolved about how this may actually evolve. More direct evidence for how the loss of TET, through mutations in these genes, or through the inhibition in the setting of IDH mutations, actually generates abnormal DNA methylation during tumour progression must be provided. Also, it remains to be precisely clarified how the proteins cause DNA demethylation. Current evidence is that this is not solely due to the formation of 5-hydroxymethylcytosine, which instead may be an intermediate that must subsequently be converted to cytosine through the action of the DNA repair processes that are mediated by proteins such as activation-induced cytidine deaminase (AID; also known as AICDA) and apolipoprotein B mRNA-editing enzyme 1 (APOBEC1)[105,106]. It is clear that future research will concentrate on all of these events

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over the next few years and the result will be a much clearer understanding of at least some of the events that contribute to the cancer epigenome.

Mutations in DNMT3A have also been recently defined with high frequency in aggressive forms of acute myelogenous leukaemia (AML), although altered DNA methylation patterns have not yet been found to correlate with this[107,108]. Intriguingly, one mutational hotspot is at a CpG site in *DNMT3A*, suggesting that the enzyme might be causing its own demise by methylating this site and increasing the likelihood for an inactivating C to T transition creating a base mismatch, which is not repaired.

Mutations in the histone-modifying enzyme EZH2, the catalytically active component of the PRC2 complex, have also been reported[109,110]. Initially, this was a surprising finding as EZH2 was considered to be an oncogene and the mutations were thought to be inactivating. However, follow-up studies reveal that several of the mutations actually lead to increased levels of H3K27me3, which is consistent with the predicted role of EZH2 in cancer[111]. Chromosomal translocations that lead to the oncogenic misstargeting of the mixed lineage leukaemia (MLL) histone methyltransferase are increasingly being characterized[112–115]. Interestingly, leukaemias can have hypermethylation of DNA sequences targeted by MLL owing to the absence of H3K4me3 (REF. 116), which, as discussed above, may repel DNA methyltransferases during *de novo* methylation. Importantly, some of the MLL translocations seem to occur *in utero* as they are observed in newborns, suggesting that this is the driver event in this type of cancer.

It has been known for some time that a component of the chromatin remodelling SNF5 complex BAF47, encoded by *SMARCB1*, is altered in particularly aggressive cases of rhabdomyosarcomas[117,118]. A recent mouse knockout model for this mutation has shown a tumour phenotype that seems to be entirely dependent on the activity of the PcG pathway for downstream effects[119]. Perhaps most excitingly, frequent mutations in related chromatin remodelling-encoding genes are increasingly being found in ovarian tumours, kidney tumours and leukaemias (FIG. 2). The frequency of these mutations strongly hints at their having a major causative or driver role. These new discoveries firmly establish that interference in epigenetic processes can lead to cancer and add credence to the idea that epigenetics is a major player in the disease process. A final exciting development linking genetics, epigenetics and cancer origins has been findings that firmly suggest that the inheritance of certain single nucleotide promoter variants, or other regional genetic alterations, can increase the probability of the *de novo* methylation of key genes such as *MLH1*, thus contributing to familial cancers and early onset disease[120–123].

Translational advances

Biomarker development

In the 1990s, the detection of abnormal promoter CpG island DNA hypermethylation emerged as a potential biomarker strategy for assessing cancer risk, early detection, prognosis and predicting therapeutic responses[124]. During the past decade, such strategies have moved towards actual clinical practice. The list of potential marker genes, knowledge of their position in cancer progression, and the development of ever more sensitive detection strategies, including nanotechnology approaches are all expanding[125,126]. Recent key developments include the use of hypermethylated genes in stool and blood DNA as highly sensitive and specific markers for colon cancer risk and detection[127,128], as well as the detection of glutathione *S*-transferase PI (*GSTP1*) hypermethylation in tumour biopsy samples and urine samples for prostate cancer[129,130]. Assays for these are commercially offered to clinicians, although the final realization of their value awaits approval by regulatory agencies, such as the US Food and Drug Administration (FDA). Mapping the

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patterns of DNA methylation has also recently been proposed to help in the identification of cancers of unknown primary site[131].

DNA methylation biomarkers can be used for the molecular prognosis of potentially curable, stage I non-small-cell lung cancer[132]. The concurrent hypermethylation of four genes, *CDKN2A* and *CDH13* in particular, in primary tumour and mediastinal lymph node biopsy samples, strongly correlates with early recurrence and death[132]. The validation of these findings might lead to a potentially powerful molecular re-staging strategy and might thus identify high risk patients who require special adjuvant treatments.

DNA methylation patterns can robustly predict response to chemotherapy. Importantly, hypermethylation marks that silence the DNA repair gene *MGMT*, which removes alkyl groups added to guanine in DNA, predict the best response and survival times after standard-of-care treatment with the alkylating agent temozolomide and radio-therapy in patients with glioma[133,134]. This clinical utility has recently been documented in a large, international Phase III cooperative group trial[135], and these data should now lead to FDA approval for using this marker in clinical practice. One caution engendered from recent findings in The Cancer Genome Atlas (TCGA) project, is that gliomas that harbour *MGMT* methylation in patients who have received alkylating agent treatment have a markedly increased frequency of mutations of the type predicted by DNA alkylation[136]. It seems that selection in treated tumours for mismatch repair deficiency allows cells to survive with a hypermutator phenotype[136].

Other chromatin abnormalities in cancer that we have discussed are also emerging as having important biomarker potential for cancer. For example, global and specific changes in patterns of histone acetylation and methylation have been reported to be hallmarks of cancer and/or to predict risk of tumour recurrence[137,138]. These changes are presumably due to abnormalities in histone-modifying proteins, which can sometimes be linked to the important problem of treatment resistance, as discussed below. The degree to which these potential biomarkers progress towards the clinic will crucially depend on work over the next several years to expand on these initial findings.

