DISEASE MECHANISMS

Mutations in regulators of the epigenome and their connections to global chromatin patterns in cancer

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Abstract | Malignancies are characterized by extensive global reprogramming of epigenetic patterns, including gains or losses in DNA methylation and changes to histone marks. Furthermore, high-resolution genome-sequencing efforts have discovered a wealth of mutations in genes encoding epigenetic regulators that have roles as 'writers', 'readers' or 'editors' of DNA methylation and/or chromatin states. In this Review, we discuss how these mutations have the potential to deregulate hundreds of targeted genes genome wide. Elucidating these networks of epigenetic factors will provide mechanistic understanding of the interplay between genetic and epigenetic alterations, and will inform novel therapeutic strategies.

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Cancer genome analysis has spurred the identification of genetically altered genes that drive tumorigenesis. Identifying these cancer genes is important for understanding pathways and gene functions in normal and cancer tissues, and such identification is a necessary prerequisite in the development of biomarkers and targeted therapies for cancer. Approaches to find cancer genes have evolved from studies of candidate genes to genome-screening protocols. Driven by large consortia (such as the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA)) and the rapid development and implementation of next-generation sequencing platforms, genetic studies have recently entered a new phase of uncovering large catalogues of gene mutations and structural variations.

Alterations in the epigenetic regulation of genome activity are as important to tumorigenesis as alterations in the genomic coding information itself. Epigenetic modifications of DNA and histones, and/or alterations in chromatin-remodelling processes, determine active and repressive chromatin states of genes and of chromosomal regions and thus operate as switches either to turn gene expression 'on' or 'off' (such as by promoter methylation), or to modulate gene expression levels (such as by enhancer methylation). Each of these epigenetic pathways involves enzymes that transfer the modification ('writers'), enzymes that modify or revert a modification ('editors') and enzymes that mediate the interactions of proteins or protein complexes with the modification ('readers') (FIG. 1). DNA methylation changes have been extensively investigated in cancer as a reflection of aberrant epigenetic regulation of genes in human disease, followed by studies of histone modifications[1,2]. Numerous reports showed that cancer genomes exhibit frequent alterations to the epigenome. These include epigenetic silencing of various tumour suppressor genes with functions in almost all cancer-relevant signalling pathways, such as apoptosis, cell proliferation, cell migration and DNA repair[3].

Currently, the integration of epigenetic profiles with genetic profiles of cancer genomes is underdeveloped. The reasons for the lack of such comprehensive studies were mainly due to the limited ability of methodologies to specifically and quantitatively assess epigenetic alterations, the complexity of epigenetic alterations in cancer genomes and a lack of mechanistic knowledge of how epigenetic deregulation of the genome occurs. However, this picture is becoming clearer owing to the recent identification of cancer-specific genetic mutations in various proteins that are involved in establishing epigenetic patterns, thus providing mechanistic insights into the interplay between genetic and epigenetic alterations in cancer. Furthermore, recently developed high-throughput and quantitative assays have been used to measure and integrate genome-wide

DNA methylation profiles, histone modification profiles and the resultant gene expression patterns, which have eased the analysis of multiple data sets across various cancer and normal tissue samples.

In this Review, we discuss the interconnection between genetic and epigenetic alterations in tumour genomes, highlight current high-profile analyses, and call for an integrative genomic and epigenomic approach

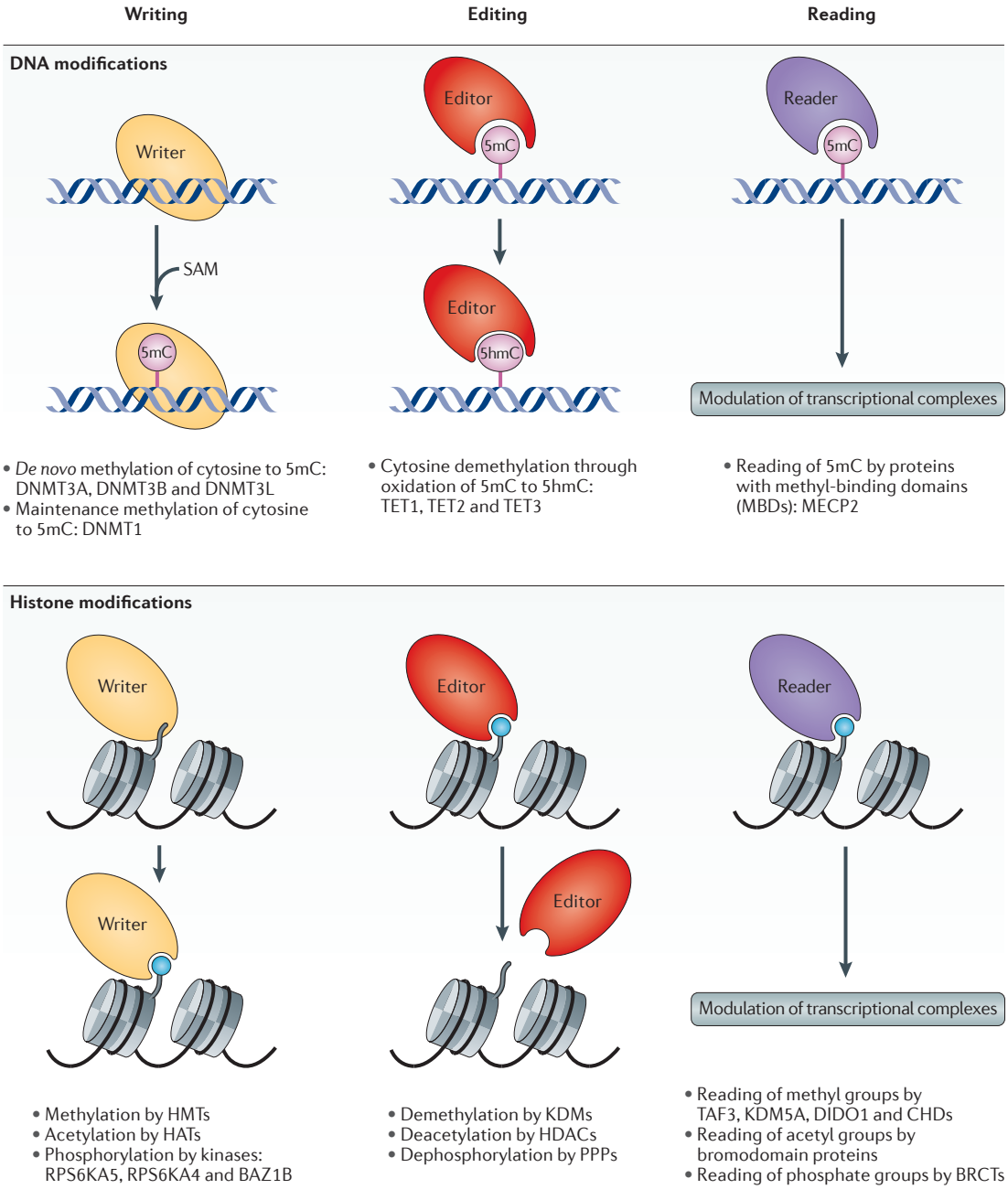


Figure 1 | **Enzymes involved in DNA and histone modification pathways.** Enzymes that establish a mark on either DNA or the histone tail are termed 'writers'. These modifications can be removed or modified by 'editing' enzymes. The third class of enzymes includes the 'readers' of an epigenetic mark, which mediate the interaction of the mark with a protein complex to exert effects on transcription. The top panel depicts DNA modifications, such as DNA methylation and demethylation, and the enzymes involved; the bottom panel shows histone modifications and the enzymes involved. Examples for each class of enzyme are given. 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; BAZ1B, tyrosine protein kinase BAZ1B; BRCT, BRCT domain-containing protein; CHD, chromodomain helicase DNA-binding protein; DIDO1, death-inducer obliterator 1; DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; KDM, lysine-specific histone demethylase; MECP2, methyl-CpG-binding protein 2; PPP, serine/threonine protein phosphatase; RPS6K, ribosomal protein S6 kinase; SAM, S-adenosyl-L-methionine, TAF3, transcription initiation factor TFIID subunit 3; TET, TET 5mC hydroxylase.

Epigenetic modifications
Modifications of DNA and histones that do not change the genetic code but have an effect on gene expression or chromatin condensation; these modification patterns are stably transmitted to daughter cells after cell division.

Epigenome
The entire epigenetic modifications of DNA and histones in the genome of a tissue.

to understand the mechanisms of epigenetic deregulation. We use recent discoveries of mutations in *H3F3A* (which encodes the histone H3 variant H3.3) in paediatric glioblastoma, as well as isocitrate dehydrogenase 1 (*IDH1*) mutations in gliomas, as examples of a successful integration of genetic and epigenetic data in the understanding of tumorigenesis (BOX 1). We aim to illuminate the principles and pathways that may affect epigenetic patterning rather than to provide complete lists of mutated epigenetic factors, which have recently been reported elsewhere in the literature[4–6]. We also describe the latest progress in targeting epigenetic enzymes as an anticancer therapeutic strategy.

