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Chromatin remodeling and cancer, part I: covalent histone modifications

Gang G. Wang[1], C. David Allis[1] and Ping Chi[1,2]

[1] Laboratory of Chromatin Biology, The Rockefeller University, New York, NY 10021, USA
[2] Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

Dynamic chromatin remodeling underlies many, if not all, DNA-templated biological processes, including gene transcription; DNA replication and repair; chromosome condensation; and segregation and apoptosis. Disruption of these processes has been linked to the development and progression of cancer. The mechanisms of dynamic chromatin remodeling include the use of covalent histone modifications, histone variants, ATP-dependent complexes and DNA methylation. Together, these mechanisms impart variation into the chromatin fiber, and this variation gives rise to an 'epigenetic landscape' that extends the biological output of DNA alone. Here, we review recent advances in chromatin remodeling, and pay particular attention to mechanisms that appear to be linked to human cancer. Where possible, we discuss the implications of these advances for disease-management strategies.

Introduction

Chromatin is the physiological template of our genome. Its fundamental unit, the nucleosome core particle, consists of 146 DNA base pairs organized around an octamer consisting of two copies of each highly conserved core histone protein – H2A, H2B, H3 and H4. Dynamic modulation of chromatin structure, that is, chromatin remodeling, is a key component in the regulation of gene expression, apoptosis, DNA replication and repair and chromosome condensation and segregation. Disruption of these processes is intimately associated with human diseases, including cancer [1].

Nature has evolved elaborate mechanisms to dynamically modulate chromatin structure, including chromatin remodeling by ATP-dependent complexes, covalent histone modifications, utilization of histone variants and DNA methylation. Because DNA methylation has been extensively reviewed elsewhere [2–5], this review will discuss the mechanisms and clinical implications of chromatin remodeling. Part I will focus on covalent histone modifications and histone variants, and part II will focus on chromatin remodeling by ATP-dependent complexes involved in cancer.

Mechanisms of covalent histone modifications

There are at least eight different classes of covalent modifications involving more than 60 distinct modification sites within the major core histones characterized to date; these modifications include lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, lysine ubiquitylation, glutamate poly-ADP ribosylation, lysine sumoylation, arginine deimination and proline isomerization [6].

Molecular mechanisms underlying the use of each individual histone modification can be generalized into two categories, '*cis*' mechanisms (Figure 1a) and '*trans*' mechanisms (Figure 1b and Figure 2). *Cis* mechanisms achieve alteration of intra- and internucleosomal contacts via changes of steric or charge interactions, and prominent examples include histone acetylation and deacetylation. It has been proposed that histone acetylation, a modification associated with transcriptional activation, unfolds chromatin via neutralization of the basic charges of lysines [6]. Indeed, recent studies with recombinant nucleosomal arrays have demonstrated that the acetylation of H4K16 inhibits the formation of compact 30 nm fibers and higher-order chromatin structures [7,8] (Figure 1a). *Trans* mechanisms involve utilization of non-histone 'readers' that bind specific histone modifications and lead to corresponding functional consequences. Prominent examples include H3K4 methylation, H3K9 methylation and H3K27 methylation, which are recognized by inhibitor of growth (ING) proteins, heterochromatin protein 1 (HP1) and polycomb proteins, respectively (see below) (Figure 1b and Figure 2).

It is hypothesized that the combination of specific histone modifications signifies a 'histone or epigenetic code' that is written by some enzymes ('writers') and removed by others ('erasers') and is readily recognized by proteins ('readers') that are recruited to modifications and bind via specific domains [9,10] (e.g. Figure 2). These 'writing', 'reading' and 'erasing' activities, in turn, establish the optimal local environment for chromatin-templated biological processes, such as transcriptional regulation and DNA-damage repair. For example, tri-methylation of histone H3, lysine 4 (H3K4me3), a modification associated with transcriptional activation [6], is 'written' by the histone methyltransferase (HMT) MLL (mixed lineage leukemia) and 'erased' by the jumonji, AT-rich interactive domain 1 (JARID1) demethylases (Figure 2a). ING proteins 'read' the H3K4me3 mark via their plant homeodomain (PHD) finger. The downstream consequence of ING binding depends on the specific cellular scenario. Whereas YNG1 (yeast homolog of ING) recruits histone acetylatransferases (HAT) and translates H3K4me3 into active transcription [11] (Figure 2a), ING2 recruits histone deacetylases (HDAC) and translates the

Corresponding authors: Allis, C.D. (alliscd@mail.rockefeller.edu); Chi, P. (chip@mskcc.org).