The potential for epigenetic therapy for cancer

There is growing excitement regarding the reversal of epigenetic abnormalities for cancer therapy[139,140]. 5-azanucleosides, which have long been known to have DNA demethylating activities[141], inhibit all three biologically active DNA methyltransferases, and initially proved too toxic for clinical use. However, pioneering clinical work by Silverman and colleagues[142], Issa and colleagues[143], and others[144] generated a remarkable oncology paradigm — therapeutic efficacy could be achieved at low drug doses. Such reduced doses were used in a large trial in patients with myelodisplastic syndrome (MDS), which can lead to leukaemia, and showed an increase in the time of conversion of MDS to frank leukaemia, as well as increased overall survival[144]. Now, two inhibitors, azacitidine (Vidaza; Celgene) and decitabine (Dacogen; Eisai), have gained recent approval by the FDA for MDS, and this paves the way for refining the use of low-dose regimens not only for leukaemia but also for solid tumours.

Histone deacetylase (HDAC) inhibitors are receiving intense trial activity[145]. Although HDACs targeted by these compounds affect many protein targets, they clearly also inhibit histone deacetylation that accompanies gene silencing states[145]. A milestone in therapy using agents that modify the epigenome is the recent FDA approval of vorinostat (Zolinza; Merck) and romidepsin (Istodax; Celgene) for their remarkable efficacy in cutaneous T cell lymphoma[146,147]. However, the precise molecular mechanisms for response in these patients have yet to be determined. The concept of combination epigenetic therapy has evolved from

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laboratory findings in 1999 that an HDAC inhibitor, which alone had little heritable effects on reversing the silencing of genes with dense CpG island DNA methylation, could augment gene reactivation following cell treatment with DNA methyl-transferase inhibitors[148]. Combination of these two classes of drugs is receiving attention in clinical trials for many cancers, but final efficacy is still to be determined[149,150].

Another recently raised fascinating possibility regarding the use of HDAC inhibitors is in overcoming the overwhelming problem of resistance to cancer therapy[41]. Multiple HDAC inhibitors were shown to reverse therapeutic resistance in selected subpopulations of cancer stem-like cells in culture, which are characterized by the overexpression of the histone demethylase *JARID1A*, which removes the active transcription mark H3K4me3 (REF. 41). A related protein has also just been shown to be overexpressed in stem-like subpopulations of melanoma cells[32].

This link between epigenetic changes and drug resistance, and the *in vitro* findings that an HDAC inhibitor can reverse the process, is important for the future development of drugs that are currently in use for epigenetic therapy. Epigenetic therapy used alone, even in diseases such as MDS and AML, in which efficacy has been greatest to date, is usually not curative. The best use of such drugs may be in combination with other chemotherapeutic strategies. Both HDAC inhibitors, and inhibitors of DNA methylation, could theoretically be used in patients to sensitize responses to standard agents that are being used in cancer management and/or to delay or reverse resistance to such agents and to targeted therapies. Much preclinical work is needed to sort out these possibilities and to derive the best design for future clinical trials to test these possibilities.

Finally, there is growing excitement that some of the mutated enzymes, such as EZH2 in the PcG system and the JARID histone demethylases, or other overexpressed proteins associated with the chromatin abnormalities discussed in this Perspective could be therapeutic targets for specific DNA methylation and histone deacetylation changes. One proof of principle linking genetics and epigenetics, involves translocations in leukaemia that result in altered chromatin landscapes and resultant gene expression changes. For example, translocation-mediated fusion of MLL with the protein AF10, and other partners, in infant leukaemias, leads to the abnormal recruitment of the histone methyltransferase DOT1L to gene targets such as *HOXA9* (REF. 114). These are leukaemia-causing events that are dependant on the DOT1L interaction with AF10 and other fusion partners with resultant targeting of hypermethylation of H3K79 and overactivation of HOXA9 and other MLL target genes[114,115]. Very recently, selective inhibitors of DOT1L have been developed that inhibit the H3K79 methylation, block overexpression of leukaemogenic genes and selectively inhibit, *in vitro* and *in vivo*, leukaemic cells harbouring the MLL gene translocations[114]. Most recently, translocations and/or overexpression of the bromodomain protein BRD4 have been linked to a driver role in a lethal form of paediatric epithelial cancer and to AML[151–153]. This factor seems to be intimately involved in the overactivation of the *MYC* oncogene in these settings[151–153]. Inhibiting BRD4 with small molecules blunts MYC overactivity and has antitumour effects[151–153]. This provides a potentially unique way of targeting MYC through an epigenetic abnormality and could accomplish the long sought-after goal of suppressing this oncogene. All of the above findings are tremendously compelling for the development of selective cancer therapies that are based on reversing chromatin abnormalities.

Conclusions

The past decade has seen exponential increases in interest and progress in the field of cancer epigenetics. Basic work on defining the structure of the mammalian epigenome in both

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normal and diseased states is leading to an unprecedented definition of the cancer epigenome. The field is now ripe for the elucidation of the topography of these epigenomes with respect to the precise cellular origins of cancer and how to use epigenetic classifications to better define cancer subcategories. Key remaining questions relate to how enhancer structures are regulated; the role of non-coding RNAs in specifying chromatin structure and the control of normal and abnormal gene expression; how boundaries are maintained and abnormally lost between regions of active versus repressed transcription; and in defining the three-dimensional maintenance and regulation of chromatin regions and how these function in cancer. The full range of epigenetic abnormalities in cancer will be clarified by this research. Rather than separating genetics from epigenetics, or trying to decide which is more important for cancer initiation and progression, the past few years have emphasized that these fields are merging. This is leading to an understanding of how mutations and epigenetic alterations work together to cause this disease. The next challenge lies not only in chronicling the mutations but also in defining the phenotypic ramifications for each of the epigenetic abnormalities in cancer. Exciting doors have been opened for translating all of the emerging knowledge for developing cancer biomarkers and new prevention and therapeutic strategies. Challenges for the next decade include bringing DNA methylation markers to full clinical use and mining the latest knowledge of chromatin abnormalities to obtain new biomarkers. Epigenetic therapies, although clearly now a reality, must be markedly refined and we must better understand how currently available agents are actually producing their clinical efficacies. As we have discussed, we still have much to learn about the best strategies for maximally using such drugs in terms of what combinations to use, how to synchronize them with other treatment modalities and especially whether they can sensitize patients to other agents or be used to overcome the paramount issue of drug resistance. Finally, the many new epigenetic therapeutic targets that are emerging must be intensely and creatively pursued. The future is exciting in terms of the benefits that may emerge for the prevention and management of all types of cancer.