Global epigenetic alterations in cancer

Overview of DNA methylation alterations in cancer. Epigenetic alterations in cancer have been investigated for more than 25 years, both on the single-gene level[7,8] and on the genome-wide level[9]. Most studies have analysed 5-methylcytosine (5mC), although it is now known that cytosines can be further modified to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC)[10,11]. Hypermethylation of a CpG island promoter is associated with gene silencing, which has been demonstrated for numerous tumour suppressor genes[1,2]. However, cancer genomes are also characterized by the global loss of 5mC (hypomethylation), which mainly affects repetitive and gene-body sequences, including gene regulatory sequences[12]. In addition, hypomethylation loosens the chromatin structure, leading to chromosomal instability such as translocations or deletions[13,14].

Global profiling: detailed insights into DNA methylation and histone modifications. Novel profiling technologies for epigenetic alterations have revolutionized the precision and comprehensiveness of mapping epigenetic alterations (FIG. 2). Profiling studies found 2,000–3,000 aberrantly methylated gene promoters per cancer genome, most of which are hypermethylated and associated with gene silencing[15,16]. By contrast, the number of protein-coding genes that are affected by genetic mutations per cancer genome is one to three orders of magnitude smaller. Thus, although some genes are inactivated by genetic mutations in cancer, most inactivated genes are silenced by epigenetic alterations or a combination of epigenetic and genetic events. A challenge in the cancer epigenetics field is to identify the epigenetic events that drive tumorigenesis (that is, driver events) and to distinguish them from passenger events, which are not causally linked to tumorigenesis.

An early study reported recurrent patterns of aberrant CpG island methylation across tumours of similar tissue types. Such nonrandom patterns indicate the presence of specific molecular mechanisms that lead to these patterns[16]. Additionally, differences in DNA methylome patterns are found among tumour types[16], which suggests that tumour-specific methylome patterns are partly representative of the cell of origin of a given tumour type. The different methylome profiles can be used to further stratify tumours into subtypes of different histopathological groups or clinical outcomes[17–19]. This has been exemplified by cancer-specific methylation patterns in selected gene promoter sequences, which are known as CpG island methylator phenotypes[18,20].

Altered DNA methylation patterns in cancer genomes correlate with altered patterns of histone modifications. DNA and histone modifications collectively determine the overall chromatin state, which maintains whether genes are transcriptionally active or inactive. An interesting observation was that many hypermethylated promoters in cancer are in lineage-commitment genes that display bivalent chromatin states in normal multipotent cells[21,22]. Bivalent chromatin is characterized by both active (such as H3K4me3) and repressive (such as H3K27me3) chromatin marks and seems to 'poise' genes for either transcriptional activation or inactivation upon differentiation[23]. Hypermethylation of the promoter DNA of these genes in cancer cells may suggest that stem cells and progenitor cells, in particular, are prone to cancer initiation[22,24,25].

Global profiling techniques have provided further insights into the overall DNA hypomethylation state in cancer cells. Partially methylated domains (PMDs) are large stretches of partially methylated DNA (that is, <70% of cytosines are methylated). Despite this hypomethylation, PMDs of up to 10 Mb in size are associated with repressive histone marks (including H3K9me3 and H3K27me3) and gene silencing. PMDs were first detected in somatic tissues by applying whole-genome bisulphite sequencing (WGBS)[26]; interestingly, these loci are nearly completely methylated in pluripotent stem cells[27]. WGBS analyses in breast, colon, lung and thyroid cancer cells identified PMDs with tissue-specific patterns[12]. The functional consequences of PMDs are unknown but it is possible that gene regulatory elements such as enhancers are affected. PMDs are associated with regions of low gene content or silenced genes, and with repressive histone marks, sometimes in an allele-specific manner[12,27,28]. PMDs were recently shown to occur at the same locations as nuclear lamina-associated DNA domains[29]. These domains are transcriptionally silent regions that are attached to the nuclear membrane, and thus PMDs may reflect cell type-specific features of three-dimensional genome architecture. The frequency and occurrence of PMDs in cancer need to be determined but PMDs could have an even more important effect on global gene expression than gene silencing by promoter methylation.

Mutations in regulators of the epigenome

Novel sequencing technologies are now enabling the resequencing of thousands of cancer genomes. Large consortia (for example, the ICGC, TCGA or the International Human Epigenome Consortium (IHEC)) have used this opportunity to sequence cancer genomes and methylomes, to unravel coding-gene mutations using whole-exome sequencing[30], to discover large genetic rearrangements using paired-end mapping[31–33], to identify both gene mutations and DNA copy-number alterations using whole-genome sequencing, and to characterize alterations in either regulatory sequences[34]

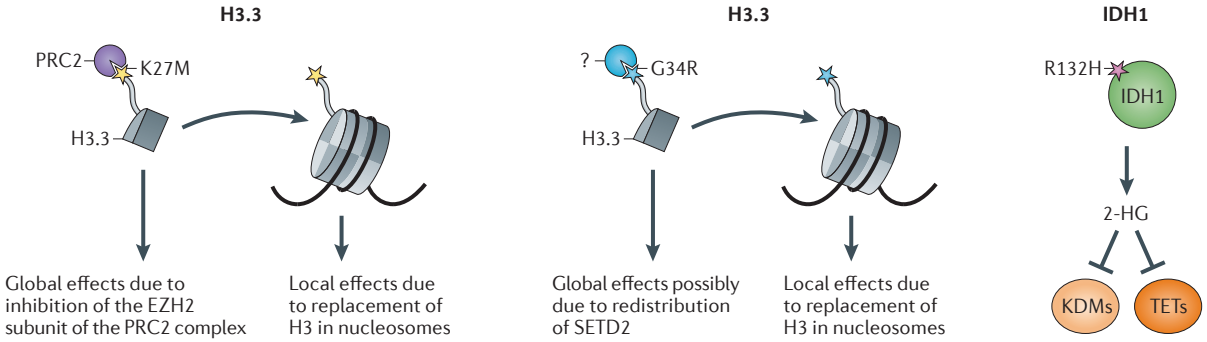
CpG island methylator phenotypes
Enrichments for the methylation of GC-rich promoter sequences that were initially defined in a colon cancer study.

Box 1 | The epigenetic effects of H3.3-K27M, H3.3-G34R/V and IDH1-R132H mutations in glioblastoma

Glioblastoma is a common and heterogeneous brain tumour with defined characteristics. Although the initiating event remains unresolved, a combination of mutated genes seems to drive extensive angiogenesis (such as vascular endothelial growth factor (*VEGF*)), trigger proliferation (such as genes encoding receptor tyrosine kinases (RTKs)), disrupt metabolism (such as isocitrate dehydrogenase 1 (*IDH1*)), and promote migration (such as neurotrophic tyrosine kinase receptor type 1 (*TRKA*)) and invasion (such as hepatocyte growth factor (*HGF*) or its receptor (*MET*))[134]. It has recently been discovered that mutations in histone variants have an important role in global defects in chromatin architecture, leading to aberrant gene expression[42]. Mutations in *H3F3A*, one of three genes encoding histone variant H3.3, have been recognized as being especially important in chromatin remodelling[18] (see the figure). The H3.3 mutations result in Lys27Met (K27M) and Gly34Arg or Gly34Val (G34R/V) substitutions, which promote global histone modification changes, most notably H3K27me3 and H3K36me3, respectively (see the table). Both K27M and G34R/V tumours have a general DNA hypomethylation phenotype, which indicates a dynamic relationship between histone modifications and DNA methylation (5-methylcytosine (5mC)). The aberrant gene expression in these tumours is presumably the result of a multitude of epigenetic changes that drive tumorigenesis together. K27M inhibits the enhancer of zeste homologue 2 (EZH2) subunit of Polycomb repressive complex 2 (PRC2), leading to extensive global loss of H3K27me3. Previously repressed genes become upregulated followed by a substantial redistribution of H3K27me3, creating a bivalent state with previously placed H3K4me3 (REFS 94,95). The bivalent state could potentially mimic the poised condition that is reminiscent of embryonic stem cells and provide the origin of tumorigenesis. G34R/V mainly leads to the redistribution of H3K36me3, possibly by redirecting its enzyme SET domain-containing 2 (SETD2; also known as KMT3A), leading to enhanced expression of genes such as *MYCN*[135]. Interestingly, a potential overlap of the effects of G34R/V and K27M may occur, as it has been shown that H3K36me3 binds the SUZ12 subunit of PRC2, reducing its activity and leading to loss of H3K27me3 (REFS 136,137). Furthermore, both mutations exhibit the tumour-specific alternative lengthening of telomeres (ALT) phenotype that may contribute to gliomagenesis. However, other lines of evidence suggest separate disease aetiologies for H3.3-K27M- versus H3.3-G34R/V-mutant tumours. These include the finding of H3.3-K27M and H3.3-G34R/V mutations in distinct positions of the brain, and different DNA methylation and overall gene expression patterns. For example, the gene expression pattern in H3.3-K27M tumours is biased towards mid-to-late cortical development and includes a characteristic upregulation of oligodendrocyte lineage transcription factor 2 (*OLIG2*), whereas in G34R/V tumours the gene expression pattern is biased towards early neocortical development and includes a characteristic upregulation of forkhead box G1 (*FOXG1*)[18,135]. Important roles for these epigenetic processes in human development and maturation are suggested by the enrichment for H3.3 mutations in younger patients with glioblastoma.