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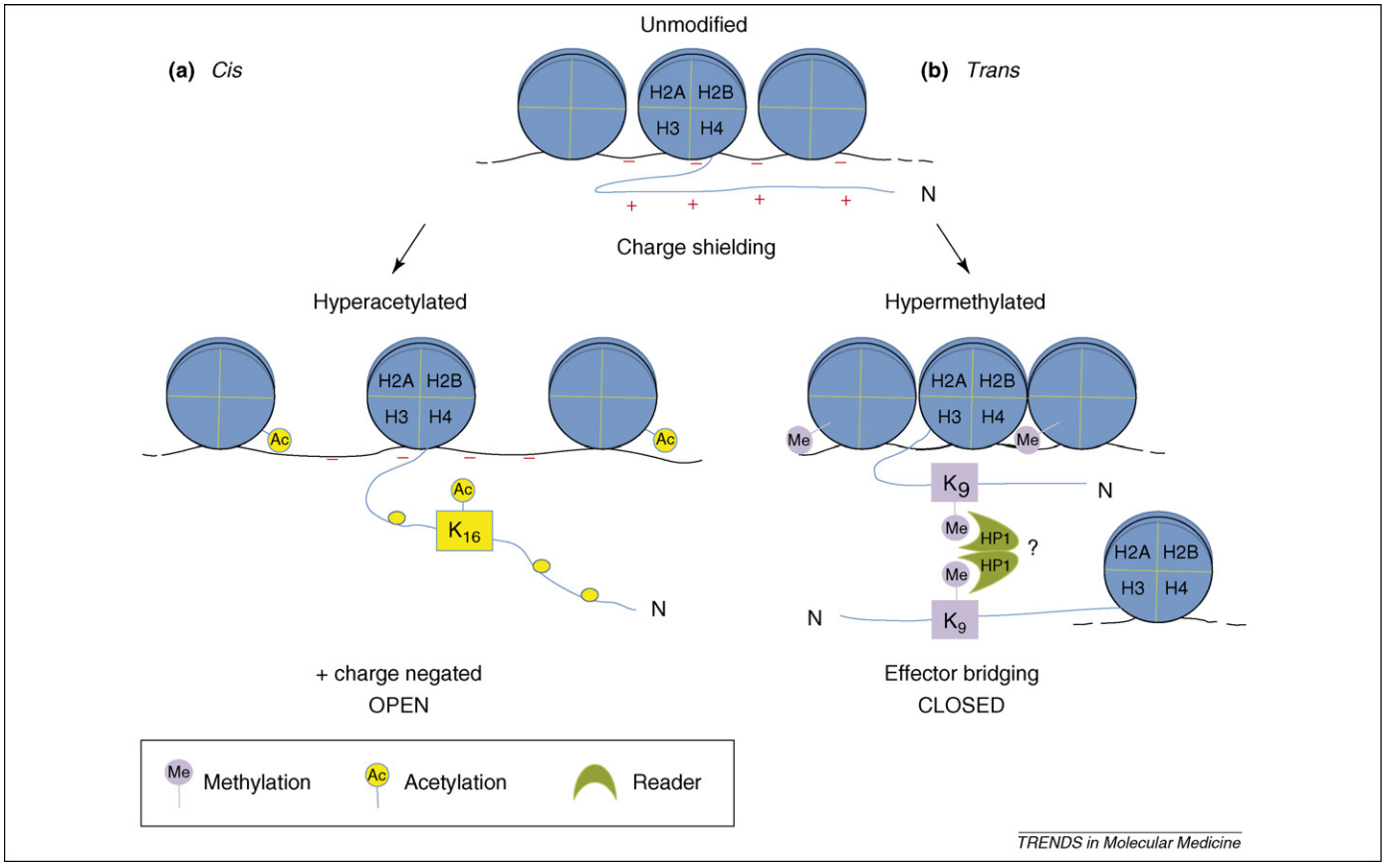

Figure 1. Schematic depiction of two basic mechanisms utilizing covalent histone modifications, '*cis*' and '*trans*'. Nucleosomes with unmodified histone N-terminal tails contain a high density of positive charges (lysine and arginine residues), which might provide 'charge shielding' for the close association of these tails with negatively charged DNA and lead to chromatin compaction. **(a)** *Cis* mechanisms alter intra- or internucleosomal contacts by changing electrostatic interactions. One prominent example shown here is the acetylation of lysines on histone H4 N-terminal tails, especially at K16; this leads to neutralization of the positive charges of lysines and unfolds chromatin into a more 'OPEN' structure. This 'OPEN' chromatin structure creates an optimal local environment for transcriptional activation. **(b)** Trans mechanisms involve utilization of non-histone effector proteins ('readers') that recognize and bind specific histone modifications; this binding leads to downstream functional consequences. The example shown here depicts heterochromatin protein 1 (HP1) recognizing and binding to methylated K9 on histone H3 tails; this binding facilitates the formation of a 'CLOSED' chromatin state and leads to heterochromatin assembly and subsequent gene silencing. '?' depicts a possible intra- or internucleosomal HP1–HP1 dimer formation ('bridging'), which might lead to further chromatin compaction, but this remains to be validated.

mark into transcriptional repression during DNA damage [12,13].

Cross-talk between existing histone modifications adds another level of complexity in chromatin remodeling; during cross-talk, the status of one histone modification regulates that of another. It occurs either within the same histone tail or among different histone tails involving one or multiple nucleosomes. The intra-tail examples include cross-talk between methyl-H3K9 and phospho-H3S10 in the regulation of chromosomal stability (Figure 3b) and potential cross-talk between phospho-H2BS14 and acetyl-H2BK15 during apoptosis (Figure 3c). As an example of trans-tail crosstalk, the ubiquitylation of H2BK123 is required for methylation of H3K4 and H3K79 [6].

Covalent histone modifications and cancer

Roles of selective covalent histone modification in tumorigenesis are emerging. Global loss of trimethylation of H4K20 (H4K20me3) and loss of acetylation of H4K16 (H4K16Ac) have been observed along with DNA hypomethylation at repetitive DNA sequences in various primary tumors [14]. Distinct patterns of global alternations in histone modifications have also been correlated with the risk of prostate cancer recurrence [15]. Despite these discoveries, most of our understanding of histone modifications and cancer stems from research specifically examining the 'writers', 'readers' and 'erasers' of specific histone modifications in cancer related cellular processes. Here, we will focus on histone lysine methylation and acetylation and their involvement in transcriptional regulation in oncogenesis (Table 1 and Figure 2). We will discuss emerging evidence of histone phosphorylation in non-transcriptional cellular processes, including DNA-damage repair, chromosome stability and apoptosis (Figure 3). As reviewed below, numerous genetic disruptions (e.g. mutations and deletions) of the protein machinery involved in histone modifications have been clearly implicated in oncogenesis. Yet, direct evidence that points to a causal role for covalent histone modifications resulting from the above genetic events in oncogenesis largely remains to be established.