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References

1. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nature Rev Genet. 2002; 3:415–428. [PubMed: 12042769]
2. Allis, C.; Jenuwein, T.; Reinberg, D. Epigenetics. Caparros, M., editor. Cold Spring Harbor Laboratory Press; 2007.
3. Mikkelsen TS, et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature. 2007; 448:553–560. [PubMed: 17603471]
4. Kouzarides T. Chromatin modifications and their function. Cell. 2007; 128:693–705. [PubMed: 17320507]
5. Bernstein BE, et al. The NIH Roadmap Epigenomics Mapping Consortium. Nature Biotech. 2010; 28:1045–1048.
6. Guenther MG, Young RA. Transcription. Repressive transcription Science. 2010; 329:150–151.
7. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007; 128:683–692. [PubMed: 17320506]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

8. Esteller M. Epigenetics in cancer. N Engl J Med. 2008; 358:1148–1159. [PubMed: 18337604]
9. Mikkelsen TS, et al. Dissecting direct reprogramming through integrative genomic analysis. Nature. 2008; 454:49–55. [PubMed: 18509334]
10. Cui K, et al. Chromatin signatures in multipotent human hematopoietic stem cells indicate the fate of bivalent genes during differentiation. Cell Stem Cell. 2009; 4:80–93. [PubMed: 19128795]
11. Kelly TK, et al. H2A.Z. maintenance during mitosis reveals nucleosome shifting on mitotically silenced genes. Mol Cell. 2010; 39:901–911. [PubMed: 20864037]
12. Lister R, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature. 2009; 462:315–322. [PubMed: 19829295]
13. Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA. A chromatin landmark and transcription initiation at most promoters in human cells. Cell. 2007; 130:77–88. [PubMed: 17632057]
14. Maunakea AK, et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature. 2010; 466:253–257. [PubMed: 20613842]
15. Heintzman ND, et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature. 2009; 459:108–112. [PubMed: 19295514]
16. Lin JC, et al. Role of nucleosomal occupancy in the epigenetic silencing of the MLH1 CpG island. Cancer Cell. 2007; 12:432–444. [PubMed: 17996647]
17. Bernstein BE, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell. 2006; 125:315–326. [PubMed: 16630819]
18. Chi AS, Bernstein BE. Developmental biology. Pluripotent chromatin state. Science. 2009; 323:220–221. [PubMed: 19131621]
19. Fujimura Y, et al. Distinct roles of Polycomb group gene products in transcriptionally repressed and active domains of Hoxb8. Development. 2006; 133:2371–2381. [PubMed: 16687444]
20. Azuara V, et al. Chromatin signatures of pluripotent cell lines. Nature Cell Biol. 2006; 8:532–538. [PubMed: 16570078]
21. Meshorer E, et al. Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. Dev Cell. 2006; 10:105–116. [PubMed: 16399082]
22. Gal-Yam EN, et al. Frequent switching of Polycomb repressive marks and DNA hypermethylation in the PC3 prostate cancer cell line. Proc Natl Acad Sci USA. 2008; 105:12979–12984. [PubMed: 18753622]
23. Kondo Y, et al. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nature Genet. 2008; 40:741–750. [PubMed: 18488029]
24. Ohm JE, et al. A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. Nature Genet. 2007; 39:237–242. [PubMed: 17211412]
25. Widschwendter M, et al. Epigenetic stem cell signature in cancer. Nature Genet. 2007; 39:157–158. [PubMed: 17200673]
26. Schlesinger Y, et al. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for *de novo* methylation in cancer. Nature Genet. 2007; 39:232–236. [PubMed: 17200670]
27. Guelen L, et al. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature. 2008; 453:948–951. [PubMed: 18463634]
28. Klose RJ, Kallin EM, Zhang Y. JmjC-domain-containing proteins and histone demethylation. Nature Rev Genet. 2006; 7:715–727. [PubMed: 16983801]
29. Allis, CD.; Jenuwein, T.; Reinberg, D. Overview and concepts. Cold Spring Harbor Laboratory Press; New York: 2007.
30. Agger K, Christensen J, Cloos PA, Helin K. The emerging functions of histone demethylases. Curr Opin Genet Dev. 2008; 18:159–168. [PubMed: 18281209]
31. Whetstine JR, et al. Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. Cell. 2006; 125:467–481. [PubMed: 16603238]
32. Roesch A, et al. A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. Cell. 2010; 141:583–594. [PubMed: 20478252]
33. Shi Y, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell. 2004; 119:941–953. [PubMed: 15620353]

