EPIGENETICS AND GENETICS

Covalent histone modifications — miswritten, misinterpreted and mis-erased in human cancers

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Abstract | Post-translational modification of histones provides an important regulatory platform for processes such as gene transcription and DNA damage repair. It has become increasingly apparent that the misregulation of histone modification, which is caused by the deregulation of factors that mediate the modification installation, removal and/or interpretation, actively contributes to human cancer. In this Review, we summarize recent advances in understanding the interpretation of certain histone methylations by plant homeodomain finger-containing proteins, and how misreading, miswriting and mis-erasing of histone methylation marks can be associated with oncogenesis and progression. These observations provide us with a greater mechanistic understanding of epigenetic alterations in human cancers and might also help direct new therapeutic interventions in the future.

Chromatin
The composition of DNA and proteins (mainly histones) that form chromosomes. It is organized as repeating subunits of nucleosomal core particles that comprise approximately 147 base pairs of DNA wrapped around a histone octamer containing two copies each of histones H2A, H2B, H3 and H4.

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Modulation of chromatin through covalent histone modification is one fundamental way of regulating DNA accessibility during processes such as gene transcription, DNA replication and DNA damage repair. According to the 'histone code hypothesis' (BOX 1), the biological outcome of histone modifications is manifested by direct physical modulation of nucleosomal structure or by providing a signalling platform to recruit downstream 'reader' or 'effector' proteins[1,2]. Emerging evidence suggests that both genetic alterations and epigenetic aberrations contribute to the initiation and progression of human cancers[3]. For example, aberrant DNA methylation is a common mechanism used by tumour cells to silence tumour suppressor genes[4]. In this Review, we focus on the recent advances that link oncogenesis to histone methylation events, with those occurring at histone H3 lysine 4 (H3K4) and H3 lysine 27 (H3K27) as paradigmatic examples (TABLE 1). We propose that epigenetic alterations involving histone modifications lead to the misregulation of gene expression and consequently tumorigenesis.

Methylation of histones can occur at both lysine and arginine resides and is now appreciated as a reversible process. Its homeostasis is mediated by two antagonizing groups of enzymes, histone methylation 'writers' and 'erasers', which install and remove histone methylation marks, respectively, in a site-specific manner[1,5]. For example, H3K4 methylation is established by the SET1 and mixed lineage leukaemia (MLL) family of histone methyltransferases (HMTs)[5] (FIG. 1a), and removed by the lysine-specific histone demethylase 1 (LSD1) and jumonji AT-rich interactive domain 1 (JARID1) family of histone demethylases (HDMs)[6] (TABLE 1).

For histone H3, methylation has been observed at multiple lysine sites, including H3K4, K9, K27, K36 and K79, and the addition of up to three methyl groups at each lysine produces a total of four methyl states: unmethylated, monomethylated, dimethylated or trimethylated. These histone methylation states exhibit a distinct distribution pattern in the mammalian genome[7]. H3K4 trimethylation (H3K4me3) is strongly associated with transcriptional competence and activation, with the highest levels observed near transcriptional start sites of highly expressed genes, whereas H3K27 trimethylation (H3K27me3) is frequently associated with gene silencing, especially the repression of unwanted differentiation programmes during lineage specification[7–9]. The distribution patterns of H3K4me3, H3K27me3 and their associated histone marks underlie the diversity of cellular states for pluripotency and lineage differentiation. For example, in embryonic stem cells, active and repressive (bivalent) histone modifications coexist on developmentally crucial genes. By contrast, monovalent active or repressive histone marks are often found on these genes in differentiated cell lineages[8,9]. The bivalent chromatin state has been suggested as a mechanism for retaining chromatin and cellular plasticity at early stages of development[8,9]. As epigenetics and histone modification have crucial roles in cell fate determination, it has been proposed that they