Histone lysine methylation and cancer

With a few exceptions, trimethylations of H3K9, H3K27 and H4K20 are enriched in heterochromatin and are associated with transcriptional repression, whereas methylations of H3K4, H3K36 and H3K79 are enriched

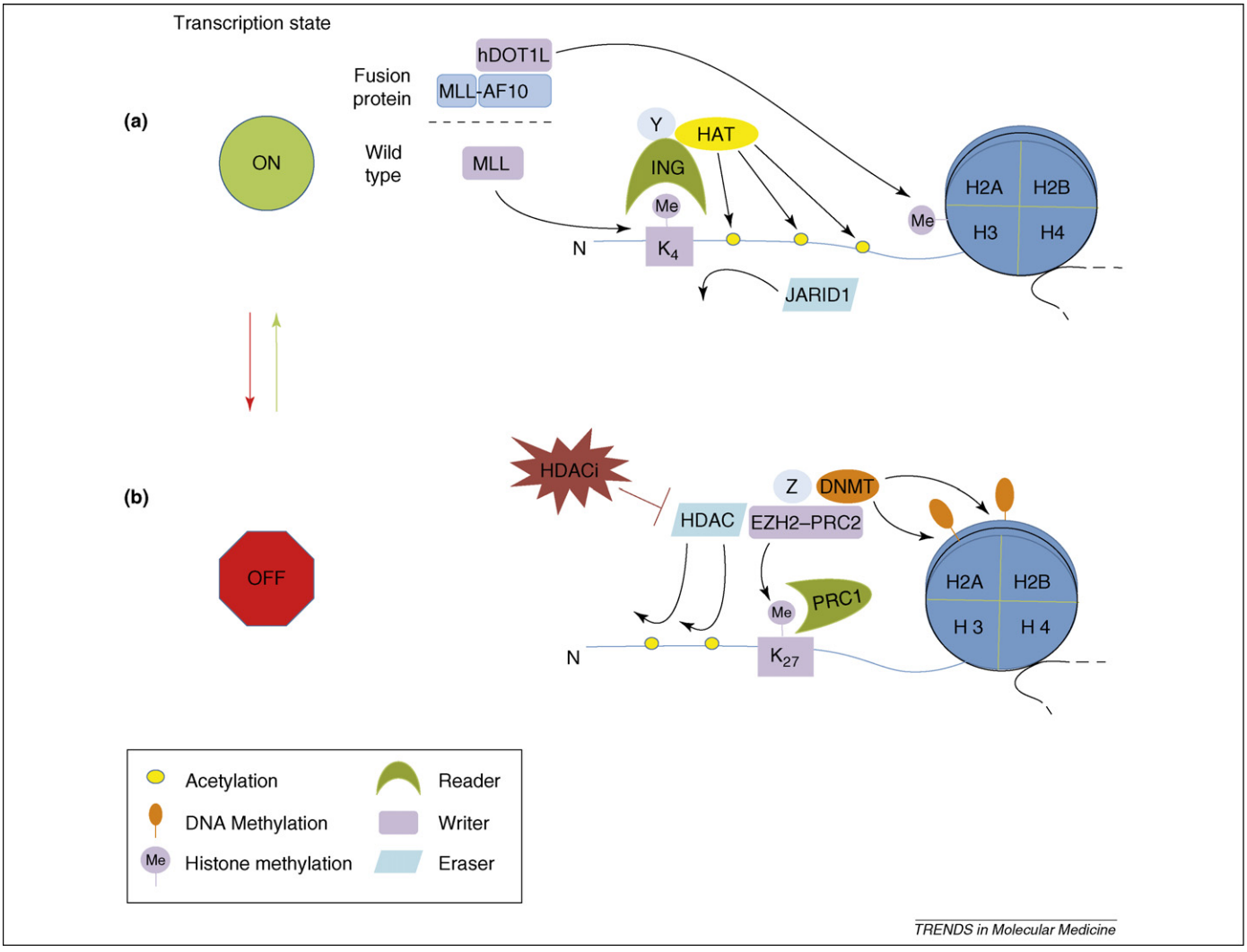

Figure 2. Schematic depiction of transcriptional regulation by post-translational histone modifications. **(a)** Histone H3K4me3 is often associated with transcriptional activation. The H3K4me3 mark is established by the MLL methyltransferase ('writer') and can be erased by JARID1 family demethylases ('erasers') [6]. In human leukemia with the MLL-AF10 oncogenic fusion protein, hDOT1L, a H3K79 methyltransferase, is mistargeted to the *Hox* locus via interaction with AF10; subsequent hypermethylation at H3K79 is responsible for leukemogenesis [55]. One mechanism of H3K4me3-mediated transcriptional activation is by association with ING proteins ('readers') via a plant homeodomain (PHD) finger domain in a context-dependent manner [11,12]. ING proteins are part of complexes containing the MYST family HATs, which in turn mediate acetylation of histone tails and subsequent transcriptional activation [11]. **(b)** H3K27me3 is often associated with transcriptional silencing. The H3K27me3 mark is established by the EZH2 ('writer')–PRC2 complex. The EZH2–PRC2 complex can also recruit DNMTs and HDACs to mediate DNA methylation and histone deactylation [4,16]. The PRC1 complex recognizes the H3K27me3 mark through chromodomains of the human polycomb (HPC) subunit and mediates ubiquitylation (Ub) of H2AK119 (not shown in figure) [16]. The coordinated activities of the H3K27Me3 'writer' and 'reader' collectively mediate gene transcriptional silencing. Y and Z represent other interacting proteins not specified in the schematic. For clarity, not all known modifications, writers, erasers and readers are shown in Figures 2 and 3. See text for details and references.

in euchromatin and are associated with transcriptional activation [6]. The following section will review emerging connections that the 'writers', 'readers' and 'erasers' of these histone modifications have with cancer (summarized in Table 1).

H3K27 methylation

The H3K27 methylation mark is primarily governed by the polycomb group (PcG) proteins that are initially genetically defined in *Drosophila melanoganster* [16]. Two polycomb repressor complexes (PRC1 and PRC2) have been characterized. PRC1, comprising core components of BMI-1, Ring-1, HPH and HPC, recognizes trimethylated H3K27 and mediates the maintenance of the silent state [16]. PRC2, comprising of enhancer of zeste homologue 2 (EZH2), suppressor of zeste 12 (SUV12) and embryonic ectoderm development (EED), mediates the initiation of gene repression via association with HDACs and DNA methyltransferases (DNMTs) [16]. Thus, in addition to initiating a suppressive histone-modification state, PRC2 might also target DNA for methylation and collectively contribute to stable gene silencing [17,18] (Figure 2b).