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NIH-PA Author Manuscript

34. Trojer P, Reinberg D. Histone lysine demethylases and their impact on epigenetics. Cell. 2006; 125:213–217. [PubMed: 16630806]
35. Ooi SK, et al. DNMT3L connects unmethylated lysine 4 of histone H3 to *de novo* methylation of DNA. Nature. 2007; 448:714–717. [PubMed: 17687327]
36. Jeong S, et al. Selective anchoring of DNA methyltransferases 3A and 3B to nucleosomes containing methylated DNA. Mol Cell Biol. 2009; 29:5366–5376. [PubMed: 19620278]
37. Jones PA, Liang G. Rethinking how DNA methylation patterns are maintained. Nature Rev Genet. 2009; 10:805–811. [PubMed: 19789556]
38. Kriaucionis S, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science. 2009; 324:929–930. [PubMed: 19372393]
39. Williams K, et al. TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity. Nature. 2011; 473:343–348. [PubMed: 21490601]
40. Ficz G, et al. Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. Nature. 2011; 473:398–402. [PubMed: 21460836]
41. Sharma SV, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell. 2010; 141:69–80. [PubMed: 20371346]
42. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002; 16:6–21. [PubMed: 11782440]
43. Maegawa S, et al. Widespread and tissue specific age-related DNA methylation changes in mice. Genome Res. 2010; 20:332–340. [PubMed: 20107151]
44. Issa JP. CpG-island methylation in aging and cancer. Curr Top Microbiol Immunol. 2000; 249:101–118. [PubMed: 10802941]
45. Easwaran HP, et al. Aberrant silencing of cancer-related genes by CpG hypermethylation occurs independently of their spatial organization in the nucleus. Cancer Res. 2010; 70:8015–8024. [PubMed: 20736368]
46. Frigola J, et al. Epigenetic remodeling in colorectal cancer results in coordinate gene suppression across an entire chromosome band. Nature Genet. 2006; 38:540–549. [PubMed: 16642018]
47. Coolen MW, et al. Consolidation of the cancer genome into domains of repressive chromatin by long-range epigenetic silencing (LRES) reduces transcriptional plasticity. Nature Cell Biol. 2010; 12:235–246. [PubMed: 20173741]
48. Wood LD, et al. The genomic landscapes of human breast and colorectal cancers. Science. 2007; 318:1108–1113. [PubMed: 17932254]
49. Jones S, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science. 2008; 321:1801–1806. [PubMed: 18772397]
50. Leary RJ, et al. Integrated analysis of homozygous deletions, focal amplifications, and sequence alterations in breast and colorectal cancers. Proc Natl Acad Sci USA. 2008; 105:16224–16229. [PubMed: 18852474]
51. Chan TA, et al. Convergence of mutation and epigenetic alterations identifies common genes in cancer that predict for poor prognosis. PLoS Med. 2008; 5:e114. [PubMed: 18507500]
52. Schuebel KE, et al. Comparing the DNA hypermethylome with gene mutations in human colorectal cancer. PLoS Genet. 2007; 3:1709–1723. [PubMed: 17892325]
53. Yi JM, et al. Genomic and epigenomic integration identifies a prognostic signature in colon cancer. Clin Cancer Res. 2011; 17:1535–1545. [PubMed: 21278247]
54. Akiyama Y, et al. GATA-4 and GATA-5 transcription factor genes and potential downstream antitumor target genes are epigenetically silenced in colorectal and gastric cancer. Mol Cell Biol. 2003; 23:8429–8439. [PubMed: 14612389]
55. Suzuki H, et al. Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. Nature Genet. 2004; 36:417–422. [PubMed: 15034581]
56. Suzuki H, et al. Frequent epigenetic inactivation of Wnt antagonist genes in breast cancer. Br J Cancer. 2008; 98:1147–1156. [PubMed: 18283316]
57. Barekati Z, et al. Methylation profile of TP53 regulatory pathway and mtDNA alterations in breast cancer patients lacking TP53 mutations. Hum Mol Genet. 2010; 19:2936–2946. [PubMed: 20466735]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

58. Saito Y, et al. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell. 2006; 9:435–443. [PubMed: 16766263]
59. Lujambio A, et al. A microRNA DNA methylation signature for human cancer metastasis. Proc Natl Acad Sci USA. 2008; 105:13556–13561. [PubMed: 18768788]
60. Toyota M, et al. Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. Cancer Res. 2008; 68:4123–4132. [PubMed: 18519671]
61. Fabbri M, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci USA. 2007; 104:15805–15810. [PubMed: 17890317]
62. Garzon R, et al. MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. Blood. 2009; 113:6411–6418. [PubMed: 19211935]
63. Varambally S, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science. 2008; 322:1695–1699. [PubMed: 19008416]
64. Friedman JM, et al. The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. Cancer Res. 2009; 69:2623–2629. [PubMed: 19258506]
65. Lujambio A, et al. CpG island hypermethylation-associated silencing of non-coding RNAs transcribed from ultraconserved regions in human cancer. Oncogene. 2010; 29:6390–6401. [PubMed: 20802525]
66. Ren X, Kerppola TK. REST interacts with Cbx proteins and regulates polycomb repressive complex 1 occupancy at RE1 elements. Mol Cell Biol. 2011; 31:2100–2110. [PubMed: 21402785]
67. Yap KL, et al. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. Mol Cell. 2010; 38:662–674. [PubMed: 20541999]
68. Yu W, et al. Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. Nature. 2008; 451:202–206. [PubMed: 18185590]
69. Gupta RA, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature. 2010; 464:1071–1076. [PubMed: 20393566]
70. Carter MG, et al. Mice deficient in the candidate tumor suppressor gene Hic1 exhibit developmental defects of structures affected in the Miller-Dieker syndrome. Hum Mol Genet. 2000; 9:413–419. [PubMed: 10655551]
71. Chen WY, et al. Heterozygous disruption of Hic1 predisposes mice to a gender-dependent spectrum of malignant tumors. Nature Genet. 2003; 33:197–202. [PubMed: 12539045]
72. Chen W, et al. Epigenetic and genetic loss of Hic1 function accentuates the role of p53 in tumorigenesis. Cancer Cell. 2004; 6:387–398. [PubMed: 15488761]
73. Briggs KJ, et al. Cooperation between the Hic1 and Ptch1 tumor suppressors in medulloblastoma. Genes Dev. 2008; 22:770–785. [PubMed: 18347096]
74. Zhang W, et al. A potential tumor suppressor role for Hic1 in breast cancer through transcriptional repression of ephrin-A1. Oncogene. 2010; 29:2467–2476. [PubMed: 20154726]
75. Mohammad HP, et al. Loss of a single Hic1 allele accelerates polyp formation in Apc(Delta716) mice. Oncogene. 2011; 30:2659–2669. [PubMed: 21297660]
76. Teng IW, et al. Targeted methylation of two tumor suppressor genes is sufficient to transform mesenchymal stem cells into cancer stem/initiating cells. Cancer Res. 2011; 71:4653–4663. [PubMed: 21518779]
77. Cui H, Horon IL, Ohlsson R, Hamilton SR, Feinberg AP. Loss of imprinting in normal tissue of colorectal cancer patients with microsatellite instability. Nature Med. 1998; 4:1276–1280. [PubMed: 9809551]
78. Steenman MJ, Rainier S, Dobry CJU, Grundy P, Horon IL, Feinberg AP. Loss of imprinting of IGF2 is linked to reduced expression and abnormal methylation of H19 in Wilms' tumour. Nature Genet. 1994; 7:433–439. erratum 8, 203 (1994). [PubMed: 7920665]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