At a glance

- Post-translational modifications of histones introduce meaningful variations into chromatin and provide a regulatory platform for controlling and/or fine-tuning many important DNA-templated processes, including gene transcription, the repair of DNA damage and DNA replication.
- Histone modifications, together with factors responsible for adding ('writing'), interpreting ('reading') and removing ('erasing') histone modifications, regulate specific and distinct functional outputs of our genomes, which constitute the basis of the 'histone code hypothesis'.
- As recent evidence starts to link the miswriting, misinterpretation and mis-erasing of histone modifications to oncogenesis, we further propose that misregulation of the histone code leads to deregulated gene expression and perturbation of cellular identity, and is therefore a major contributor to cancer initiation, progression and/or metastasis.
- Mixed lineage leukaemia (MLL) and enhancer of zeste 2 (EZH2) catalyse the addition of methylation of histone H3 lysine 4 (H3K4) and H3 lysine 27 (H3K27), respectively, which are arguably two of the most important histone methylation marks. *MLL* rearrangement and deregulation of *EZH2* are among the most common mutations in leukaemia and solid tumours, respectively.
- Several plant homoedomain (PHD) finger-containing proteins have recently been identified as reading factors of trimethylation of H3K4 (H3K4me3). Misinterpretation of H3K4me3 by leukaemia-associated translocations of PHD finger factors (NUP98–JARID1A or NUP98–PHF23) is crucial for the induction of myeloid leukaemia; somatic mutations of ING1, a PHD finger factor, interfere with the reading of H3K4me3 and associate with the development of oesophageal squamous cell carcinoma, head and neck squamous cell carcinoma and melanoma.
- Deregulation or mutations of the recently identified H3K4- or H3K27-specific histone demethylases have been observed in solid tumours. However, their involvement in cancer development and underlying mechanisms are largely unclear.

may help establish tumour-initiating cell populations in early tumorigenesis[10]. Indeed, changes in global histone modification patterns have been observed in several types of cancer, and these studies have provided us with the first glimpse of how the epigenetic landscape and cellular context might be altered in tumorigenesis[11,12].

Histone methylation: a chromatin index

One important issue in chromatin biology and epigenetics is how the pattern of a potential histone code or epigenetic code (BOX 1) is translated into meaningful biological consequences, and the identification of factors that specifically recognize or read histone modifications has greatly contributed to our mechanistic understanding. A recent breakthrough was the discovery of a specialized group of protein modules, plant homeodomain (PHD) fingers (BOX 2), which function as the specific reading motif for trimethylated and dimethylated H3K4 (H3K4me3/2), with H3K4me3 as the preferred ligand[13–16] (TABLE 2). Although many PHD finger motifs are encoded by the human genome, only a subset contains the crucial hydrophobic and aromatic residues that can form a specialized structural pocket to accommodate the H3K4me3 side chain[5] (BOX 2). These PHD histone modification reading modules have been reviewed in detail elsewhere[5,17,18]. Here, we focus only on how these histone modification reading factors are involved in normal cellular processes, such as transcriptional regulation, as well as in oncogenesis.

Around 15 PHD finger-containing readers for H3K4me3/2 have been experimentally confirmed (TABLE 2), and these include an RNA polymerase II-associated general transcription machinery component TFIID subunit 3 (also known as TAF3), a V(D)J recombinase RAG2, and several key chromatin-modifying and remodelling factors. The H3K4me3 mark could be a crucial chromatin 'index' mechanism, allowing specific genomic regions to be readily recognized by their downstream readers and/or associated effectors. For example, the targeting of TFIID to H3K4me3 at promoters has been suggested to help anchor and/or recruit TFIID and associated machinery for active transcriptional initiation[19,20] (FIG. 2a). The recognition of H3K4me3 by the RAG2 PHD finger at V(D)J gene segments is crucial for efficient V(D)J recombination during B and T cell development and maturation, and deleterious germline mutations that abrogate such recognition of H3K4me3 lead to severe immunodeficiency syndromes[21] (FIG. 2b). Now, emerging evidence also reveals that deregulation in the reading of H3K4me3 contributes to cellular transformation and tumorigenesis, for example, in acute myeloid leukaemia that is induced by chromosomal translocation of the H3K4me3-reading PHD finger of PHF23 or JARID1A (also known as KDM5A and RBBP2)[22] (TABLE 1). Furthermore, many enzymes that mediate the writing and erasing of histone methylation are strongly associated with oncogenesis (TABLE 1). Notably, some histone methylation writers and erasers also contain the methyl-reading module (for example, MLL and JARID1A (TABLES 1,2)), indicating potential coordination between the reading, writing and erasing events of histone modification.