EZH2 – an H3K27 methylation 'writer'

EZH2, a SET domain methyltransferase for H3K27, plays essential roles in embryonic development and stem cell renewal [16,17]. Overexpression of EZH2 has been observed and positively correlates with the progression of multiple malignancies, including prostate cancer, breast cancer, lymphoma, myeloma, colorectal cancer, endometrial cancer, bladder cancer and melanoma [4]. Ectopic overexpression of EZH2 leads to increased cell proliferation and transformation independent of growth factors in

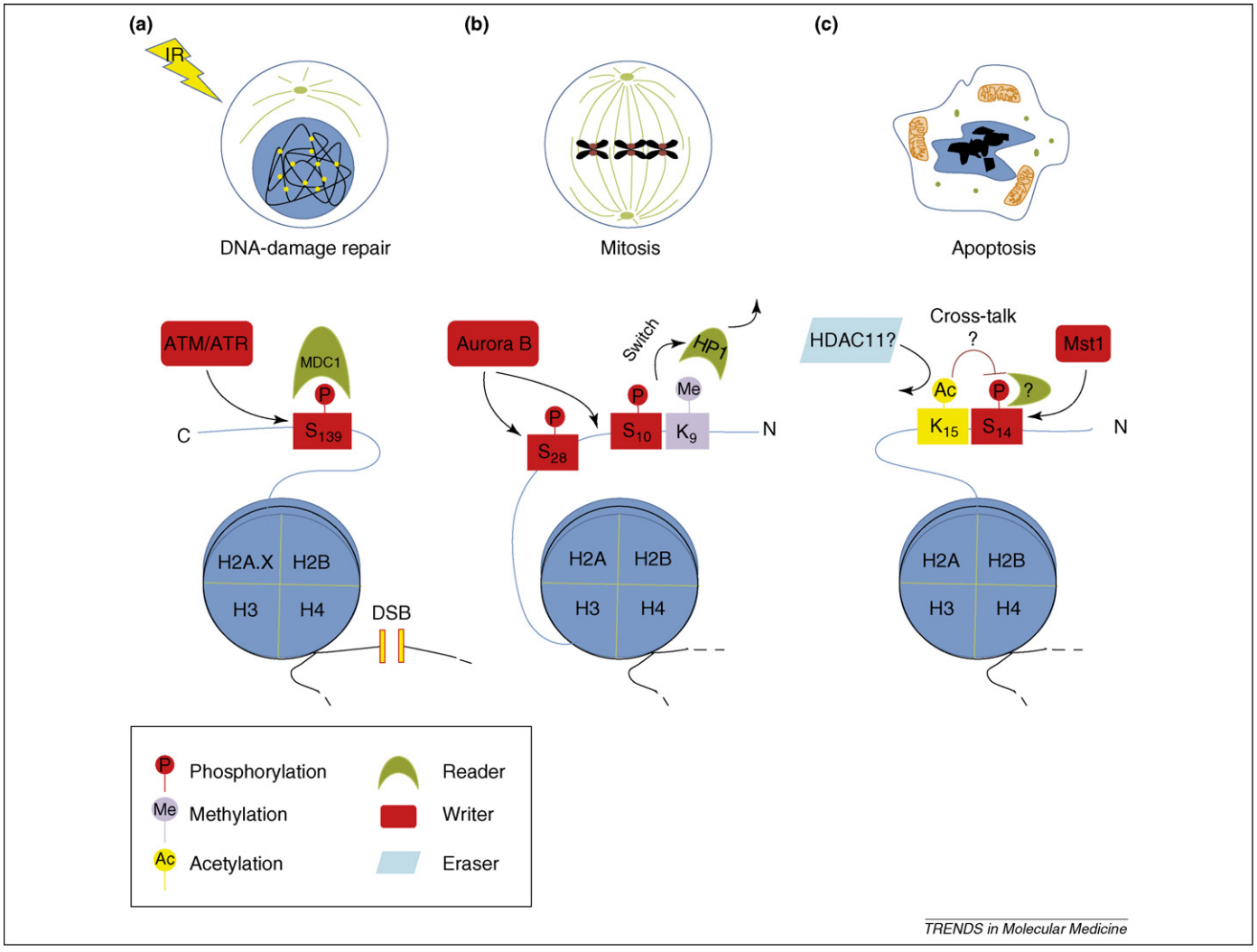

Figure 3. Schematic of non-transcriptional regulation of DNA-damage repair, chromosome segregation and apoptosis by chromatin remodeling. **(a)** DNA double-strand breaks (DSBs) induced by ionizing irridation (IR) can lead to rapid phosphorylation of H2A.X at Ser139 by ATM/ATR ('writer'). MDC1 ('reader') recognizes phosphorylated H2A.X and mediates accumulation of DNA-damage-response proteins, including 53BP1, NBS1 and phosphorylated ATM, at the site of damaged chromatin for proper DNA-damage repair [65]. **(b)** HP1 recognizes H3K9me3 and mediates heterochromatin formation. During mitosis, aurora B kinase ('writer') phosphorylates H3S10, which in turn leads to the dissociation of HP1 from heterochromatin while maintaining H3K9 in a trimethylated state to allow proper chromosome segregation. This represents a 'phos-methyl switch' mechanism for the regulation of HP1 ('reader') association with H3K9me3 [71]. **(c)** Mammalian histone H2B undergoes dynamic phosphorylation at Ser14 by Mst1 ('writer') during apoptosis. Yeast histone H2B also undergoes dynamic phosphorylation at a serine site at the N-terminal tail during programmed cell death. Phosphorylation of yeast H2B requires deacetylation of an adjacent lysine residue by Hos3, a yeast homologue of mammalian HDAC11, signifying a unidirectional 'cross-talk' pathway. Whether an exact parallel 'cross-talk' pathway exists in the mammalian system remains to be determined. See text and [77] for details and references.

multiple myeloma [19], whereas decreased expression of EZH2 by RNAi leads to growth arrest in prostate cancer cells [20]. The methyltransferase activities of both EZH2 and HDAC recruited by EED are required for cell proliferation and invasion mediated by EZH2 [4,20,21]. The fact that the PRC2 complex recruits DNMTs to the promoters of EZH2 target genes and induces their silencing in tumor cells suggests that EZH2 might selectively induce the silencing of tumor-suppressor genes during cancer cell evolution [17,18] (Figure 2b). However, direct evidence supporting EZH2-mediated silencing of tumor-suppressor genes remains to be established. Alternative mechanisms of oncogenesis have been proposed. For example, cytoplasmic EZH2 controls actin polymerization and cell signaling upstream of the small GTPase CDC42 [22], indicating that EZH2 might contribute to the observed increase in the metastatic capacity of breast and prostate cancer cells [20,21] via regulation of actin-dependent cell adhesion and migration.