79. Bell AC, Felsenfeld G. Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature. 2000; 405:482–485. [PubMed: 10839546]
80. Schoenherr CJ, Levorse JM, Tilghman SM. CTCF maintains differential methylation at the Igf2/H19 locus. Nature Genet. 2003; 33:66–69. [PubMed: 12461525]
81. Moulton T, et al. Epigenetic lesions at the H19 locus in Wilms' tumour patients. Nature Genet. 1994; 7:440–447. [PubMed: 7920666]
82. Sparago A, et al. Microdeletions in the human H19 DMR result in loss of IGF2 imprinting and Beckwith-Wiedemann syndrome. Nature Genet. 2004; 36:958–960. [PubMed: 15314640]
83. Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. Nature Rev Genet. 2006; 7:21–33. [PubMed: 16369569]
84. Sakatani T, et al. Loss of imprinting of Igf2 alters intestinal maturation and tumorigenesis in mice. Science. 2005; 307:1976–1978. [PubMed: 15731405]
85. Doi A, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. Nature Genet. 2009; 41:1350–1353. [PubMed: 19881528]
86. Irizarry RA, et al. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. Nature Genet. 2009; 41:178–186. [PubMed: 19151715]
87. Issa JP. Epigenetic variation and cellular Darwinism. Nature Genet. 2011; 43:724–726. [PubMed: 21792236]
88. Hansen KD, et al. Increased methylation variation in epigenetic domains across cancer types. Nature Genet. 2011; 43:768–775. [PubMed: 21706001]
89. Feinberg AP, Tycko B. The history of cancer epigenetics. Nature Rev Cancer. 2004; 4:143–153. [PubMed: 14732866]
90. Berman BP, et al. Regions of focal DNA hypermethylation and long-range 1 hypomethylation in colorectal cancer coincide with nuclear lamina-associated domains. Nature Genet. (in the press).
91. Ohm JE, Baylin SB. Stem cell chromatin patterns: an instructive mechanism for DNA hypermethylation? Cell Cycle. 2007; 6:1040–1043. [PubMed: 17457052]
92. Meissner A, et al. Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature. 2008; 454:766–770. [PubMed: 18600261]
93. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nature Rev Genet. 2009; 10:295–304. [PubMed: 19308066]
94. Bartke T, et al. Nucleosome-interacting proteins regulated by DNA and histone methylation. Cell. 2010; 143:470–484. [PubMed: 21029866]
95. Tiwari VK, et al. PcG proteins, DNA methylation, and gene repression by chromatin looping. PLoS Biol. 2008; 6:2911–2927. [PubMed: 19053175]
96. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002; 420:860–867. [PubMed: 12490959]
97. Meng X, Riordan NH. Cancer is a functional repair tissue. Med Hypotheses. 2006; 66:486–490. [PubMed: 16290925]
98. Baylin SB. Stem cells, cancer, and epigenetics. Stembook. 2009:2–14.
99. Rideout WM, Coetzee GA, Olumi AF, Jones PA. 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. Science. 1990; 249:1288–1290. [PubMed: 1697983]
100. Figueroa ME, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell. 2010; 18:553–567. [PubMed: 21130701]
101. Noushmehr H, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell. 2010; 17:510–522. [PubMed: 20399149]
102. Issa JP. CpG island methylator phenotype in cancer. Nature Rev Cancer. 2004; 4:988–993. [PubMed: 15573120]
103. Tahiliani M, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009; 324:930–935. [PubMed: 19372391]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

104. Wu H, et al. Dual functions of Tet1 in transcriptional regulation in mouse embryonic stem cells. Nature. 2011; 473:389–393. [PubMed: 21451524]
105. Carey N, Marques CJ, Reik W. DNA demethylases: a new epigenetic frontier in drug discovery. Drug Discov Today. 2011; 15–16:683–690.
106. Cortellino S, et al. Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. Cell. 2011; 146:67–79. [PubMed: 21722948]
107. Ley TJ, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med. 2010; 363:2424–2433. [PubMed: 21067377]
108. Yamashita Y, et al. Array-based genomic resequencing of human leukemia. Oncogene. 2010; 29:3723–3731. [PubMed: 20400977]
109. Ernst T, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nature Genet. 2010; 42:722–726. [PubMed: 20601953]
110. Nikoloski G, et al. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nature Genet. 2010; 42:665–667. [PubMed: 20601954]
111. Yap D, et al. Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. Blood. 2011; 117:2451–2459. [PubMed: 21190999]
112. Stumpel DJ, et al. Specific promoter methylation identifies different subgroups of MLL-rearranged infant acute lymphoblastic leukemia, influences clinical outcome, and provides therapeutic options. Blood. 2009; 114:5490–5498. [PubMed: 19855078]
113. Schafer E, et al. Promoter hypermethylation in MLL-r infant acute lymphoblastic leukemia: biology and therapeutic targeting. Blood. 2010; 115:4798–4809. [PubMed: 20215641]
114. Daigle SR, et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. Cancer Cell. 2011; 20:53–65. [PubMed: 21741596]
115. Okada Y, et al. hDOT1L links histone methylation to leukemogenesis. Cell. 2005; 121:167–178. [PubMed: 15851025]
116. Erfurth FE, et al. MLL protects CpG clusters from methylation within the Hoxa9 gene, maintaining transcript expression. Proc Natl Acad Sci USA. 2008; 105:7517–7522. [PubMed: 18483194]
117. Versteege I, et al. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. Nature. 1998; 394:203–206. [PubMed: 9671307]
118. Uno K, et al. Aberrations of the hSNF5/INI1 gene are restricted to malignant rhabdoid tumors or atypical teratoid/rhabdoid tumors in pediatric solid tumors. Genes Chromosomes Cancer. 2002; 34:33–41. [PubMed: 11921280]
119. Wilson BG, et al. Epigenetic antagonism between polycomb and SWI/SNF complexes during oncogenic transformation. Cancer Cell. 2011; 18:316–328. [PubMed: 20951942]
120. Hitchins MP, et al. Dominantly inherited constitutional epigenetic silencing of MLH1 in a cancer-affected family is linked to a single nucleotide variant within the 5′UTR. Cancer Cell. 2011; 20:200–213. [PubMed: 21840485]
121. Hesson LB, Hitchins MP, Ward RL. Epimutations and cancer predisposition: importance and mechanisms. Curr Opin Genet Dev. 2010; 20:290–298. [PubMed: 20359882]
122. Chan TL, et al. Heritable germline epimutation of MSH2 in a family with hereditary nonpolyposis colorectal cancer. Nature Genet. 2006; 38:1178–1183. [PubMed: 16951683]
123. Ligtenberg MJ, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3′ exons of TACSTD1. Nature Genet. 2009; 41:112–117. [PubMed: 19098912]
124. Laird PW. The power and the promise of DNA methylation markers. Nature Rev Cancer. 2003; 3:253–266. [PubMed: 12671664]
125. Bailey VJ, et al. MS-qFRET: a quantum dot-based method for analysis of DNA methylation. Genome Res. 2009; 19:1455–1461. [PubMed: 19443857]
126. Li M, et al. Sensitive digital quantification of DNA methylation in clinical samples. Nature Biotech. 2009; 27:858–863.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