Another known gain-of-function mutation with a strong influence on chromatin remodelling in glioblastoma is the *IDH1* R132H mutation (and equivalent mutations in *IDH2*), producing the oncometabolite 2-hydroxyglutarate (2-HG)[138]. A series of studies have shown that 2-HG inhibits the activity of deaminases — for example, the TET 5mC hydroxylases (TETs) and the lysine-specific histone demethylases (KDMs)[19,60]. The inhibition of these enzymes collectively affects chromatin remodelling, leading to DNA hypermethylation, increased histone lysine methylation and aberrant gene expression. However, the *IDH1* R132 mutations are associated with a better prognosis than non-mutated *IDH1* in glioblastoma, presumably through increased repressive modifications that slow cellular proliferation and invasion.

Together, these gain-of-function mutations are the archetypes of proteins or compounds that interfere with other master regulatory chromatin proteins, such as PRC2 or the TET proteins.



	H3.3-K27M mutation	H3.3-G34R mutation	IDH1-R132H mutation
Targeted enzyme	EZH2 in PRC2	SETD2?	KDMs and TETs
5mC	Hypomethylation	Hypomethylation	Hypermethylation
Histone modifications	H3K27me3 decreased and redistributed	H3K36me3 redistributed	H3K9me3 increased, H3K27me3 increased, H3K4me3 increased and H3K36me3 increased
Developmental gene-expression pattern	Neurogenic and mid-to-late cortical development	Neurogenic and early-to-late neocortical development	Neurogenic and neural progenitor cell bias
Gene expression owing to 5mC changes	PRC2-target genes upregulated, *FOXG1* downregulated and *OLIG2* upregulated	*MYCN* upregulated, *FOXG1* upregulated and *OLIG2* downregulated	Genes involved in differentiation downregulated
ALT	Yes	Yes	Not known
Prevalence in childhood glioblastoma	19%	15%	<10%
Prevalence in adult glioblastoma	0%	0%	77%

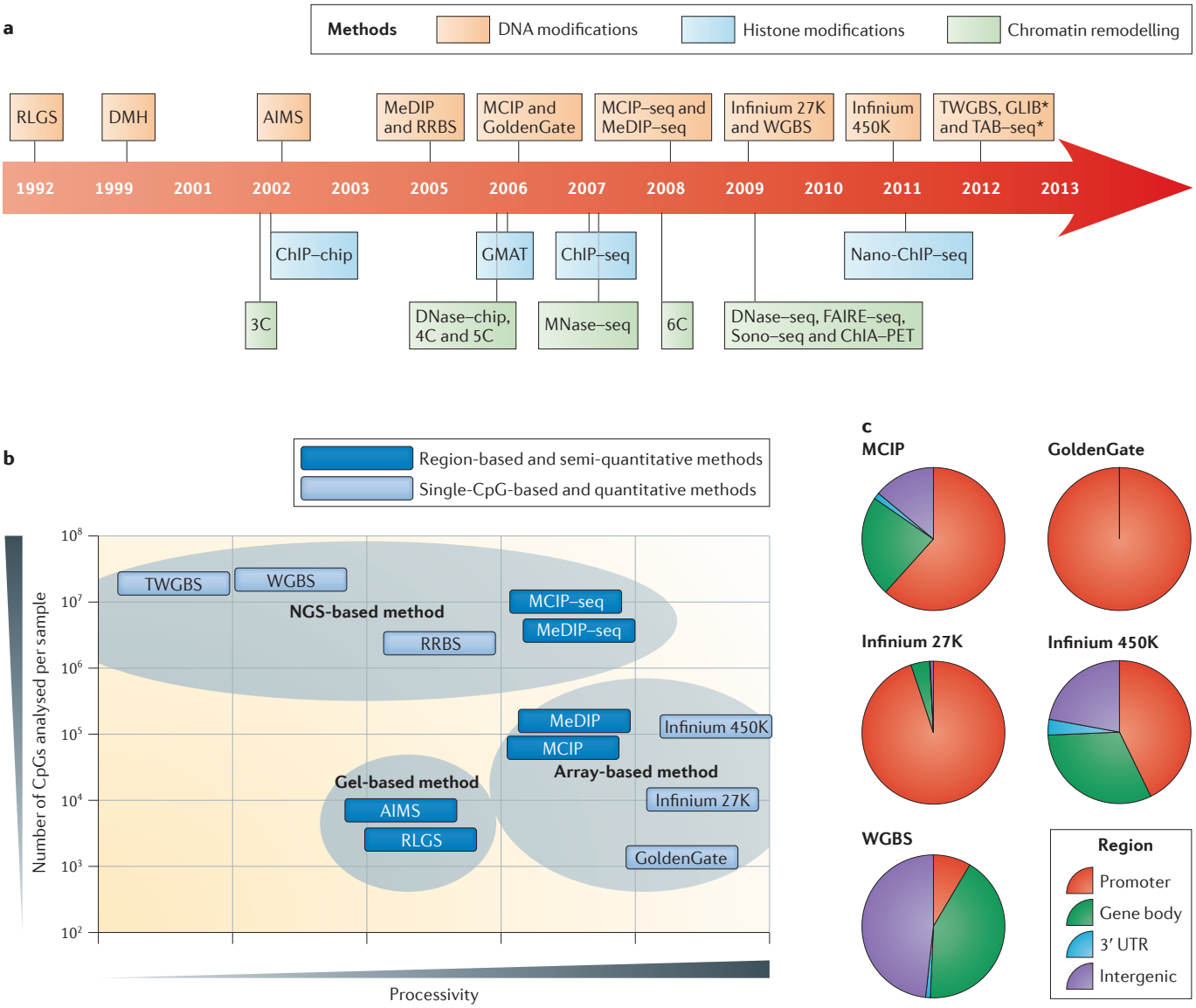


Figure 2 | **Evolution of global epigenetic data in cancer.** **a** | Examples of techniques that were developed for the profiling of DNA modifications, histone modifications and chromatin remodelling are shown. The timeline indicates the year in which a technique was used for the first time in a cancer study. First-generation profiling technologies allowed the screening of thousands of CpG island sequences using rare-cutting, methylation-sensitive restriction sites for either restriction landmark genomic scanning (RLGS)[139] or differential methylation hybridization (DMH)[140]. Second-generation profiling technologies allowed the interrogation of substantial subsets of CpG sites in the genome, which include GoldenGate[141], methyl-CpG immunoprecipitation (MCIP)[142], methylated DNA immunoprecipitation (MeDIP)[143], reduced representation bisulfite sequencing (RRBS)[144] and Infinium arrays[145]. Third-generation profiling technologies using whole-genome bisulphite sequencing (WGBS)[26] or tagmentation-based WGBS (TWGBS)[146] allow the quantitative analysis of almost every CpG in the genome. Glucosylation, periodate oxidation and biotinylation (GLIB), and TET-assisted bisulphite sequencing (TAB–seq) were developed to map 5-hydroxymethylcytosine (indicated by the asterisks)[147,148]. Chromatin immunoprecipitation (ChIP)-based assays are followed by either microarrays (for ChIP–chip) or sequencing (for ChIP–seq). Nano-ChIP–seq is a second-generation ChIP–seq method that requires as few as 10,000 cells. Formaldehyde-assisted isolation of regulatory elements (FAIRE)–seq, Sono–seq[149], micrococcal nuclease (MNase)–seq and DNase I-hypersensitive site mapping (DNase–seq) are used to identify regulatory sequences in the genome. Chromosome conformation capture (3C), circular chromosome conformation capture (4C), chromosome conformation capture carbon copy (5C) and combined chromosome conformation capture ChIP cloning (6C) are used to map global chromatin interactions. Chromatin interaction analysis by paired-end tag sequencing (ChIA–PET) is used to map long-range chromatin interactions[150]. Genome-wide mapping of histone modifications (GMAT) was developed to map protein targets and histone modification patterns[150–156]. **b** | DNA methylation profiling technologies ordered based on the 'processivity' of a technique (*x* axis), as measured by an estimate of the total number of samples analysed, and the number of CpGs that can be analysed per sample (*y* axis). Processivity was measured based on published data, but it also reflects the cost per assay, the time for post-processing of data and the ease of handling. **c** | The distribution of CpGs covered in various sequence compartments (promoter, 3′ untranslated region (UTR), gene body and intergenic region) by selected assays as calculated from published data sets is shown[26,142,145]. Although the older assays such as GoldenGate or Infinium 27K had a strong bias towards the analysis of promoter CpGs, this is shifting towards a more comprehensive analysis of the genome including intragenic and intergenic regions. AIMS, amplification of intermethylated sites; NGS, next-generation sequencing.