BMI1

BMI1 is recruited to trimethylated H3K27 (H3K27me3) as part of the PRC1 complex via the interaction between HPC and H3K27me3. It was originally identified as a proto-oncogene that cooperates with Myc to promote B-cell lymphoma development in mouse models [23,24]. It is also overexpressed in lymphoma, leukemia, medulloblastoma, neuroblastoma and non-small-cell lung cancer (NSCLC) [16]. BMI1 inhibits Myc-induced apoptosis through repression of the *CDKN2A* locus [25,26]. In addition, BMI1 harbors crucial activities in maintaining proliferative capacities of both normal stem cells and leukemic stem cells [27], suggesting that BMI1 might promote tumorigenesis by misdirecting tumor cells toward a stem cell fate [28].

H3K9 methylation

HP1 – an H3K9 methylation 'reader'

HP1 proteins (HP1α, HP1β, HP1γ) bind to methyl-H3K9 and are crucial for the formation of heterochromatin and

Table 1. Examples of histone methylation and cancer

Classes of histone modification	Histone-modifying enzymes and binding partners involved in cancer		Cellular defects and/or deregulation observed in cancer	Refs
'Writers' – HMTs	H3K4	MLL	Tumor promotion Transcription regulation; oncogenic fusion proteins of MLL; amplification; tandem duplication in AML, ALL and MDS	[6,44,45]
		SMYD3	Tumor promotion Transcription regulation; cell-cycle defects; upregulation in colorectal and haptocellular carcinoma cell lines; overexpression in cell lines promotes cell growth and transformation	[31,46]
	H3K9	SUV39H1	Tumor suppression Cell-cycle defects; chromosome instability; transcription regulation; B cell lymphoma in knockout mice (mouse model); no known mutations reported in human cancers yet	[31–36]
		RIZ1/PRDM2	Tumor suppression Transcription regulation; frame shift, missense mutations and epigenetic silencing; LOH in human hepatocellular carcinoma, colon, melanoma, sarcoma, breast and gastric cancers; B cell lymphoma and stomach cancer in mouse model	[31,37–39]
	H3K27	EZH2	Tumor promotion Cell-cycle defects; overexpression and amplification in human prostate cancer, breast cancer, lymphoma, myeloma, colorectal cancer, endometrial cancer, bladder cancer and melanoma	[4,16–22]
	H3K36	NSD1	Tumor suppression Heterozygous germline mutations in Sotos syndrome increases risk for human hepatocellular carcinoma, leukemia and neuroblastoma	[50,52,53]
	H3K79	hDOT1L	Tumor promotion Transcription regulation; leukemogenesis in association with AF10 fusion proteins	[55,56]
'Readers'	H3K4	INGs	Tumor suppression Transcription regulation; DNA-damage repair; DNA replication; mutations, LOH and downregulation in breast cancer, gastric cancer, melanoma, glioma, and head and neck squamous cell carcinoma	[47–49]
	H3K9	HP1	Tumor suppression Transcription regulation; frame shift, missense mutations and epigenetic silencing; LOH in human hepatocellular carcinoma, colon, melanoma, sarcoma, breast and gastric cancers; B-cell lymphoma and stomach cancer in mouse model	[4,29,30]
	H3K27	BMI1/PRC1	Tumor promotion Cell-cycle defects; overexpression in lymohoma, leukemia, medulloblastoma, neuroblastoma and NSCLC	[16,23–28]
'Erasers' – Demethylases	H3K9 H3K36	JMJD2C/GASC1	Tumor promotion Promotes AR-dependent transcription and prostate cancer cell proliferation; overexpressed in human esophageal squamous cell carcinoma, lung sarcomatoid carcinoma and desmoplastic medulloblastoma	[40–43]

gene silencing [29]. Decreased HP1α and HP1β expression has been observed in metastatic breast tumors, melanoma and other metastatic tumors [4,30]. Ectopic expression of HP1α reduces invasion of breast cancer cells, whereas inhibition of HP1α leads to increased invasion without affecting cell growth [30], implicating its role in tumor metastasis suppression. However, either an increase or reduction in HP1 levels can lead to chromosome instability and aneuploidy [4]. It remains unclear whether the metastasis-suppressive effect of HP1 is derived from maintenance of chromosomal stability, from transcriptional regulation, or both.

SUV39H and RIZ1 – H3K9 methylation 'writers'

SUV39H1 and SUV39H2, mammalian homologues of *Drosophila* SU(VAR)3–9, are SET domain HMTs for H3K9 [31]. Whereas SUV39H1$^{-/-}$ and SUV39H2$^{-/-}$ mice are viable with no clear phenotype, SUV39H1$^{-/-}$/SUV39H2$^{-/-}$ mice exhibit severely compromised viability, increased chromosomal instability and increased risk of B-cell lymphoma [32]. These phenotypes were associated with chromosomal mis-segregation, abnormally long telomeres and significant reduction of di- and tri-methylation of H3K9 and loss of HP1 binding at pericentric heterochromatin and telomeres [32–34]. Biochemically, SUV39H1 and HP1 interact with pRb and mediate gene silencing of –pRb targets [35]. SUV39H1 also prevents Ras-induced tumorigenesis by promoting senescence [36]. Despite these observations, the role of SUV39H1 in human cancer is not defined because there have not been any SUV39H1 mutations or losses reported in human cancers.

RIZ1/PRDM2, another family of SET domain H3K9 methyltransferase, was originally identified as a pRb-binding protein [31]. Inactivation of RIZ1 by mutations and silencing via promoter hypermethylation are observed in many human cancers, including hepatocellular, colon, breast and gastric cancers [37,38], suggesting a tumor-suppressive role. Furthermore, missense mutations that abolish the HMT activity of RIZ1 are found in human cancers [37,39], suggesting a direct causal effect

of the HMT activity in tumor suppression. Indeed, ectopic expression of RIZ1 induces G2/M cell-cycle arrest and apoptosis in tumor cell lines [38]. RIZ1$^{-/-}$ mice are prone to developing B cell lymphoma and stomach cancer [39].