127. Glockner SC, et al. Methylation of TFPI2 in stool DNA: a potential novel biomarker for the detection of colorectal cancer. Cancer Res. 2009; 69:4691–4699. [PubMed: 19435926]
128. Lofton-Day C, et al. DNA methylation biomarkers for blood-based colorectal cancer screening. Clin Chem. 2008; 54:414–423. [PubMed: 18089654]
129. Cairns P, et al. Molecular detection of prostate cancer in urine by GSTP1 hypermethylation. Clin Cancer Res. 2001; 7:2727–2730. [PubMed: 11555585]
130. Rosenbaum E, et al. Promoter hypermethylation as an independent prognostic factor for relapse in patients with prostate cancer following radical prostatectomy. Clin Cancer Res. 2005; 11:8321–8325. [PubMed: 16322291]
131. Fernandez AF, et al. A DNA methylation fingerprint of 1628 human samples. Genome Res. May 25.2011 10.1101/gr.119867.110
132. Brock MV, et al. DNA methylation markers and early recurrence in stage I lung cancer. N Engl J Med. 2008; 358:1118–1128. [PubMed: 18337602]
133. Esteller M, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med. 2000; 343:1350–1354. [PubMed: 11070098]
134. Hegi ME, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med. 2005; 352:997–1003. [PubMed: 15758010]
135. Gilbert, MR., et al. ASCO Annual Meeting 2011 Abstr. University of Texas M. D. Anderson Cancer Center; Chicago: 2006. p. 2011
136. Network CGAR. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008; 455:1061–1068. [PubMed: 18772890]
137. Seligson DB, et al. Global histone modification patterns predict risk of prostate cancer recurrence. Nature. 2005; 435:1262–1266. [PubMed: 15988529]
138. Fraga MF, et al. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nature Genet. 2005; 37:391–400. [PubMed: 15765097]
139. Kelly TK, De Carvalho DD, Jones PA. Epigenetic modifications as therapeutic targets. Nature Biotech. 2010; 28:1069–1078.
140. Issa JP, Kantarjian HM. Introduction: emerging role of epigenetic therapy: focus on decitabine. Semin Hematol. 2005; 42:S1–S2. [PubMed: 16015499]
141. Jones PA, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. Cell. 1980; 20:85–93. [PubMed: 6156004]
142. Silverman LR, Mufti GJ. Methylation inhibitor therapy in the treatment of myelodysplastic syndrome. Nature Clin Pract Oncol. 2005; 2:S12–S23. [PubMed: 16341236]
143. Issa JP, Kantarjian H. Azacitidine. Nature Rev Drug Discov. 2005; 5:S6–S7. [PubMed: 15962522]
144. Fenaux P, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009; 10:223–232. [PubMed: 19230772]
145. Yoo CB, Jones PA. Epigenetic therapy of cancer: past, present and future. Nature Rev Drug Discov. 2006; 5:37–50. [PubMed: 16485345]
146. Duvic M, et al. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood. 2007; 109:31–39. [PubMed: 16960145]
147. Olsen EA, et al. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. J Clin Oncol. 2007; 25:3109–3115. [PubMed: 17577020]
148. Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nature Genet. 1999; 21:103–107. [PubMed: 9916800]
149. Gore SD, et al. Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. Cancer Res. 2006; 66:6361–6369. [PubMed: 16778214]
150. Griffiths EA, Gore SD. DNA methyltransferase and histone deacetylase inhibitors in the treatment of myelodysplastic syndromes. Semin Hematol. 2008; 45:23–30. [PubMed: 18179966]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

151. Schwartz BE, et al. Differentiation of NUT midline carcinoma by epigenomic reprogramming. Cancer Res. 2011; 71:2686–2696. [PubMed: 21447744]

152. Zuber J, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature. Aug 3.2011 10.1038/nature10334

153. Filippakopoulos P, et al. Selective inhibition of BET bromodomains. Nature. 2010; 468:1067–1073. [PubMed: 20871596]

154. Caligiuri MA, et al. Molecular rearrangement of the ALL-1 gene in acute myeloid leukemia without cytogenetic evidence of 11q23 chromosomal translocations. Cancer Res. 1994; 54:370–373. [PubMed: 8275471]

155. Jiao Y, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. Science. 2011; 331:1199–1203. [PubMed: 21252315]

156. Pasqualucci L, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature. 2011; 471:189–195. [PubMed: 21390126]

157. Banine F, et al. SWI/SNF chromatin-remodeling factors induce changes in DNA methylation to promote transcriptional activation. Cancer Res. 2005; 65:3542–3547. [PubMed: 15867346]

158. Jones S, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science. 2010; 330:228–231. [PubMed: 20826764]

159. Varela I, et al. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. Nature. 2011; 469:539–542. [PubMed: 21248752]