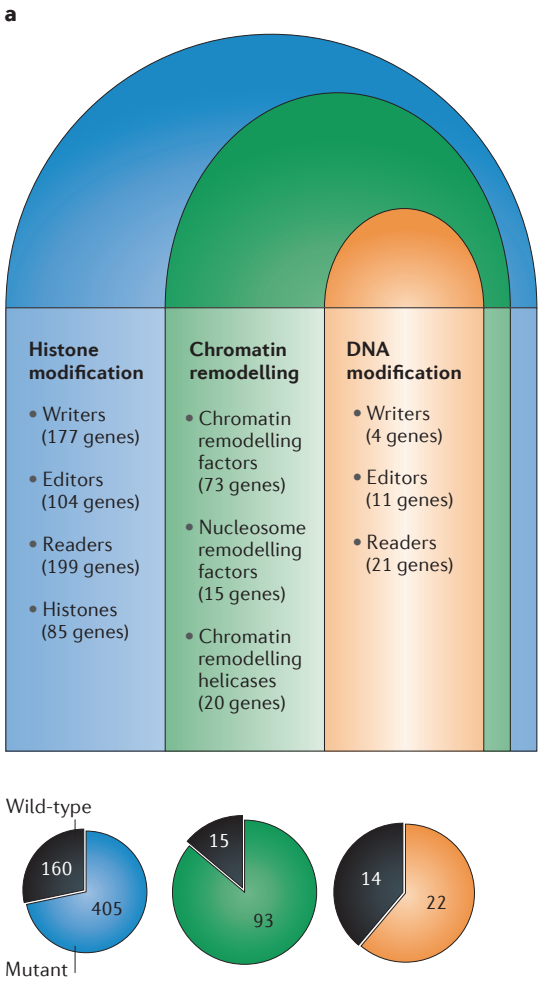
or epigenetic patterns[35]. Besides known cancer genes, these studies identified defects in epigenetic enzymes and pathways, including components that are responsible for chromatin packaging, DNA methylation or demethylation, histone modification and chromatin remodelling (FIG. 3a). Surprising findings and novel insights into tumour genomes have been provided by uncovering recurrent gene mutations that were previously unknown, such as *IDH1* or DNA methyltransferase 3A (*DNMT3A*) in acute myeloid leukaemia (AML)[36,37]; mitochondrial succinate dehydrogenase genes in paragangliomas[38] or gastrointestinal stromal tumours[39]; AT-rich interactive domain 1A (*ARID1A*) in non-small-cell lung cancer[40]; CREB-binding protein (*CREBBP*), E1A-binding protein p300 (*EP300*) and mixed-lineage leukaemia (*MLL*) in small-cell lung cancer[41]; *H3F3A* in paediatric glioblastoma[42]; and *MLL2* and SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin (*SMARCA4*) in medulloblastoma[43–45]. There are current efforts and criteria that will help to decide whether these mutations are drivers in tumorigenesis[46,47]. The discovery of novel mutations has uncovered previously unknown molecular pathways of mutated genes leading to altered epigenetic patterns in cancer genomes, some of which are highlighted below.

DNA methylation pathways. The addition of a methyl group to cytosine is mediated by DNMT1, DNMT3A and DNMT3B using the methyl donor *S*-adenosyl-L-methionine (SAM). DNMT1 preferentially methylates the unmethylated strand of hemimethylated DNA during DNA replication, whereas DNMT3A and DNMT3B catalyse *de novo* methylation of both strands. The importance of these enzymes in normal developmental processes has been demonstrated in mouse models lacking DNMT function[48,49]. Passive loss of DNA methylation occurs as a result of the absence of maintenance methylation by DNMT1. Pathways for active DNA demethylation have been described, including the oxidation of 5mC by TET 5mC hydroxylases; these enzymes catalyse the sequential conversion of 5mC to 5hmC[50,51], to 5fC and then to 5caC[10,11], which is then excised by thymidine-DNA glycosylase[10]. Alternative mechanisms to oxidative demethylation of cytosines include processes based on deamination and subsequent base-excision repair (BER)[52] or nucleotide-excision repair[53]. These demethylation pathways are replication-independent processes and allow rapid conversions from methylated to unmethylated states, as seen in early embryonic development when waves of genome-wide losses and gains of 5mC occur[54].

Genetic defects were identified in enzymes that are involved in the establishment and removal of DNA methylation patterns (FIG. 3b). So far, mutations in human DNMTs have been predominantly reported in leukaemias. Whole-exome sequencing identified *DNMT3A* mutations in the M5 subgroup of AML and, less frequently, in the AML-M4 subgroup (that is, acute myelomonocytic leukaemia)[55], in addition to *DNMT3A* mutations in some solid tumours, according to the Catalogue of Somatic Mutations in Cancer (COSMIC) database. Reported mutations in *DNMT3A* either showed reduced catalytic activity — thus leading to the global activation of more than 800 genes, including HOX family genes and *IDH1* — or they had an effect on the binding affinity of DNMT3A to histone H3. Tumours harbouring mutated *DNMT3A* have an intermediate-risk cytogenetic profile that is independently associated with poor outcome[37,56]. A pilot study showed global differences in DNA methylation patterns between tumours with or without the *DNMT3A* R882H mutation[55]; however, this could not be confirmed in a larger cohort[56].

As the loss of 5mC is a hallmark of almost all cancers, pathways of active DNA demethylation, such as those involving the TET enzymes (TET1, TET2 and TET3), have key roles in the establishment of DNA methylation patterns and, possibly, of other epigenetic marks. Mutations in the TET gene family have been discovered in myeloproliferative neoplasms and in myelodysplastic syndromes (MDSs). *TET1* is a known fusion partner of *MLL* in rare t(10;11) translocations found in AML[57]. Missense, frameshift and nonsense mutations in *TET2* occur in 7.6% of AML samples[58] but are rarely found in solid tumours, according to the COSMIC database. Mutation rates in myeloproliferative neoplasms are higher (>20%), but the number of tested samples is smaller and thus these rates may be biased[59]. Interestingly, mutations in *TET2* and those in *IDH1* or *IDH2* are mutually exclusive[19]. Dominant-negative *IDH1* or *IDH2* mutations are frequently found in both AML and glioblastoma; these mutations produce the metabolite 2-hydroxyglutarate (2-HG), which is structurally similar to the α-ketoglutarate substrate for both TET enzymes and Jumonji-C domain-containing lysine-specific histone demethylase (KDM) proteins (BOX 1). Consequently, 2-HG acts as an inhibitor of TET or KDM protein activity[19,60]. This apparent ability of *IDH1* and *IDH2* mutations to partially phenocopy the loss of TET function may explain the mutual exclusivity of these mutations in cancer. Non-invasive magnetic resonance imaging detection of 2-HG may become a valuable diagnostic tool for detecting and monitoring disease progression and treatments[61,62]. Cases with mutations in these enzymes are characterized by distinct DNA methylation patterns, as seen in both AML[19] and glioblastoma[18,35,63]. Experiments using immortalized primary human astrocytes and isogenic cells expressing either wild-type *IDH1* or the *IDH1* R132H mutant have provided strong evidence that this single mutation leads to the accumulation of changes to global DNA methylation, as well as to H3K9me2, H3K27me3 and H3K36me3 patterns, over time[63]. Epigenetic alterations primarily occur in genes targeted by Polycomb repressive complex 2 (PRC2) (BOX 1); however, the detailed molecular mechanisms that lead to epigenetic repatterning remain unknown.

