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

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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. 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 of the 1980s of DNA methylation abnormalities. 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, resulting in an emerging view of what may now be called ‘the cancer epigenome’, which harbours myriad abnormalities that are based on somatically inheritable alterations that are not due to primary DNA sequence changes (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 (iPSCs), have revolutionized our knowledge of chromatin states, nucleosome positioning and how alterations in these contribute to disease. Intriguingly, as discussed below, these gene promoters are prone to undergoing an epigenetic switch and become de novo DNA methylated in cancer and pre-cancerous cells.

There has also been an explosion of knowledge regarding the molecular determinants of global-scale chromatin architecture. Insulator proteins such as CTCF and others, together with PcG occupancy, 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 specific covalent marks, including H3K36me3, which may facilitate transcriptional elongation. These regions normally have dense lysine methylation, even in downstream CpG islands, which might also promote transcription elongation rather than repress transcription initiation — as methylation does in promoter regions. Importantly, structural features of enhancers are being defined, including deoxyribonuclease 1 (DNase1) sensitivity, nucleosome depletion, and the presence of H3K4me1 and H3 acetylated on lysine 27 (H3K27ac) in the active state. 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. 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.

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. In contrast to the strong localization of active marks to TSSs, the H3K27me3 inhibitory mark applied by the Polycomb group proteins (PcG) 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. 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 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. Intriguingly, as discussed below, these gene promoters are prone to undergoing an epigenetic switch and become de novo DNA methylated in cancer and pre-cancerous cells.

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defined that can neatly surround a nucleosome and presumably initiate methylation in this context. This complex comprises two molecules of the de novo DNA methyltransferase DNMT3A and two of DNMT3L, a catalytically inactive isomer that is expressed in ESCs. Nucleosomes containing the active histone mark H3K4me3 cannot be encompassed by this complex, 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. 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.

Excitingly, enzyme systems that remove DNA methylation, such as the TET family proteins, which form 5-hydroxymethyl cytosine from methylated cytosine, have been discovered to be crucially important during development and tumorigenesis. The concept of bivalent chromatin and balanced chromatin for the control of key genes in embryonic stem cells is introduced.

### 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, and these findings are providing a greater understanding of the importance of these abnormalities to tumorigenesis. Juxtaposing these changes with other abnormalties 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. Rapid advances that have enabled the precise mapping of DNA methylation across normal and cancer genomes confirms that almost all cancers harbour hundreds of genes with these abnormal gains in methylation, affecting some 5–10% of the thousands of promoter CpG islands that never normally contain DNA methylation from embryonic development onwards. Some genes do, however, show changes in CpG island DNA methylation with ageing. Although most genes that become DNA hypermethylated in cancer are usually affected on an individual basis, subchromosomal domains with multiple hypermethylated loci have recently been discovered.

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, 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 CDK2NA (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.

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, their importance may be their aggregation in the same signalling pathways, thus helping in the derivation of the cancer phenotype. Importantly, epigenetic changes can be much more common for many of the same infrequently mutated genes. 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. 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. DNA hypermethylation also affects multiple genes that negatively regulate the WNT pathway from the cell membrane to the nucleus in this same tumour type. 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. Additionally, DNA hypermethylation of a range of genes can collectively contribute to the disruption of the p53 pathway.
PERSPECTIVES

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 miRNAs, such as BCL-6 (REF 58), and the constitutive activation of signalling that promotes invasiveness and metastatic activities59–62. Intriguingly, downregulation of the miRNA-29 family has also been linked to overexpression of DNA methyltransferases60,61, possibly creating a scenario that is permisive for gene promoter DNA hypermethylation. miR-101 is often downregulated in cancer, leading to overexpression of enhancer of zeste 2 (EZH2)62–64 – a key component of the PcG system, which, as previously noted, marks genes that are prone to cancer-specific DNA hypermethylation65,66,67. Cancer-specific DNA hypermethylation has also been reported to silence other non-coding RNAs68. 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 changes69–72.