160. Wiegand KC, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med. 2010; 363:1532–1543. [PubMed: 20942669]

161. Yan H, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009; 360:765–773. [PubMed: 19228619]

162. van Haaften G, et al. Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. Nature Genet. 2009; 41:521–523. [PubMed: 19330029]

163. Jankowska AM, et al. Mutational spectrum analysis of chronic myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX, EZH2 and DNMT3A. Blood. Aug 9.2011 10.1182/blood-2010-10-311019

164. Gui Y, et al. Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. Nature Genet. 2011; 43:875–878. [PubMed: 21822268]

165. Wartman LD, et al. Sequencing a mouse acute promyelocytic leukemia genome reveals genetic events relevant for disease progression. J Clin Invest. 2011; 121:1445–1455. [PubMed: 21436584]

166. Schones DE, Zhao K. Genome-wide approaches to studying chromatin modifications. Nature Rev Genet. 2008; 9:179–191. [PubMed: 18250624]

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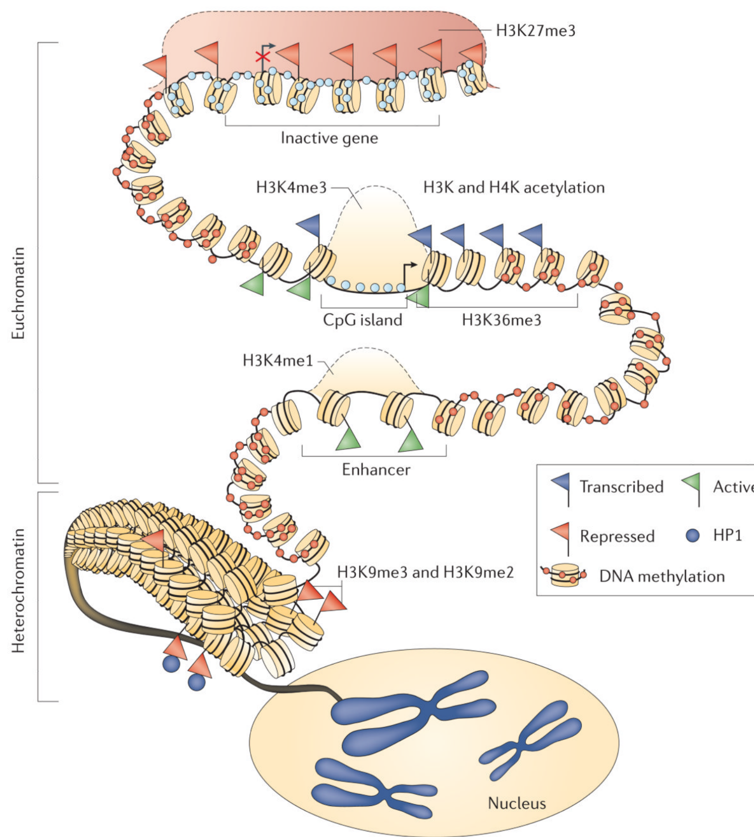

Figure 1. Model of the overall structure of the epigenome in normal human cells

This diagram shows the balanced state of chromatin, nucleosome positioning and DNA methylation, which maintains the normal packaging state of DNA. A silenced gene (indicated by a red X over the transcription start site designated by the arrow) at the top of the figure has its promoter CpG island occupied by a Polycomb group (PcG) complex (indicated by a red shaded area) that mediates chromatin changes that include the repressive histone modification trimethylation of lysine 27 on histone 3 (H3K27me3). There is no CpG DNA methylation within the gene promoter CpG island (shown by pale blue circles) and nucleosomes are positioned over the transcription start site. Sites upstream from the promoter are heavily DNA methylated (shown by red circles). The gene promoter illustrated below the silenced gene has been signalled to adopt a fully active transcription state and retains the active H3K4me3 marks at the promoter. It also has acetylation of key H3 and H4 lysines, the presence of the variant histone, H2A.Z (not shown) and H3K36me3 in the gene body to facilitate transcriptional elongation. The transcription start region (indicated by an arrow) is not occupied by nucleosomes. Just below, a distal enhancer is shown for this gene with an active nucleosome configuration, and the signature histone modification for enhancers, H3K4me1, is present. Finally, towards the bottom of the figure, the packaging of the majority of the cellular DNA into a transcriptionally repressed configuration is depicted, with compacted nucleosomes, the presence of H3K9me2 and H2K9me3, which are signature repressive marks for constitutive heterochromatin, the presence of heterochromatin protein 1 (HP1; also known as CBX5) and extensive DNA methylation. The folding of the heterochromatin into chromosomal locations in the nucleus is shown. Image is adapted, with permission, from REF. 166© (2008) Macmillan Publishers Ltd. All rights reserved.

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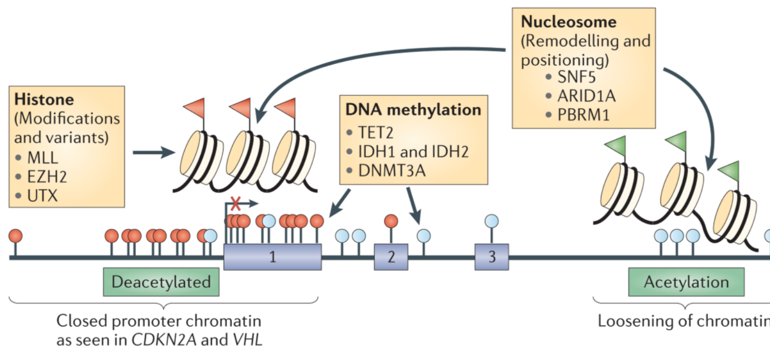