Histone-modifying enzymes. Histone tails are marked by multiple modifications, which are recognized by reader proteins that subsequently translate the information into distinct transcriptional profiles through alterations



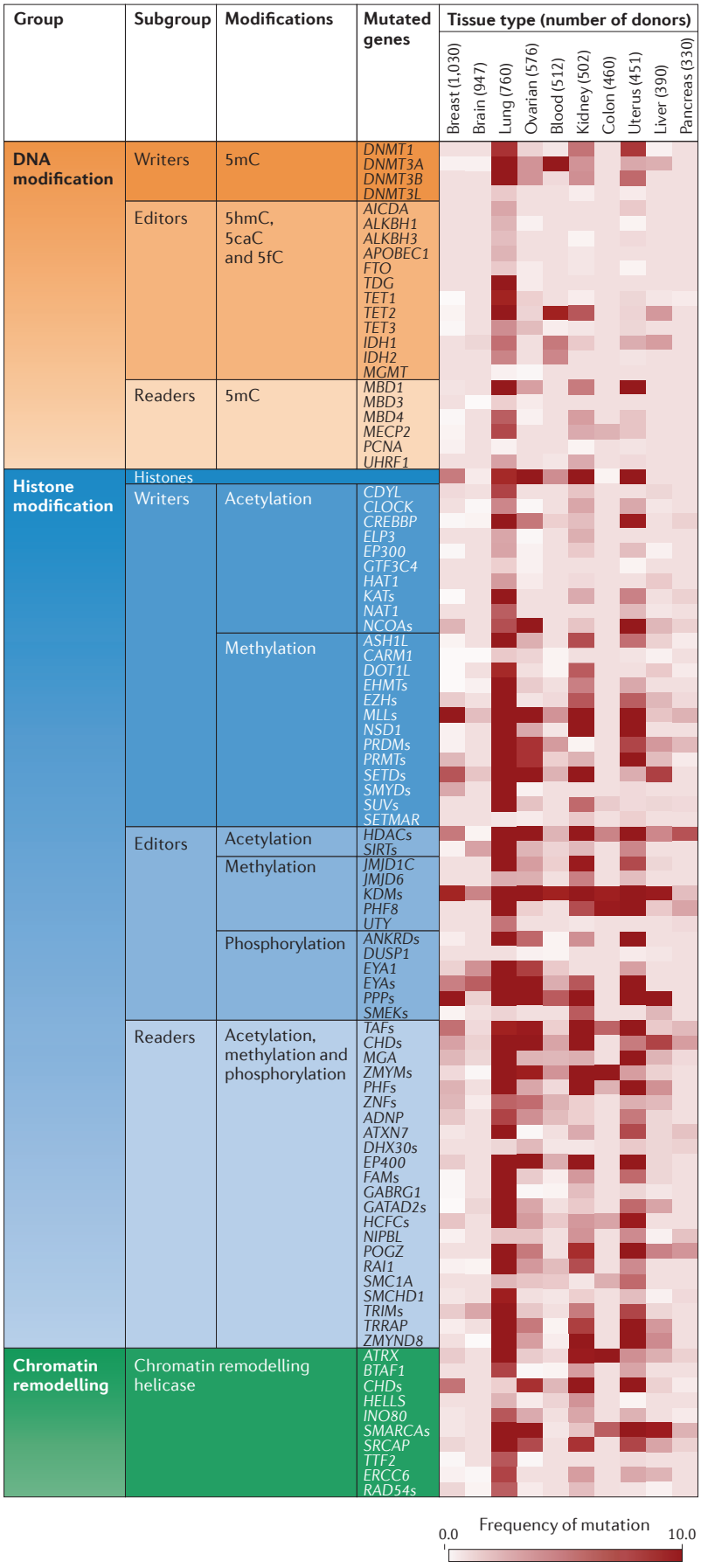


Figure 3 | **Mutations in regulators of the epigenome identified in cancer.** **a** | Using several data browsers, ~709 epigenetic enzymes that were identified can be grouped into three major categories: histone modification, chromatin remodelling and DNA modification. Each group is divided into subgroups based on their functions. The total number of genes in each subgroup is given in brackets. Each pie chart lists the number of genes that were found to be mutated in at least two tumour samples (indicated in blue, green or orange) and the number of unmutated genes (black) for each group of epigenetic enzymes. **b** | Examples of mutated genes in the groups of DNA modification, histone modification and chromatin remodelling enzymes, based on data as of January 2013 in the International Cancer Genome Consortium (ICGC) data set; see the ICGC Data Portal for the latest report. The number of analysed tumour tissues is given. Several tumour entities have high frequencies of mutations in epigenetic enzymes. Note that these data are not adjusted for chromosomal instability or mutator phenotypes, hence the frequencies reflect a combination of probable driver mutations in epigenetic regulators, in addition to the background mutation rate for the tumour type. 5caC, 5-carboxylcytosine; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine.

of chromatin states. Both histone modifications and their readers determine whether a chromosomal region is accessible for the binding of transcription factors or other regulatory molecules; that is, whether a gene locus is active or silent. The modification patterns are established by histone acetyltransferases (HATs) or histone methyltransferases (HMTs) and can be removed by histone deacetylases (HDACs) or histone demethylases (HDMs). These enzymes are usually specific to particular amino acid motifs and target both core histones (such as H3) and histone variants (such as H3.3)[64]. The modification and exchange of core histone proteins allow the generation of rapid, replication-independent changes to the chromatin state.

Disturbances to histone modification patterns have a global effect on transcriptional deregulation in multiple regions of the genome (FIG. 3b). One of the first mutations was described in *MLL*, which encodes an H3K4me3 HMT[65]. More than 50 different translocations or partial tandem duplications involving *MLL* on chromosome 11q23 have been described and are found to be associated with poor prognosis in acute lymphoblastic leukaemia or normal-karyotype AML[66–68]. Although the partial tandem duplication in *MLL* leads to increased H3K4me3 levels at its target genes, MLL–AF4 or MLL–AF10 fusion oncoproteins are associated with increased H3K79 methylation through their interaction with DOT1-like histone H3 methyltransferase (DOT1L)[69,70]. Another example of altered HMT activity is represented by mutations in enhancer of zeste homologue 2 (*EZH2*), a core component of PRC2 that is required for H3K27 trimethylation. *EZH2* mutations are mainly found in myeloid leukaemias and lymphomas but are rarely found in solid tumours. In myeloid leukaemias, both inactivating mutations and deletions of *EZH2* are associated with poor prognosis[71]. Additionally, multiple reports have demonstrated the overexpression of *EZH2* and its effects on the trimethylation of H3K27 in several human malignancies, including prostate cancer, breast cancer and medulloblastoma[72–75]. In contrast to these loss-of-function mutations, mutations in the SET domain of *EZH2* in diffuse large B cell lymphoma lead to increased catalytic activity of the protein[76]. The consequences of the mutations in *EZH2* are most probably determined by the target genes in the respective tissues.

Histone methylation patterns can also be altered by HDMs, two types of which have been reported: lysine-specific histone demethylase 1A (KDM1A; also known as LSD1), which demethylates monomethylated or dimethylated lysines through an amine oxidation reaction using FAD as a cofactor; and Jumonji domain proteins, which are able to demethylate monomethylated, dimethylated or trimethylated lysines. In this group, genetic mutations have been reported in the genes encoding KDM5A (also known as JARID1A); KDM5C (also known as JARID1C), which affects H3K4 methylation; and KDM6A (also known as UTX), which affects H3K27 methylation[40,44,45,77].

HDACs are important proteins that influence the activity state of chromatin. Mutations have been described in tumour genomes for all members of this protein family (except HDAC9) but these seem to be rare events with no dramatic epigenetic alterations described. However, changes in the expression level of HDACs might have a more important effect than mutations of HDAC-encoding genes, at least when considering the phenotypic effects of HDAC inhibition that result from treatment with clinically tested HDAC inhibitors (see below). However, when HDAC mutations occur in cancer, they might have other important functional consequences. For example, it was recently reported that specific *HDAC1* mutations are associated with high *HDAC5* expression and with a sensitization of cells to the drug panobinostat, which inhibits multiple HDACs[78]. Thus, the potential of using HDAC mutations as predictive biomarkers should be further investigated.

Chromatin remodelling factors. Chromatin remodelling complexes are multisubunit components that use ATP hydrolysis to disrupt the contact between nucleosomes and the DNA, to shuffle nucleosomes around and to replace or remove them from chromatin[79]. There are four classes of chromatin remodellers: SWI/SNF, chromodomain helicase DNA-binding proteins (CHDs), ISWI and INO80, all of which carry the typical two-part ATPase domains DExx and HELICc[80]. The other domains of these proteins are essential for selective targeting to the genome and consequently give them functional locus-specific properties. Recent genome-wide sequencing projects in tumours have identified recurrent mutations in chromatin remodelling components[81]. Immuno-histolocalization and chromatin immunoprecipitation (ChIP) experiments have shown that most of these components act on large portions of the genome; hence, we suspect that mutations in these components cause serious chromatin aberrations and strongly influence tumour progression. Most chromatin remodellers are classified as tumour suppressors but their mutations might also result in a change of function rather than in a loss of function[82]. One of the most commonly mutated chromatin remodellers is the ARID1A subunit of the SWI/SNF chromatin remodelling complex, which has been implicated in the control of proliferation and differentiation in epithelial cells. *ARID1A* has been found to be frequently mutated in multiple human malignancies[40,81,83–88]. Biallelic inactivation of *SMARCB1* is the only recurrent mutation in rhabdoid tumours, which are otherwise characterized by low frequencies of gene mutations[89]. Conceptually, this highlights the importance of proper chromatin remodelling in the coordination of gene expression, particularly to regulate retinoblastoma (RB), p53, Polycomb, Sonic hedgehog, MYC and nuclear hormone receptor signalling, as well as stem cell programmes[81], higher-order chromatin structure and proper packaging of the genome before mitosis[90]. We anticipate that the recent identification and subsequent characterization of the function of chromatin remodellers will further illuminate the interactive network of the respective proteins and its importance in tumorigenesis.