Demethylation of histones and beyond

Although histone modification writers and erasers were originally identified as enzymes that modify histones, recent evidence suggests that they may also target non-histone proteins, which might confound attempts to infer the function of histone modifications. For example, LSD1 not only targets its canonical substrate, histone H3, but also demethylates the tumour suppressor p53 at lysine 370 and represses p53 activities[23,24]. Similarly, the histone methyltransferases G9a, SET7 and SET9 induce the methylation of many non-histone proteins[24,25]. To our knowledge, none of the histone methyltransferases and demethylases listed in TABLE 1 has been shown to function on non-histone substrates; however, this remains a formal possibility. Bearing in mind these potential caveats, in the following sections, we use mutations affecting H3K4me3-reading PHD finger readers, and mutations affecting chemical modification of H3K4me3 and H3K27me3, as instructive examples to discuss recent evidence that links the miswriting, misinterpretation and mis-erasing of the histone code to oncogenesis.

Histone methylation miswritten in cancer

The establishment of an appropriate pattern of histone methylation is not only crucial for normal development and differentiation, but is also intimately associated with tumour initiation and development (TABLE 1).

Epigenetic
Refers to the study of mechanisms underlying inheritable phenotypic variations caused by DNA sequence-independent alterations. This term has been more loosely used to describe alterations caused by a change of chromatin structure, which often have unclear heritability.

DNA methylation
A type of chemical modification of DNA that involves the addition of a methyl group to the number 5 carbon of the cytosine pyrimidine ring.

Histone methylation
A chemical modification involving the addition of one, two or three methyl groups on the lysine or arginine residues in a histone protein.

General transcription machinery
A large protein complex including RNA polymerase II and general transcription factors such as TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH.

V(D)J recombinase
An enzyme that carries out V(D)J recombination, a specialized DNA rearrangement that randomly selects and assembles the Variable (V), Diversity (D) and Joining (J) gene fragments of immunoglobulin (Ig)- or T cell receptor (TCR)-encoding loci, thus generating a repertoire of Ig or TCR molecules in lymphocytes and a diverse immune response.

Gene rearrangement
Alteration of chromosomes such as a chromosomal translocation that causes changes in gene structural composition or organization. It can occur as a normal developmental event such as during V(D)J recombination, but is more commonly found in cancer cells as chromosomal abnormalities.

Since the discovery of the first transcription-related histone-modifying enzyme, GCN5 (a histone acetyltransferase (HAT)), in 1996 (REF. 26), many new enzymatic activities have been discovered. Some of these genes already have well-established roles in oncogenesis. Conversely, recent genomic studies have identified new recurrent mutations in some of these enzymes in human cancer, implicating a potential causal role in oncogenesis.

MLL gene rearrangement in leukaemia. The *MLL* (also known as *ALL-1* and *KMT2A*) gene was initially identified through its involvement in recurrent translocations of chromosomal band 11q23 in human myeloid and lymphoid leukaemias[27], and was later shown to encode a major H3K4-specific HMT enzyme[28,29]. MLL forms a large macromolecular nuclear complex with the core complex components (WDR5, RBBP5 and ASL2) and induces H3K4me3 for efficient transcription[5,28,30] (FIG. 1a). Other MLL-associated factors, menin (also known as MEN1) and LEDGF, tether the MLL complex to appropriate targets[31] (FIG. 1a). *MLL* gene rearrangement is one of the most common chromosomal abnormalities in human leukaemia, accounting for around 80% of infant leukaemia and 5–10% of adult acute myeloid leukaemia (AML) or lymphoid leukaemia[27,32].