Figure 2. The cancer epigenome and relevant gene mutations

The cancer epigenome is characterized by simultaneous global losses in DNA methylation (indicated by pale blue circles) with hundreds of genes that have abnormal gains of DNA methylation (indicated by red circles) and repressive histone modifications (indicated by red flags) in promoter region CpG islands. The hypomethylated regions have an abnormally open nucleosome configuration and abnormally acetylated histone lysines (indicated by green flags). Conversely, abnormal DNA hypermethylation in promoter CpG islands is associated with nucleosomes positioned over the transcription start sites of the associated silenced genes (indicated by an arrow with a red X). Recent whole-exon sequencing of human cancers has shown a high proportion of mutations in genes in leukaemias, lymphomas, and ovarian, renal and pancreatic cancers, and rhabdomyosarcoma[109–111,154–156] (indicated in yellow boxes), which are depicted as helping to mediate either abnormal DNA methylation, histone modifications and/or nucleosome remodelling[100,107,108,118,155,157–165]. ARID1A, AT-rich interactive domain-containing protein 1A; DNMT3A, DNA methyltransferase 3A; EZH2, ehancer of zeste 2; IDH1, isocitrate dehydrogenase 1; MLL, mixed lineage leukaemia; PBRM1, protein polybromo 1; SNF5, SWI/SNF-related, matrix associated, actin-dependent regulator of chromatin, subfamily B, member 1; VHL, Von Hippel–Lindau.

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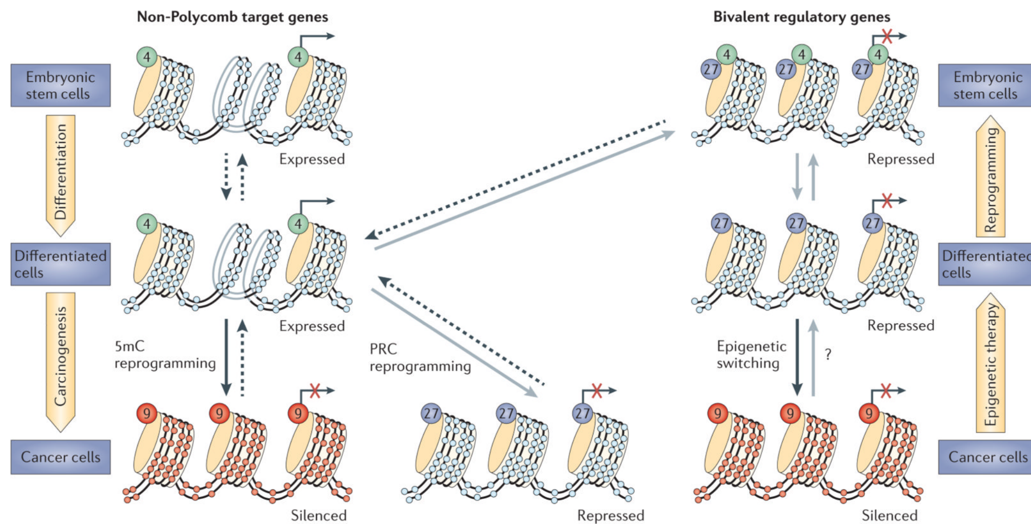

Figure 3. Modes of abnormal gene silencing in cancer

The currently suggested routes to abnormally silenced genes in cancer are shown. Genes that are active in cells throughout development and adult cell renewal initially have active promoter chromatin that is characterized by the presence of the histone modification, H3K4me (indicated by green circles and dashed arrows), and a lack of DNA methylation (indicated by pale blue circles). Genes that become silenced (indicated by a red X) can do so either by the acquisition of DNA methylation (indicated by red circles) and the presence of the repressive mark, H3K9me (indicated by orange circles and black arrows), or by the presence of Polycomb-mediated repressive chromatin (PRC) marks, H3K27me (purple circles and grey arrows). DNA methylation and H3K9me marks during tumour progression are shown. The wide yellow arrows at the sides of the figure depict movements that link stem and progenitor cells and differentiated cells and which can be impeded by epigenetic abnormalities in cancer or which can be corrected by epigenetic therapy.

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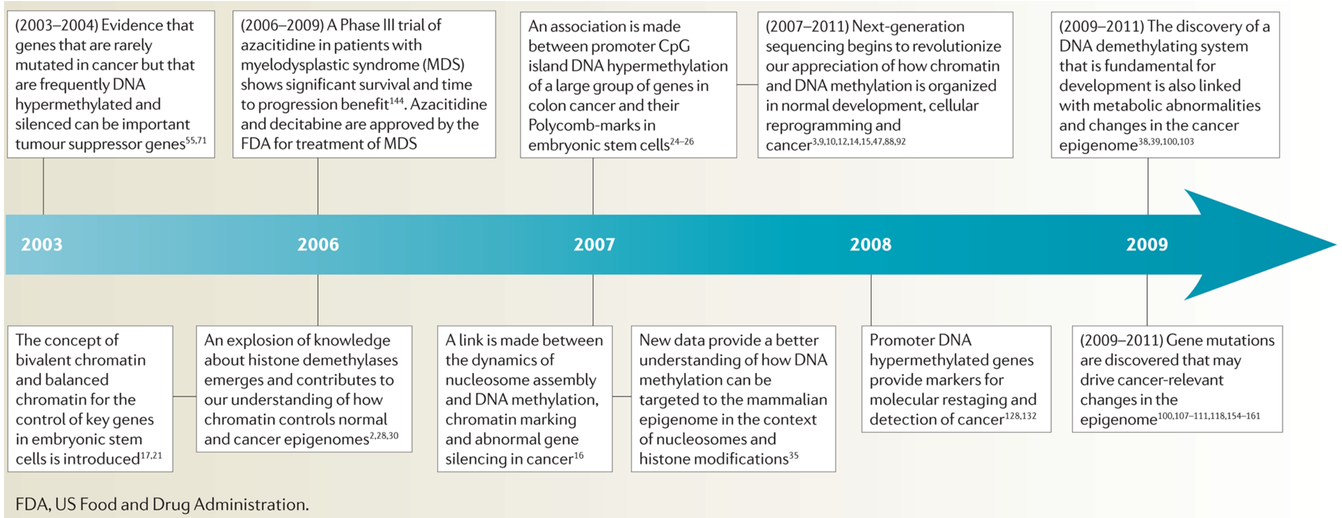

Timeline. Example of key advances in epigenetics and cancer over the past decade