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 effects70 but heterozygotes develop a range of tumour types that are common in humans as they age71. In these cancers, the wild-type Hic1 allele almost always acquires promoter DNA methylation72. 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 targets73–75. Most recently, targeted DNA hypermethylation of the HIC1 promoter has been found to transform human mesenchymal stem cells76.

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 region79,80. 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 enhancer78,81. 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

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 Polycromb 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.
epigenetic changes can be the earliest initiating factor in a human cancer\textsuperscript{41}. More recently, this change in JGF2 has been linked to the expansion of abnormal stem and progenitor cells in the progression of colon cancer\textsuperscript{44}.

**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\textsuperscript{85,86}. Alterations of this methylation pattern in cancer cells have been reported to be more frequent than those in nearby CpG islands\textsuperscript{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\textsuperscript{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\textsuperscript{1,2,3,89}. 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\textsuperscript{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\textsuperscript{47,88,90}. Also, this change and the methylation losses that occur within the bodies of many genes could be functionally related to reduced expression in cancer\textsuperscript{41}. 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\textsuperscript{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\textsuperscript{21}. A working model\textsuperscript{91} 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\textsuperscript{92,93}. 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\textsuperscript{22}. In others, once DNA methylation evolves, the PcG complex and the H3K27me3 histone mark may be quantitatively and/or qualitatively replaced\textsuperscript{21,93} (FIG. 3). These possibilities are consistent with recent data that methylated DNA in a nucleosomal context actually repels components of PcGs\textsuperscript{41}. 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\textsuperscript{95}.

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\textsuperscript{86,87}. 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\textsuperscript{96}. The exploration of these possibilities provides fertile ground for future research.
**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.

**Genetics meets epigenetics.** We have previously reviewed how CpG methylation directly causes genetic changes in cancer by generating mutational hotspots in somatic tissues\(^9\) 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\(^100\), 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\(^103,104\). 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-methylcytosine 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\(^103,104\)) make this an attractive hypothesis. Furthermore, TET proteins may help to direct transcriptional repression\(^103,104,105\) 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 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. 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. 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. Chromosomal translocations that lead to the oncogenic mis-targeting of the mixed lineage leukaemia (MLL) histone methyltransferase are increasingly being characterized. 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. 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. 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.

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. During the past decade, such strategies have moved towards actual clinical application. 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. 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, as well as the detection of glutathione S-transferase P1 (GSTP1) hypermethylation in tumour biopsy samples and urine samples for prostate cancer. 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 patterns of DNA methylation has also recently been proposed to help in the identification of cancers of unknown primary site.

DNA methylation biomarkers can be used for the molecular prognosis of potentially curable, stage I non-small-cell lung cancer. The concurrent hypermethylated of four genes, CDKN2A and CDH13 in particular, in primary tumour and mediastinal lymph node biopsy samples, strongly correlates with early recurrence and death. 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 radiotherapy in patients with glioma. This clinical utility has recently been documented in a large, international Phase III cooperative group trial, 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. It seems that selection in treated tumours for mismatch repair deficiency allows cells to survive with a hypermutator phenotype.

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. 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. 5-azanucleosides, which have long been known to have DNA demethylating activities, inhibit all three biologically active DNA methyltransferases, and initially proved too toxic for clinical use. However, pioneering clinical work by Silverman and colleagues, Issa and colleagues, and others 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 myelodysplastic 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. 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. Although HDACs targeted by these compounds affect
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 dependent 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 genes114,115. 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 translocations114. 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 AML149–151. This factor seems to be intimately involved in the overactivation of the MYC oncogene in these settings114,115. Inhibiting BRD4 with small molecules blunts MYC overactivity and has antitumour effects149–151. 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 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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methylated DNA.


PERSPECTIVES


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Competing interests statement

The authors declare competing financial interests. See Web version for details.

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