Box 1 | **The histone code hypothesis**

The histone code hypothesis, initially proposed by Allis and colleagues[1,2], refers to an epigenetic marking system using different combinations of histone modification patterns to regulate specific and distinct functional outputs of eukaryotic genomes.

The histone code hypothesis in gene regulation and development

The histone code hypothesis proposes several layers of regulation in the interpretation of the genome. First, the establishment of homeostasis of a combinatorial pattern of histone modification — the histone code — in a given cellular and developmental context is brought about by a series of 'writing' and 'erasing' events carried out by histone-modifying enzymes. Here, the writer of histone modification refers to an enzyme (for example, a histone methyltranferase) that catalyses a chemical modification of histones in a residue-specific manner, and the eraser of histone modification refers to an enzyme (for example, a histone demethylase) that removes a chemical modification from histones[1,2,5]. Second, the specific interpretation or the 'reading' of the histone code; this is accomplished by reader or 'effector' proteins that specifically bind to a certain type or a combination of histone modification and translate the histone code into a meaningful biological outcome, whether transcriptional activation, silencing or other cellular responses[1,2,5]. In addition to such a recruitment or *trans* mechanism, the manifestation of histone modification can be also achieved by direct physical modulation of chromatin structure or alteration of intra-nucleosomal and inter-nucleosomal contacts through steric or charge interaction (for example, neutralization of the positive charges of histones by acetylation of lysines)[1–3]. All these regulatory mechanisms function broadly to set up an epigenetic landscape that determines cell fate decision-making during embryogenesis and development[9], and fine-tunes gene transcriptional output at a few gene loci during DNA damage repair[14] or other DNA-templated contexts.

The histone code hypothesis extended to oncogenesis

In the contexts of tumorigenesis and cancer epigenetics, we further propose that alteration in the homeostasis between epigenetically regulated gene-on versus gene-off chromatin states leads to inappropriate expression or silencing of transcriptional programmes that consequently alter the states of cellular identity. In certain developmental cell lineages, these alterations lead to undesirable outcomes: proliferation versus senescence and/or differentiation during tumorigenesis.

The partial tandem duplication of *MLL* (*MLL-PTD*), the most frequent form of MLL rearrangement in AML[32,33], contains an in-frame duplication of *MLL* exon 4 to 11 (or exon 4 to 12) and retains the H3K4 HMT activity[27] (FIG. 1a). Dorrance *et al.*[34] have recently generated an *MLL-PTD* knock-in mouse model and found that MLL-PTD causes aberrant elevation of H3K4 dimethylation and histone acetylation of the HOXA gene cluster[34,35] (FIG. 1a). Overexpression of Hox genes promotes leukaemia induction[36]. Normally, the expression of HOXA genes such as *Hoxa9* is developmentally restricted: they are highly expressed in early haematopoietic precursors and silenced following differentiation[37] (FIG. 1a). In the *MLL-PTD* knock-in mice, increased histone methylation and acetylation correlates with a significant increase in the colony formation potentials of erythroid, myeloid and pluripotent haematopoietic progenitors *in vitro*, as well as a drastic increase in *Hoxa9* expression among terminally differentiated blood cells (increased by ~100–250-fold) and unsorted haematopoietic tissues (by ~4–150-fold)[34,35]. However, these *MLL-PTD* knock-in mice fail to develop frank leukaemia, indicating that additional alterations are required for malignant transformation.