Structural chromosomal proteins and associated components. A recent study found recurrent mutations in *H3F3A*, *HIST1H3B* (which encodes the canonical histone H3.1) or their associated protein complexes in paediatric glioblastoma, thus highlighting the importance of nucleosomal integrity in coordinated gene expression in brain tumours[42,91,92] (BOX 1). Histone H3.3 is frequently affected by a Lys27Met (H3.3-K27M) mutation that inhibits PRC2 by dominant-negative interactions, leading to a substantial global loss of H3K27me3 (REFS 93–95). An almost equally frequent mutation produces a Gly34Arg or Gly34Val (H3.3-G34R/V) substitution[42]. Furthermore, tumours with H3.3-K27M and H3.3-G34R/V mutations lack the previously characterized genetic alterations, such as mutated *IDH1* or *IDH2*, have minimal epithelial growth factor receptor (*EGFR*) and platelet-derived growth factor receptor, α-polypeptide (*PDGFRA*) amplification, and have minimal chromosome 7 gain and chromosome 10 loss, although such tumours frequently harbour mutated *TP53* (which encodes p53)[18]. Mutations of H3.3 in the mutated *TP53* background are consequently sufficient to reprogramme cells into a state of high proliferation, which obstructs neuronal network formation in the developing brains of younger patients. Although these studies have provided valuable mechanistic insights into the effects of *H3F3A* mutations in glioblastoma, it is currently unknown whether they are driver mutations. Various lines of evidence support a gain-of-function effect of these mutations: their heterozygous occurrence in tumours, the specific amino acids that are recurrently affected (H3.3-K27M, H3.3-G34R/V and H3.1-K27M) and the observed PRC2-inhibitory effects of H3.3-K27M when expressed in the presence of wild-type H3.3 (BOX 1). Interestingly, cancer-associated mutations have not been found in the other H3.3 family member *H3F3B*, although it encodes an identical protein to *H3F3A*. Although the global epigenetic changes that result from H3.3 mutations remain to be fully characterized, it is easy to conceive that extensive loss and gain of H3K27me3 have dramatic effects on gene expression and genome integrity, leading to aberrant gene expression and epigenomic abnormalities.

Deep-sequencing projects have also identified mutations in the H3.3 chaperone complex members α-thalassaemia/mental retardation syndrome X-linked (ATRX) and death domain-associated protein (DAXX) in multiple human tumours[42,96,97]. ATRX and DAXX are part of a complex that is responsible for the deposition of histone H3.3 to subtelomeric regions and to other chromosomal locations[98,99]. The interaction of ATRX with H3.3 is mediated by the ATRX–DNMT3–DNMT3L (ADD) domain[100–102]. The loss of *ATRX* in pancreatic neuroendocrine tumours and glioblastoma is associated with alternative lengthening of telomeres, possibly through the lack of normal H3.3 deposition in subtelomeric regions[42,103].

From epigenetic patterns to molecular mechanisms

Despite the observation of multiple genetic mutations in epigenetic regulators, the underlying mechanisms leading to global changes in epigenetic patterns are mostly unknown. The nonrandom patterns of DNA methylation seen in tumour tissues could be the result of a targeted mechanism that leads to the epigenetic silencing of certain groups of genes while leaving other sequences unmethylated. Different targeted mechanisms could operate in different tissues, thus generating tissue type-specific aberrant DNA methylation patterns. Alternatively, control of defined epigenetic patterns may break down and genes would be globally affected through epigenetic activation or silencing as a result of stochastic-, ageing- or differentiation-related phenomena. In the case of global epigenetic alterations, cells with an epigenetic pattern that favours cell growth would then obtain a selective advantage within a tumour cell population, potentially acquiring a less differentiated and more motile phenotype that facilitates dissemination and metastasis. Thus, such selection for particular tumour-associated phenotypes could also result in the observed nonrandom epigenetic profiles.

Possible mechanisms. The idea of a targeted mechanism stems from work in leukaemias, in which there is some evidence to suggest that oncogenic fusion proteins or activated oncogenes may have the ability to recruit DNMT activity (DNMT1 and DNMT3A) to previously unmethylated loci. This was shown *in vitro* for the oncogenic fusion protein PML–RAR (promyelocytic leukaemia–retinoic acid receptor), which is generated by a t(15;17) translocation in acute promyelocytic leukaemia[104] and interacts with DNMT3A through PML. Additionally, the translocation t(8;21) in AML results in the expression of the oncogenic fusion protein runt-related transcription factor 1 (RUNX)–MTG8, which recruits DNMT1 and leads to aberrant silencing of RUNX1-target genes[105].

Alternatively, mutations in genes encoding epigenetic factors that are involved in the establishment or maintenance of global epigenetic patterns could lead to epigenetic alterations either genome wide or within distinct subchromosomal regions. A good example is the *H3F3A* G34 mutation in paediatric glioblastomas, which is associated with hypomethylation at telomere ends[18]. This multiplicity of a single genetic event affecting multiple loci could have important consequences for tumorigenesis, as discussed below.

An integrated view into cancer genomes is needed. In the past, tumour genome studies mainly focused on either genetic or epigenetic events, and only a few groups attempted an integrated analysis of genetic and epigenetic alterations based on available data[106–109]. This is now shifting to a more comprehensive analysis of single cancer genomes that includes information on mutations, copy-number aberrations, structural variations, epigenetic patterns and expression changes in both mRNAs and non-coding RNAs. These data are now available for an integrated genome, epigenome and transcriptome analysis (FIG. 4). The importance of a cancer gene should not be solely evaluated by the

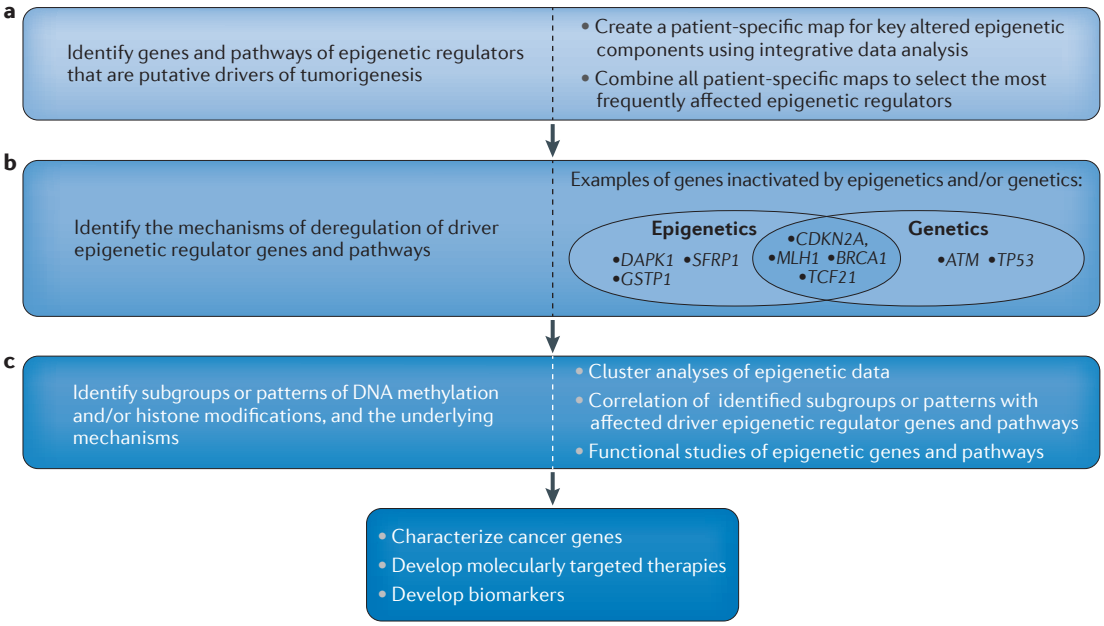


Figure 4 | **Workflow of integrative analysis for molecular profiling of cancer.** The workflow of an integrative analysis includes three main steps outlined on the left; a more detailed description of these steps is given on the right. **a** | The proposed workflow starts with the identification of all enzymes (writers, editors and readers) involved in the establishment of epigenetic patterns (for example, DNA methylation and demethylation, histone modifications and chromatin remodelling). For each patient, the genetic alterations (mutations, deletions, translocations and amplifications), epigenetic alterations (hypermethylation or hypomethylation in regulatory sequences), as well as the deregulation of associated non-coding RNAs, are tabulated. These alterations are subsequently cross-referenced with the expression levels of these genes. **b** | Ultimately, this strategy will identify the most frequent alterations in genes encoding epigenetic regulators and determine whether these genes are preferential targets for genetic or epigenetic alterations. Shown on the right are general examples of genes (rather than specific genes encoding epigenetic regulators) that are known to be disrupted by genetic, epigenetic or combined mechanisms. In validation experiments (either resequencing of mutations or quantitative DNA methylation analysis (using MassARRAY or pyrosequencing)), the nature and frequency of the alterations can be confirmed. **c** | Subsequent cluster analyses of epigenetic data would help to identify tumour subgroups that are associated with defects in particular genes. Additionally, molecular and functional analyses in model systems will help to characterize the mechanisms by which the mutation of an epigenetic regulator results in the observed alterations to the epigenome and the contribution of these alterations to tumorigenesis. Such studies will facilitate the development of novel biomarkers or investigations of novel therapeutic targets. *ATM*, ataxia telangiectasia mutated; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *DAPK1*, death-associated protein kinase 1; *GSTP1*, glutathione S-transferase pi 1; *MLH1*, mutL homologue 1; *SFRP1*, secreted frizzled-related protein 1; *TCF21*, transcription factor 21; *TP53* encodes p53.