JMJD2C – an H3K9 methylation 'eraser'

Jumonji-domain-containing proteins (JMJD) have recently been characterized as histone lysine demethylases. JMJD2C, initially identified as gene amplified in squamous cell carcinoma 1 (GASC1), is also frequently amplified and overexpressed in esophageal squamous cell carcinoma, lung sarcomatoid carcinoma and desmoplastic medulloblastoma [40–42]. It removes the methyl group from tri- and di-methylated H3K9. Overexpression of JMJD2C/GASC1 induces reduction of global H3K9 tri- and di-methylation levels and delocalization of HP1, which might contribute to tumorigenesis [43].

H3K4 methylation

Initial genetic analysis in *D. melanoganster* defines the functional roles of the trithorax group (Trx-G), the 'writers' for the H3K4 methylation mark, during development. Conserved from flies to mammals, Trx-G proteins maintain the epigenetic activation of homeodomain genes, whereas PcG proteins mediate their silencing. These two antagonistic groups of proteins control important aspects of differentiation and proliferation during embryogenesis (Figure 2).

MLL and SMYD3 – H3K4 methylation 'writers'

The mixed lineage leukemia (MLL) protein, a mammalian Trx-G protein, is a SET domain H3K4 methyltransferase and maintains activation of *Hox* gene expression during development [6]. In several leukemia subtypes, several genetic events have been observed to involve the MLL gene. For example, reciprocal translocation with dozens of different partners can produce aberrant MLL fusion genes; partial internal tandem duplications can lead to production of in-frame elongated MLL proteins; or gene amplification can induce MLL overexpression [44]. Whereas MLL$^{-/-}$ mice had impaired hematopoiesis [45], mice carrying the MLL-AF9 fusion protein all developed acute myeloid leukemia (AML) [44]. Therefore, MLL fusion proteins are thought to contribute to tumorigenesis by a gain-of-function mechanism. Indeed, AF10, an MLL fusion partner, binds the H3K79 HMT hDOT1L, linking H3K79 methylation to leukemogenesis and persistent activation of Hox-A protooncogenes. However, the mechanism of MLL fusion involving other partners remains to be elucidated.

SET- and MYND-domain-containing protein 3 (SMYD3), another methyltransferase for H3K4, is found to be frequently upregulated in colorectal and hepatocellular carcinoma cell lines [46]. SMYD3 interacts with RNA helicase HELZ and RNA polymerase II and mediates transcriptional activation of targets, including oncogenes, homeobox genes and cell-cycle regulatory genes [31,46]. Overexpression of SMYD3 enhances cell growth and promotes transformation, whereas inhibition of SMYD3 expression represses cell growth in cancer cell lines [46]. Presumably, SMYD3 contributes to tumorigenesis by deregulating gene expression.

ING proteins – H3K4 methylation 'readers'

Mammalian ING family proteins, INGs 1–5, are putative tumor suppressors and have been observed to cooperate with p53 to mediate growth arrest, cellular senescence and apoptosis [47]. Reduced expression, somatic mutations and allelic loss of ING proteins (especially ING1, ING3 and ING4) are observed in breast cancer, gastric cancer, melanoma, glioma and head and neck squamous cell carcinoma (HNSCC) [47,48]. ING1$^{-/-}$ mice are sensitive to gamma radiation and are predisposed to lymphomas [49]. All ING proteins contain an important C-terminal PHD finger motif that specifically recognizes H3K4me3, and specific mutations that abolish H3K4me3-binding activities are found in tumors [47], indicating a direct involvement of H3K4 functional readout pathways during oncogenesis.

H3K36, H4K20 and H3K79 methylation

H3K36, H4K20 and H3K79 methylation 'writers'

The nuclear-receptor-binding SET-domain-containing protein 1 (NSD1), initially identified as a fusion partner of nucleoporin 98 (NUP98) in t(5;11)(q35;p15.5)-containing pediatric AML, is a methyltransferase for H3K36, and to a lesser degree for H4K20 [50]. NSD1 mediates cellular context-dependent gene silencing and activation [50]. NUP98-NSD1 translocation leads to hematopoietic transformation and leukemia, at least partly as a result of the activation of Hox-A genes via the H3K36 methyltransferase activity of NSD1 [51]. Heterozygous mutation or loss of heterozygosity (LOH) of NSD1 causes Sotos syndrome, a childhood overgrowth syndrome exhibiting increased risk (2%–7%) for hepatocellular carcinoma, leukemia and neuroblastoma [52,53].

hDOT1L is a human non-SET-domain methyltransferase for H3K79 [54]. hDOT1L has been shown to interact with AF10, one of the MLL fusion partners in AML [55]. In leukemias caused by MLL-AF10 and clathrin-assembly protein-like lymphoid-myeloid (CALM)-AF10 fusion proteins, hDOTL is mistargeted to the *Hox-A9* and *Hox-A5* loci, respectively; subsequent H3K79 hypermethylation at these loci and upregulation of Hox-A genes are thought to be responsible for leukemogenesis [55,56].

Histone lysine acetylation and cancer

Histone lysine acetylation is strongly associated with transcriptional activation, presumably through nucleosomal structure changes induced by charge neutralization. In addition, histone acetylation is able to recruit many bromodomain-containing transcriptional co-activators and mediators. Histone acetylation is also linked to other cellular functions, including DNA replication and repair and chromatin assembly [6]. The acetylation status of chromatin is governed by the opposing effects of HATs and HDACs. Disturbance of this balance through the disruption of HAT or HDAC activity can lead to cancer [57].

HATs

There are three main families of HATs: the Gcn5-related N-acetyl transferase (GNAT) family, the MOZ/YBF2/SAS2/TIP60 (MYST) family and the CBP/p300 family [6,58]. These HATs, in the forms of multisubunit complexes, usually

acetylate, with poor specificity, multiple lysine sites in the core histones and primarily promote active transcription [6,58]. They also acetylate several non-histone proteins, such as p53, pRb and E2F, and modulate their transcriptional activities on target genes [6,58].