MLL fusions, a second type of *MLL* gene rearrangement, result in the deletion of a large carboxy terminal fragment, which includes the H3K4 HMT domain, and also the acquisition of additional transformation mechanisms provided by MLL fusion partners[27,38] (FIG. 1b,c,d). More than 50 different MLL fusion partners have been identified in leukaemia[27,38]. Although the leukaemogenic mechanisms underlying many rare MLL fusion forms remain poorly understood, recent studies have unveiled a common transformation pathway for the most frequent MLL fusion forms (FIG. 1b,c). Okada *et al.* first reported that the AF10 portion of MLL–AF10 (REF. 39) and CALM–AF10 (REF. 40) fusions directly recruits DOT1L (also known as KMT4), a histone methyltransferase that writes the methylation of histone H3 lysine 79 (H3K79me)[39] (FIG. 1b), a key event in MLL–AF10-driven leukaemogenesis. Similarly, the ENL component of the MLL–ENL fusion also directly associates with DOT1L, and subsequently drives leukaemogenesis[41] (FIG. 1b). Aberrant induction of H3K79me was observed at leukaemia-promoting oncogenes (such as *Hoxa9* (FIG. 1b)) in leukaemia cells transformed by the most common MLL fusion forms, including MLL–AF10 (REF. 39), MLL–ENL[41,42], MLL–AF4 (REFS 43,44) and MLL–AF9 (REF. 45). Mutations of MLL–ENL[41] or CALM–AF10[40] that disrupt the interaction with DOT1L abolish leukaemic transformation. DOT1L and H3K79me are associated with active transcription[46], especially at MLL fusion target loci[39,43], thus providing a potential mechanism for the aberrant transcriptional activation found in leukaemia. DOT1L and by inference, H3K79me, are also involved in cell cycle progression[47], silencing of telomere-proximal genes[48] and regulation of Wnt-target genes[49]. Recent biochemical studies have further revealed that DOT1L associates with many factors that are known MLL fusion partners, including AF10 (REFS 39,49–51), ENL[41,49,51], AF9 (REFS 49–52), AF17 (REF. 49), AF4 (also known as MLLT2 and AFF1)[41,49,50], AF5q31 (also known as

Table 1 | **Deregulation of H3K4me3 and H3K27me3 is associated with cancer development**

Category	Gene ID	Deregulation in human cancer	Refs
H3K4me			
Writer	*MLL*	Rearrangement of MLL commonly found in myeloid and lymphoblastic leukaemia	27
	MLL2	Somatic mutation of MLL2 found in renal cell carcinoma	99
Reader	*ING1*, *ING2*, *ING3*, *ING4* and *ING5*	Loss-of-function mutations of putative tumour suppressor genes *ING1–5*, including somatic mutation, allelic loss, downregulation of expression and aberrant cytoplasmic sequestration, associate with various solid tumors. A subset of *ING2* somatic mutations interferes with binding to H3K4me3 specifically	71–73
	PHF23	Owing to chromosomal translocation, the H3K4me3-binding PHD finger of PHF23 is fused to NUP98 in myeloid leukaemia. It has been shown that H3K4me3 binding is crucial for leukaemogenesis induced by NUP98–PHF23 oncoproteins	22
	PYGO2	PYGO2, a component of the β-catenin signalling pathway, is crucial for self-renewal of mammary progenitor cells. Pygo levels are high in malignant breast tumours and low in non-malignant breast cells	90,91
Eraser	*LSD1*	LSD1, a component of NuRD–Mi-2 repressive complexes, demethylates H3K4me2/1 and suppresses the invasiveness and metastasis of breast cancer cells. LSD1 is downregulated in breast carcinoma tissues	94
	JARID1A	Similar to PHF23, the PHD finger of JARID1A is fused to NUP98 in a subset of myeloid leukaemia, forming an oncoprotein NUP98–JARID1A. H3K4me3 binding by the JARID1A PHD finger is crucial for leukaemogenesis	22
	JARID1B	Overexpression of JARID1B was found in advanced breast and prostate cancers	96,98
	JARID1C	Recurrent inactivating mutation of *JARID1C* was detected in around 3% of renal carcinoma	99
	*JHDM1B**	Upregulation of *JHDM1B* or a related gene *JHDM1A* is commonly found in retrovirus-induced rat T cell lymphomas	101–103
H3K27me			
Writer	*EZH2*	Overexpression of EZH2 is frequently found in various solid tumours, including prostate, breast, colon, skin, and lung cancers. Recurrent inactivating mutations and haploinsufficiency of *EZH2* are detected in around 10% of follicular lymphoma and 20% of diffuse large B cell lymphoma of germinal centre origin	56,65
Eraser	*JMJD3*	Downregulation of JMJD3 was found in lung and liver cancers	105,106
	UTX	Sporadic inactivating mutations of *UTX* were reported in a subset of multiple myeloma, oesophageal squamous cell carcinomas, renal cell carcinomas and other tumours	104