genetic mutation range, but such assessment should also consider epigenetic gene silencing that is measured by promoter methylation, genetic and epigenetic alterations in regulatory sequences such as enhancers and suppressors, or deregulation of microRNAs (miRNAs) that may target the candidate gene. In this context, pathway information is important because the deregulation of a signalling pathway may occur at different levels within that pathway. Integrative analyses still face major challenges, as novel algorithms and strategies are required. Bioinformatic tools are available to support visualization and pathway analysis of preselected cancer genes; however, a tool that encompasses all analyses is still needed. TABLE 1 provides a list of selected bioinformatic tools which allows the integration of at least two types of data sets. New data of genome structure recently published by the Encyclopedia of DNA Elements (ENCODE) consortium will provide a reference that is required for many of these studies[110].

As primary tumour tissue resources are often limited, studies on gene mutations will have to be carried out in model systems. This is particularly important for studies of mutations in epigenetic enzymes in which profiling techniques are required to evaluate the functional effects of these mutations. Using cell culture assays will allow the dissection of the resultant mechanisms, such as effects on protein interactions, the identification of affected target genes and the modulation of expression levels. A combination of biochemical assays and recently developed profiling technologies to measure effects on epigenetic patterns could be used to decipher the molecular mechanisms.

The gain of information from such analyses will be considerable and will allow genes to be designated as drivers on the basis of not only recurrent genetic mutations but also epigenetic information. Knudson's two-hit hypothesis in tumour initiation postulates the inactivation of both alleles of a tumour suppressor by

Table 1 | **Selected web-based bioinformatic tools and web services for integrative cancer genome analysis**

Bioinformatic tool or Webservices	Database used	Webservice or tool	Upload of data possible	Gene search	Chromosomal region search	mRNA expression	SNV	CNV	Methylation	miRNA expression	Protein	Pathways
cBioPortal for Cancer Genomics	TCGA	Webservice	–	✓	–	✓	✓	✓	–	–	✓	✓
PARADIGM, Broad GDAC Firehose	TCGA	Webservice	✓	✓	–	✓	✓	✓	✓	–	–	✓
WashU Epigenome Browser	ENCODE	Webservice	✓	✓	✓	✓	✓	✓	✓	–	–	✓
UCSC Cancer Genomics Browser	UCSC	Webservice	✓	✓	✓	✓	✓	✓	✓	✓	–	–
The Cancer Genome Workbench	TCGA	Webservice	–	✓	✓	✓	✓	✓	✓	–	–	–
EpiExplorer	ENCODE and ROADMAP	Webservice	✓	✓	✓	✓	✓	✓	✓	–	–	–
EpiGRAPH	ENCODE	Webservice	✓	✓	✓	✓	✓	✓	✓	–	–	–
Catalogue of Somatic Mutations in Cancer (COSMIC)	TCGA and ICGC	Webservice	–	✓	–	–	✓	✓	–	–	–	–
PCmtl, MAGIA, miRvar, CoMeTa etc*	GEO and TCGA	Webservice	✓	✓	–	✓	–	–	–	✓	–	✓
ICGC	ICGC	Webservice	–	✓	–	✓	✓	✓	–	–	–	–
Genomatix	User defined	Tool	–	✓	–	✓	✓	✓	✓	–	–	✓
Caleydo	TCGA	Tool	–	✓	✓	✓	✓	✓	✓	✓	–	✓
Integrative Genomics Viewer (IGV)	ENCODE	Tool	–	✓	✓	✓	✓	✓	✓	–	–	–
iCluster and iClusterPlus	User defined	Tool	–	✓	–	✓		✓	–	–	–	–

CNV, copy-number variation; ENCODE, Encyclopedia of DNA Elements; ICGC, the International Cancer Genome Consortium; GDAC, Genomic Data Analysis Center; GEO, Gene Expression Omnibus; miRNA, microRNA; SNV, single-nucleotide variation; TCGA, The Cancer Genome Atlas; UCSC, University of California Santa Cruz.*Website with links for integrated analysis of microRNA and mRNA expression.

two independent genetic events; hence, in the past, tumour suppressor genes were identified on the basis of the genetic loss of function of both alleles. By contrast, mutations in single epigenetic modifiers can affect epigenetic states and, in a chain-like reaction of secondary events, might trigger `pigenetic deregulation biallelically in one or more target genes that promote tumour growth. Furthermore, alleles of tumour suppressor genes can be inactivated by a combination of genetic and epigenetic events, including alterations in DNA methylation, histone modifications and non-coding RNAs. An early example was the identification of transcription factor 21 (*TCF21*) as a tumour suppressor gene located on chromosome 6q23–q24 — a frequently deleted region in many tumour types. Despite the lack of mutations in the remaining allele, *TCF21* was identified as a candidate tumour suppressor in head and neck cancer and lung cancer owing to frequent epigenetic silencing[108]. Similarly, the frequency of phosphatase and tensin homologue (*PTEN*) silencing in prostate cancer was underestimated owing to an assessment of genetic events only. A recent report demonstrates that the *PTEN* transcript is targeted by multiple miRNAs that are normally silenced by epigenetic mechanisms but become activated owing to hypomethylation of their promoter regions[111]. Furthermore, *PTEN* expression can be modulated by the expression of competitive endogenous RNAs that sequester these miRNAs owing to the presence of common miRNA binding sites[112].

Novel epigenetic therapies

In the past few years, the deciphering of individual components that make up the epigenetic machinery and their alterations in cancer has occurred simultaneously with the development and testing of a multitude of small-molecule inhibitors directed against either distinct compartments or single regulators. It is expected that these novel drug targets will allow the development of more rational epigenetic cancer therapies with increased efficiency, increased specificity and fewer risks associated with the reactivation of bystanders (such as developmental genes). The respective substances are at various stages of development. In general, one can distinguish between drugs that target regulators of epigenetic patterns (for example, DNMTs and HDACs) and drugs that target specific mutations in these genes (for example, *IDH1* R132H). The drugs targeting specific mutations may be less toxic, but they will only work in certain patient subgroups. Currently, preclinical data clearly indicate that these drugs induce distinct epigenetic changes that result in gene expression changes; however, the specific *in vivo* mechanisms that mediate therapeutic effectiveness in patients remain mostly enigmatic, as information on molecular mechanisms and target genes is missing. TABLE 2 lists some emerging novel drugs that are already in clinical trials.

Four epigenetically active substances have received approval by the US Food and Drug Administration (FDA) after demonstrating considerable clinical

Table 2 | **Selected novel drugs in preclinical or clinical development targeting components of the epigenetic machinery**

Substance	Target structure	Clinical trial	Disease
SGI110	DNMT	Phase I/II	MDS, AML, ovarian and hepatocellular cancer
AGI-5198	Mutant IDH	Preclinical	Glioma
Pivanex (also known as AN-9)	HDAC	Phase I/II	CLL, small lymphocytic lymphoma, malignant melanoma and NSCLC
ACY-1215	HDAC6	Phase I/II	Multiple myeloma
Resveratrol (SRT501)	SIRT1 and SIRT5 activation	Phase I/II	Colorectal cancer, melanoma, multiple myeloma
	SIRT3 inhibition	Phases I–III	Metabolic and cardiovascular diseases
Curcumin	HAT	Phase I/II	Breast cancer, colorectal cancer and multiple myeloma
Tranylcypromine	KDM1A	Phase II	AML
EPZ-5676	DOT1L	Phase I	Advanced haematological malignancies and acute leukaemia with 11q23 or *MLL* abnormalities
EPZ-6438	EZH2	Phase I	NHL and breast cancer
GSK126	EZH2	Preclinical	Haematological malignancies, including NHL
GSK525762	BET bromodomain	Phase I	NMC
RVX-208	BET bromodomain	Phase II	Atherosclerosis
		Preclinical	Haematological malignancies
JQ1	BET bromodomain	Preclinical	NMC, AML and multiple myeloma
PFI-1	BET bromodomain	Preclinical	B cell acute lymphoblastic leukaemia