Several lines of evidence support the connection between HAT dysregulation and oncogenesis. The viral oncoproteins E1A (adenovirus) and large T-antigen (SV40) primarily target p300, CBP and PCAF (p300/CBP-associated factor) and inhibit their function [59]. Heterozygous CBP germline mutation that inactivates its HAT activity is associated with Rubinstein-Taybi syndrome (RTS), a developmental disorder with increased risk of cancer. Bi-allelic mutations of p300 and CBP have been observed in colorectal, gastric, hepatocellular and breast cancers [59]. Knockout animal models appear to confirm the role of p300 and CBP as tumor suppressors. $CBP^{+/-}$ mice progressively develop hematological malignancies with somatic loss of the second CBP allele [59]; chimera mice containing CBP-null or p300-null cells develop hematological malignancies [59]. Although loss of HATs can be oncogenic, aberrant localization or activation of HATs can also be oncogenic. Chromosomal translocations resulting in fusion proteins of HATs, including MLL-CBP, MLL-p300, MOZ-CBP, MOZ-p300 and MOZ-TIF2 (transcriptional intermediary factor 2), have been identified in leukemia and myelodysplastic syndrome, and the leukemogenic potential has been confirmed in murine models in the case of MLL-CBP [59].

HDACs

The 18 HDACs identified to date can be categorized into four classes: class I (HDAC1–3, HDAC8), class II (HDAC4–7, 9–10), class III (Sirtuin1–7) and class IV (HDAC11). HDACs remove the acetyl groups from histone lysine tails [6,60] and are thought to facilitate transcriptional repression by decreasing the level of histone acetylation. Like HATs, HDACs also have non-histone targets, such as p53, E2F and TFIIF [57]. There is little evidence that any HDAC has specificity for a particular acetyl group or for a particular gene. Instead, specificity is mediated by interacting proteins that recruit HDACs to specific sites. Accordingly, many HDACs act in the context of multiprotein complexes, such as the repressive chromatin-remodeling complex NuRD, the DNA methyltransferase 1 (DNMT1)/pRb/E2F complex or the lysine-specific demethylase 1 (LSD1)/CoREST (REST corepressor) complex, to regulate multiple cellular processes, including gene transcription, cell-cycle progression and apoptosis [57,60].

A well-established oncogenic mechanism involving HDACs is the aberrant recruitment of HDACs to promoters through their physical association with aberrant leukemia fusion proteins, such as PML-RARα, PLZF-RARα and AML1-ETO [60]. Bcl-6 is inappropriately expressed in many non-Hodgkin's lymphomas, where the target genes of Bcl-6 are aberrantly repressed through the recruitment of HDACs [61]. In cell lines and murine models of colon cancer, HDAC2 is upregulated upon loss of adenomatosis polyposis coli (APC), and the expression of HDAC2 is required for tumorigenesis, presumably via its aberrant repressive activities [62]. Similarly, overexpression of HDAC1–3 and 6 has also been observed in prostate, gastric, breast and cervical cancer [60].

Recently, HDAC inhibitors (HDACis) have been shown to be a class of promising anti-cancer agents, exhibiting a range of observed cellular effects, including growth arrest, differentiation and apoptosis in a variety of cancers in clinical trials [60]. However, given the pleiotropic effects of HDACs on both histone and non-histone substrates (usually transcription factors), it remains challenging to dissect the specific contribution of chromatin-remodeling effect and transcription-factor-modulation effect to the observed promising results of HDAC inhibitors in clinical trials.

Histone serine phosphorylation and cancer

In addition to regulating transcription, chromatin remodeling also regulates many non-transcriptional cellular processes, including DNA-damage repair, chromosomal stability and apoptosis. Mechanisms for these regulations and their connections to oncogenesis are emerging. In particular, mounting evidence indicates that histone serine phosphorylation is crucial in the regulation of DNA-damage repair, chromosome stability and apoptosis (Figure 3).

H2A.X phosphorylation and DNA-damage repair

DNA-damage repair is among the most important mechanisms for guarding the genome and preventing tumorigenesis [63]. H2A.X, a histone H2A variant, represents 2%–25% of the total mammalian H2A pool. Its phosphorylation at serine 139 in the highly conserved C-terminal tail (–SQEY) has been shown to play important roles in DNA double-strand break (DSB) repair and tumor suppression [63]. DSBs, induced by ionizing irradiation (IR), stalled replication forks or immune receptor rearrangement, lead to rapid phosphorylation of H2A.X (γ-H2A.X) in chromatin regions flanking DSBs. H2A.X phosphorylation is mediated by phosphatidylinositol 3-kinase-like kinases (ATM, ATR and DNA-PK), and defects in these kinases usually result in cancer predisposition [63,64]. Mediator of DNA damage checkpoint protein 1 (MDC1) has been demonstrated to bind to γ-H2A.X directly via its tandem BRCA1 C-terminal (BRCT) domains, and this binding is essential for normal radiation resistance and accumulation of DNA-damage-response proteins for proper DNA repair [65] (Figure 3a). $H2A.X^{-/-}$ mice exhibit growth retardation, radiation sensitivity, chromosomal instability and DSB-repair defects [63]. Compared to $p53^{-/-}$ mice, $H2A.X^{+/-}/p53^{-/-}$ and $H2A.X^{-/-}/p53^{-/-}$ mice have an increased risk for lymphomas and solid tumors in a phosphorylation-dependent manner [66,67]. Therefore, H2A.X, along with other DNA-damage-response proteins, maintains genomic stability and suppresses tumorigenesis.

Histone H3 serine 10 phosphorylation and chromosomal stability

Aneuploidy resulting from chromosomal instability is characterized by imbalances in chromosomes or chromosome segment numbers [68]. Increased aneuploidy has been linked to high tumor grade, advanced stage of cancer and poor prognosis [68,69]. Increased aneuploidy has

recently been shown to cause spontaneous lymphomas and lung cancer in mice, suggesting a causal role in oncogenesis [70]. Aurora kinases are important regulators of mitotic entry, centrosome function, mitotic spindle formation, chromosome segregation and cytokinesis, and their dysregulation leads to aneuploidy and tumorigenesis [68]. As discussed earlier, aberrant expression of HP1, a H3K9me3 'reader', can lead to aneuploidy [4]. Aurora-kinase-B-mediated phosphorylation of H3S10 might act as a 'phos-methyl switch' (see 'Switch', Figure 3b) resulting in dissociation of HP1 from heterochromatin while maintaining H3K9 methylation during mitosis [71,72] (Figure 3b). H3S10 phosphorylation is crucial for proper chromosome condensation and segregation during mitosis; overexpression of a non-phosphorylatable mutant of H3S10 results in retention of HP1 and defects in chromosome segregation [73]. Similarly, aberrant expression of HP1 leads to aneuploidy, as discussed earlier [4]. Thus, dynamic phosphorylation of H3S10 by aurora kinase B during mitosis plays an integral part in maintaining chromosome stability.