EZH2, enhancer of zeste homolog 2; ING, inhibitor of growth; JARID1, jumonji AT-rich interactive domain 1; *JHDM1B*, jumonji C domain-containing histone demethylase 1B; *JMJD3*, jumonji domain containing 3; MLL, mixed lineage leukaemia; PHD, plant homeodomain; *PHF23*, PHD finger protein 23; Pygo, pygopus; *UTX*, ubiquitously transcribed tetratricopeptide repeat X chromosome.* JHDM1 factors exhibit dual demethylating activities towards H3K4me3 and H3K36me2 (REFS 101–103).

MCEF and AFF4)[41] and LAF4 (REF. 41). AF10, AF17 and ENL (or AF9) were identified as stable components of DOT1L-containing complexes[49]. One intriguing model is that DOT1L is responsible for aberrant transcription in many MLL fusion-induced leukaemias; however, this is complicated by the fact that DOT1L complexes are also linked to transcription elongation. Through a protein–protein interaction network, DOT1L–AF10–ENL complexes further associate with a transcription elongation-promoting complex that contains AF5q31, AF4, ELL1, ELL2 and ELL3 (also known MLL fusion partners), and the Pol II transcription elongation factor b (P-TEFb) kinase (which consists of CDK9, cyclin T1 and cyclin T2)[41,53] (FIG. 1b,c). ENL and AF5q31 are shared components of these two complexes[41,49,51,53]. In addition, two recent studies further demonstrate that MLL fusions involving components in this elongation complex — such as MLL–AF4, MLL–ENL, MLL–AF9 and MLL–ELL1 — all interact with AF5q31 and recruit P-TEFb transcription elongation complexes to promote the transcription of downstream targets such as Hox[53,54] (FIG. 1c). Therefore, mechanisms underlying aberrant transactivation in MLL leukaemia have been linked to H3K79me and also transcription elongation.

Although the activities of the P-TEFb complexes during transcription are well established, the role of H3K79 methylation in transcription is less well understood. Is H3K79me as important as P-TEFb, or does DOT1L merely bridge MLL fusions (such as MLL–ENL and MLL–AF10) to P-TEFb elongation complexes (FIG. 1b)? Several lines of evidence suggest that H3K79me is crucial in leukaemia induction. First, replacing the AF10 fragment of MLL–AF10 with the wild-type, but not the catalytically inactive, form of DOT1L led to

Transcription elongation
Efficient transcription and productive RNA processing after RNA polymerase II (Pol II) and associated factors have escaped from abortive initiation, a phenomenon in which Pol II and transcription are halted at promoter-proximal pause sites.