AML, acute myeloid leukaemia; BET, bromodomain and extraterminal family of proteins; CLL, chronic lymphocytic leukaemia; DNMT, DNA methyltransferase; DOT1L, DOT1-like histone H3 methyltransferase; EZH2, enhancer of zeste homologue 2; HAT, histone acetyltransferase; HDAC, histone deacetylase; IDH, isocitrate dehydrogenase; KDM1A, lysine-specific histone demethylase 1A; MDS, myelodysplastic syndrome; *MLL*, mixed-lineage leukaemia; NHL, Non-Hodgkin's lymphoma; NMC, NUT midline carcinoma; NSCLC, non-small-cell lung cancer; SIRT, sirtuin (a family of histone deactylases).

benefit. These are DNMT inhibitors (azacytidine (Vidaza; Celgene) and 5-aza-2′ deoxycytidine (Dacogen; Eisai)) and HDAC inhibitors (suberoylanilide hydroxamic acid (SAHA; also known as vorinostat and Zolinza; Merck) and romidepsin (Istodax; Celgene)). Both classes of drugs aim to reverse gene silencing that is mediated by DNMTs or HDACs. In preclinical models, a wealth of studies demonstrated transcriptional derepression accompanied by epigenetic remodelling and antitumour activity both *in vitro* and *in vivo*. Anticancer effects might be generally explained by the induction of cell cycle arrest, differentiation or apoptosis, or by the sensitization of tumour cells for chemotherapy or radiotherapy.

The inhibitors of DNA methylation that are in current clinical application are cytosine analogues that become incorporated into DNA during replication and subsequently trap DNMT by covalent binding. So far, both of the FDA-approved DNMT inhibitors have demonstrated significant response rates and survival benefits when used as low-dose therapies for patients with MDS[113,114] or AML[115]. DNA methylation changes were detected after one round of treatment; however, clinical responses required multiple cycles of therapy[116].

An interesting novel approach is the development of the inhibitor for mutant IDH1, AGI-5198, which selectively blocks the activity of mutant IDH1 and leads to growth suppression of cultured cells in soft agar and of mouse xenografts[117]. AGI-5198 treatment had no effect on DNA methylation patterns; however, dimethylation and trimethylation marks of H3K9 were more sensitive to such treatment, leading to changes in the expression of genes involved in astroglial differentiation.

HDAC inhibitors work by preventing histone deacetylation, thereby facilitating an open chromatin structure and resulting in gene activation. Vorinostat was the first HDAC inhibitor that was approved by the FDA (in 2006) for the treatment of cutaneous T cell lymphoma (CTCL), a fairly rare malignancy. Since then, many more HDAC inhibitors have been developed and have entered clinical trials for the treatment of various of human malignancies. The second FDA-licensed HDAC inhibitor, again for CTCL, was romidepsin. Although no other HDAC inhibitors are currently approved by the FDA, many are being evaluated in preclinical studies using animal tumour models and in clinical Phase I–III trials as monotherapies or in combination with other drugs including non-HDAC inhibitors. Panobinostat is one of the most potent HDAC inhibitors *in vitro*, and preliminary studies have demonstrated its efficacy in CTCL[118]. More recently, promising Phase I results were demonstrated in refractory Hodgkin's lymphoma and in prostate cancer[119,120].

The addition of acetyl groups by HATs has been difficult to target and such clinical studies are missing. HATs are composed of two major classes — nuclear (type A) and non-nuclear (type B) HATs — with family members of distinct structural diversity that mainly target histone but also non-histone proteins[121].

Altered expression levels of HATs have been found in various types of cancer[122], and association with viral oncoproteins or involvement in chromosomal translocations and mutations are two major ways to modulate HAT activity. Various molecules have been discovered to inhibit HATs, such as natural products (for example, anacardic acid, curcumin, garcinol, epigallocatechin-3-gallate and gambogic acid), synthetic derivatives of natural products, acetyl-CoA-derived bisubstrate inhibitors and synthetic small molecules[123,124].

For the inhibition of HMTs, EPZ-5676 is an inhibitor of DOT1L that acts as a competitive analogue of the SAM substrate. A first-in-human Phase I study has recently started to investigate the safety and tolerability of EPZ-5676 in patients with acute leukaemia bearing oncogenic MLL fusion proteins that bind to DOT1L. Furthermore, the EZH2 inhibitor GSK126 (also a SAM analogue) was recently shown, both *in vitro* and *in vivo*, to decrease global H3K27me3 levels and to reactivate silenced PRC2-target genes in lymphomas with EZH2-activating mutations[125].

Similarly, KDMs are frequently overexpressed in cancer and have become promising pharmacological targets. Two families of KDMs use different demethylating mechanisms: one by amine oxidation (for example, KDM1A) and the other by hydroxylation (for example, the Jumonji-C domain KDMs). Small-molecule inhibitors of both KDM1A and Jumonji-C domain KDMs are under preclinical development, and recent studies of KDM1A inhibition in AML (for example, by tranylcypromine) show that the inhibition does not lead to a global increase in H3K4me2, but increases local H3K4me2 levels and induces transcriptional reactivation at genes that are involved in differentiation pathways[126].

Although pharmacological approaches targeting enzymatic activity that catalyses the transfer of chemical groups are prevalent, a novel concept focuses on epigenetically active drugs that disturb protein–protein interactions in chromatin readers. The bromodomain and extraterminal (BET) family of bromodomain-containing proteins[127], comprising BRD2, BRD3, BRD4 and BRDt, can recognize acetylated lysine residues and is substantially involved in transcriptional elongation and cell cycle progression. Inhibition of the recruitment of BET family proteins to chromatin by specific small-molecule inhibitors (such as JQ1 and PFI-1) has recently been shown to be preclinically effective both *in vitro* and *in vivo* (in models of solid tumours[128] and haematological malignancies). Inhibition of BET suppresses *MYC* transcription, which is essential for the progression of AML, MLL and Burkitt's lymphoma[129–131]. In three murine models of multiple myeloma, JQ1 downregulated *MYC* transcription and caused genome-wide downregulation of MYC-dependent target genes. Similarly, in three *in vivo* models of neuroblastoma, a childhood cancer with frequent amplification of *MYCN*, interference with BET proteins inhibited *MYCN* transcription, suppressed the MYCN-regulated transcriptional programme, induced apoptosis and conferred a significant survival advantage. Thus, the inhibition of BET recruitment provides a novel therapeutic concept that is in transition from preclinical *in vitro* and *in vivo* models to clinical trials.

The complexity and distribution of the establishment and maintenance of epigenetic patterns on a global scale suggest that the administration of drugs needs to be provided in a timely manner and as close to the target tissue as possible to maximize the intended effect and to prevent off-target effects. A careful assessment of normal epigenetic patterns in various tissues becomes a necessity to predict potential outcomes that a defined drug may have on a particular tissue.

Conclusions and future perspectives

Cancer genetics is now entering an exciting time in which novel concepts of how genetic and epigenetic alterations cooperate in tumorigenesis can be studied. This is exemplified by the recent discovery of the TET-mediated oxidation of 5mC to 5hmC, 5fC and 5caC, a step in the active demethylation pathway. Recent profiling data for 5fC in mouse embryonic stem cells locate this modification in enhancers and other regulatory elements, highlighting its role in gene regulation[132]. Thus, it is intriguing to speculate about the crucial functions of cytosine modifications beyond 5mC in reprogramming cancer genomes.

The large number of mutations found in epigenetic pathways points to a mechanistic link that leads from a gene defect to alterations in epigenetic patterns. The integrated view into cancer genomes will have a substantial impact on our understanding of how epigenetic patterns are generated and maintained. The disruption of any factor involved in chromatin biology is likely to have important effects on global gene expression patterns, and we are currently still far away from deciphering the many downstream effects that will occur when any of these factors is mutated or therapeutically targeted in cancer. Pleiotropic effects are of major concern when using epigenetically active drugs, as general targeting of epigenetic mechanisms such as DNMT inhibition may lead to broad global effects on gene expression. Perturbation of the networks of interacting chromatin-modifying enzymes will help to elucidate these complex relationships and facilitate the development of specific intervention strategies with limited off-target effects on specific sets of genes. It might even become feasible to target epigenetic events at specific loci rather than to restore global epigenetic patterns. Such targeting approaches might include the use of fused gene constructs, an example of which could be the fusion of transcription activator-like effector nuclease (TALEN) motifs[133] to chromatin-modifying enzymes to exert site-specific modifications. Thus, in the long run, the concept of targeted molecular therapies, which is a successful approach for targeting altered signalling pathways in disease, might also become applicable to the highly complex processes involved in the epigenetic regulation of gene expression.

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Competing interests statement
The authors declare no competing financial interests.

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