Histone H2B serine 14 phosphorylation and apoptosis

Acquired resistance toward apoptosis is a characteristic in nearly all cancer types [74], and recent studies indicate that histone modification is involved in the signaling cascade of apoptosis. Mammalian histone H2B undergoes dynamic phosphorylation at Ser14 (H2BS14) by mammalian sterile twenty (Mst1) and yeast H2B at Ser10 (H2BS10) by sterile twenty kinase (Ste20) in response to apoptotic stimuli [75,76]. In yeast, H2BS10 phosphorylation is a necessary step for H_2O_2-induced apoptosis, suggesting a direct causal effect [75]. Intriguingly, there is a 'cross-talk' (Figure 3c) between H2BS10 phosphorylation status and the acetylation status of an adjacent residue, K11 (H2BK11Ac), in a unidirectional fashion [77]. H2BK11Ac (normally acetylated in growing yeast) needs to be deacetylated by Hos3 deacetylase before H2BS10 phosphorylaton upon H_2O_2 treatment in yeast [77]. Failure of deacetylation of H2BK11Ac results in resistance to H_2O_2-induced cell death in yeast [77]. Whether there is a parallel 'cross-talk' mechanism in mammalian apoptosis remains to be determined (see '?', Figure 3c). Nonetheless, if a similar regulatory pathway exists in mammals, clinical utilization of non-specific HDACis might be detrimental because they might inhibit H2BK15 deacetylation and induce resistance to apoptosis.

These 'cross-talk' and 'switch' mechanisms (Figures 3b,c) beg the question of whether therapies aimed at perturbing 'transcriptional mis-silencing' through HDACis only perturb transcriptional regulation. We look forward to further experiments aimed at examining whether the potential involvement of acetylation and deacetylation in other fundamental processes, such as DNA-damage repair (Figure 3a), chromosome segregation (Figure 3b) and apoptotic chromatin compaction (Figure 3c), are also impacted by perturbance of the steady-state balance of potential epigenetic marks, such as acetylation.

Concluding remarks and future directions

It is increasingly clear that alterations in covalent histone modifications and dysregulation of their 'readers', 'writers' and 'erasers' are closely linked to oncogenesis. However, as in most studies, it is impossible to perform histone mutational analysis in mammalian systems where dozens of copies of each histone gene exist. It is almost impossible to manipulate histone modification status at specific gene loci, and non-histone off-target effects are always a concern in experiments involving manipulation of histone-modifying enzymes. Thus, it remains a challenge to dissect the mechanistic details of histone modifications in oncogenesis. Nevertheless, chromatin immunoprecipitation on chip (ChIP on CHIP) and other novel techniques for querying histone modifications at a genomic level have been a huge step forward. Future research will need to focus on developing new technologies and performing carefully designed studies aimed at a better understanding of the mechanisms of histone modifications and cancer (Box 1).

Despite the difficulty of establishing a causal role of histone modifications in cancer, global alteration of histone modification patterns appears to be clearly linked to oncogenesis [14]. Loss of H4K16Ac and H4K20me3 appears to be an early event in tumorigenesis in a mouse skin cancer model, and this loss accumulates throughout tumorigenesis [14]. Clinically, it would be ideal to establish signature profiling of alterations of histone-modification patterns in each specific type of human cancer, and such signature profiles will be a useful alternative not only for early cancer screening in a general population but also for prediction of cancer prognosis, treatment failure and relapse (Box 1). A recent study has already provided a glimpse of the potential use of histone-modification patterns as markers of prostate cancer prognosis [15].

There are ongoing efforts to develop therapeutics that target epigenetic mechanisms. Suberoylanilide hydroxamic acid (SAHA), initially discovered as an antitumor agent and subsequently found to inhibit class I and II HDACs [57], was recently approved for the treatment of cutaneous T-cell lymphoma. By enhancing levels of histone acetylation, SAHA and other HDACis were hypothesized to upregulate tumor-suppressor genes, at least in part. However, recent studies exhibit similar numbers of suppressive events and activating events after HDACi treatment, and neither change in histone acetylation levels nor change in gene expression profiles provided good pharmacodynamic biomarkers of efficacy for HDACis. Because information on clinically relevant HDACi targets is lacking, it has been challenging to design correlative studies in these clinical trials. Given the pleiotropic effects of HDACs, it is likely that HDACis target different cancer-related pathways in different cancer types. As more HDACis are developed, whether HDAC-isotype specific inhibitors will

Box 1. Outstanding questions

- Is there a direct causal role for specific histone-modification patterns in oncogenesis?
- Can 'signature profiles' of histone modification patterns be defined for the early diagnosis, prognosis and detection of cancer relapse?
- What are the clinically relevant molecular targets of different HDACis in different types of cancer?
- What are the advantages, if any, of developing isotype-specific HDAC inhibitors?

be more efficacious and/or have a better toxicity profile than non-specific HDACis will remain a key question in the clinical realm (Box 1).

Recent studies have suggested that histone-modifying enzymes such as JMJD2C/GASC1 and SMYD3 can be potential drug targets in cancer treatment. Although these enzymes are expected to have pleiotropic cellular effects, the fact that HDACis have proven to harbor clinically relevant activities in certain cancers is very encouraging to clinicians, and it also suggests that certain cellular targets might represent the Achilles heel of particular cancer types. The next step toward identifying the Achilles heel of different cancer types will help in designing cancer-specific drugs and also enable clinicians to tailor specific therapeutics according to the genetic and epigenetic profile of each patient. We suspect that the dissection of 'normal' and 'abnormal' epigenetic signatures will be relevant to many, if not all, aspects of human biology and disease.

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