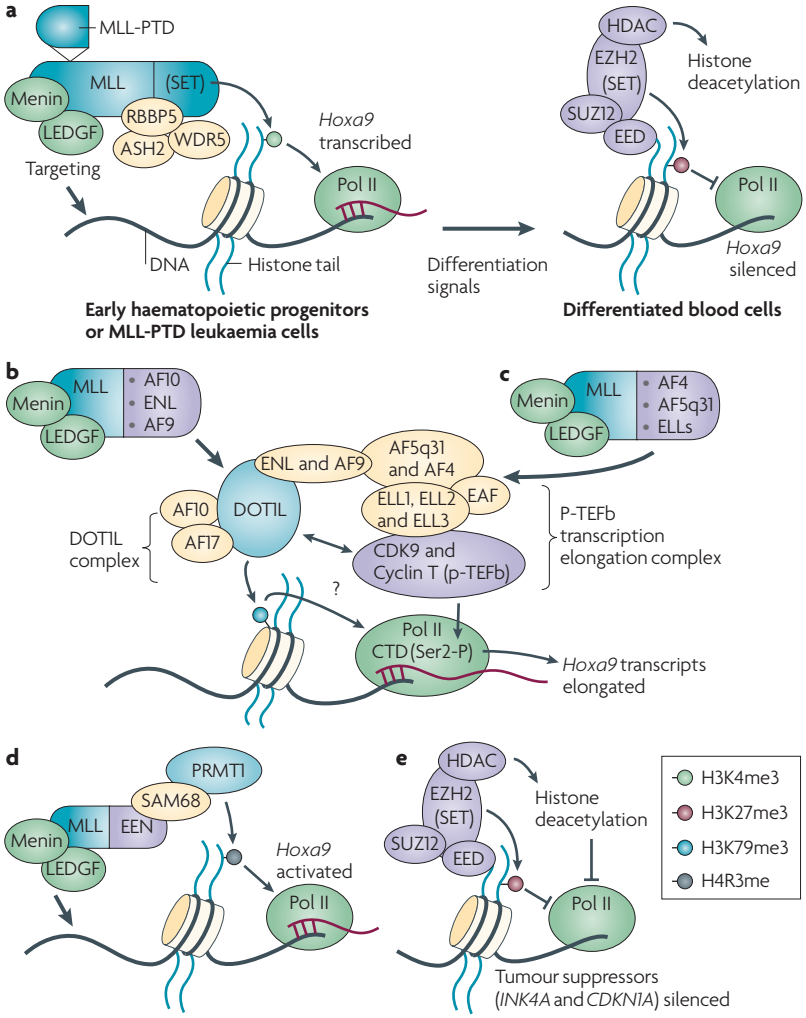

Figure 1| **Miswriting of histone methylation is associated with cancer initiation and/or progression.** Mixed lineage leukaemia (MLL)-containing complexes induce H3K4 trimethylation (H3K4me3) at Hox genes in early haematopoietic progenitor cells. After differentiation, a transition of chromatin state occurs at Hox, inducing its stable silencing. MLL-PTD, an MLL rearrangement form, causes increased H3K4 methylation and transcription of leukaemia-associated oncogenes, including *Hoxa9* (REF. 35). For simplicity, only four of the eight histone tails are shown in each nucleosome (part **a**). MLL fusion proteins, commonly found in leukaemia, lose a large carboxy-terminal portion that includes the H3K4me3-'writing' methyltransferase SET domain, retain the chromatin-targeting property and also acquire aberrant transactivation mechanisms through MLL fusion partners. A subset of MLL fusions, MLL–AF10, MLL–ENL and MLL–AF9, directly interact with DOT1L through the MLL fusion partner and induce the methylation of H3K79 at *Hoxa9* (part **b**). Some other MLL fusions, MLL–AF4, MLL–AF5q31 and MLL–ELL, interact with and recruit the P-TEFb transcription elongation complexes to *Hoxa9* (part **c**). DOT1L complexes (DOT1L–AF10–AF17–ENL (or AF9)) associate with P-TEFb complexes through the shared component ENL. Another MLL fusion partner, EEN, recruits PRMT1 and induces the methylation of H4R3 at Hox (part **d**). Hyperphosphorylation (Ser2) of the C-terminal domain (CTD) of RNA polymerase II (RNA Pol II) CTD by the P-TEFb complex H3K79me3/2 and H4R3me are associated with either transcriptional elongation or initiation. Overexpression of enhancer of zeste homolog 2 (EZH2) in tumour cells induces the silencing of the tumour suppressor genes such as *INK4A* and *CDKN1B*. EZH2-mediated repression relies on its intrinsic H3K27me3/2 writing activities and also histone deacetylation induced by associated histone deacetylases (part **e**).

leukaemic transformation[39]. Second, RNA interference (RNAi)-mediated inhibition of DOT1L significantly impaired MLL–AF4-induced transformation and the activation of Hox genes[43], despite intact association of MLL–AF4 with AF5q31 and the P-TEFb elongation complexes[53]. In addition, DOT1L directly interacts with P-TEFb[54]. Further investigations will be needed to examine the role of H3K79me during transcriptional activation and elongation in leukaemogenesis.

Another MLL fusion, MLL–EEN, recruits histone arginine methyltransferase PRMT1, and its methyltransferase activity towards histone H4 arginine 3 has been shown to be important for leukaemia transformation[55] (this also occurs through *Hoxa9* activation (FIG. 1d)). Taken together, miswriting of histone methylation marks often correlates with the aberrant transcription of oncogenes in patients with leukaemia harbouring *MLL* gene rearrangements.

EZH2 overexpression and mutation in cancers. Enhancer of zeste homolog 2 (EZH2), an H3K27-specific methyltransferase, provides another connection between miswriting histone methylation marks and oncogenesis. EZH2 is frequently found overexpressed in various solid tumours, including prostate, breast, colon, skin and lung cancer[56,57] (TABLE 1). Suppression of EZH2 by RNAi significantly decreased tumour growth in breast and prostate tumour xenograft models[58,59]. Furthermore, overexpression of EZH2 confers invasiveness to fibroblasts and immortalized benign mammary epithelial cells, and this effect is dependent on the H3K27 HMT activity of EZH2 (REFS 59–61). Mechanistically, the oncogenic function of EZH2 has been attributed to the silencing of tumour suppressor genes, including *INK4B–ARF–INK4A*[57] (FIG. 1e), E-cadherin[60,62], *p57*^{KIP2} (also known as *CDKN1C*[63]), *p27* (REF. 64), *BRCA1* (REF. 58) and adrenergic receptor β2 (REF. 59). By contrast, recurrent inactivating mutations of *EZH2* have been discovered in a recent oncogenomic study of follicular lymphoma (7.2%) and diffuse large B cell lymphoma (21.7% of the germinal centre B cell subtype)[65] (TABLE 1). The identified *EZH2* mutations specifically target a single tyrosine residue that is required for EZH2-mediated HMT activities towards H3K27me3 (REF. 65). It is tempting to speculate that the homeostasis of H3K27me3 is crucial in defining cellular context, and its perturbation through EZH2 deregulation might provide the optimal context for the development of distinct types of cancer; for example, differences in H3K27me3 homeostasis may underlie some differences in prostate cancer and follicular lymphoma. The precise

roles of EZH2 overexpression and loss-of-function mutations in distinct cancer types remain to be more rigorously validated in genetically engineered animal models. Ideally, genomic mislocalization of EZH2 and its effect on H3K27me3 and transcription need to be examined in genetically matched normal samples compared with tumour samples. Nevertheless, EZH2 inhibition has been proposed as a therapeutic strategy to inhibit tumour growth[56]. Indeed, early success for the combined use of the EZH2 inhibitor and histone deacetylase (HDAC) inhibitors has been observed in leukaemia xenograft mouse models[66]. Recent comprehensive reviews[56,57] provide further details on EZH2 in oncogenesis.

Histone methylation misread in cancer

Aberrant fusion of PHD finger motifs and misinterpretation of H3K4me3 in leukaemia. Chromosomal translocation of nucleoporin 98 (*NUP98*), a nuclear pore complex component gene, is one of the most promiscuous gene rearrangements found in various forms of haematopoietic malignancies[67]. In a subset of patients with AML, NUP98 translocation results in the fusion of the amino terminus of NUP98 to the C-terminal PHD finger motif (and also nuclear localization signals) of PHF23 or JARID1A[22,67] (FIG. 2c). Recently, leukaemia induced by NUP98–JARID1A and NUP98–PHF23 fusions has been experimentally recapitulated using *in vitro* and *in vivo* mouse models[22]. The leukaemogenic potential of these two fusion proteins relies on the ability of the PHD finger motif to recognize H3K4me3/2 (REF. 22) (BOX 2). A single point mutation in the PHD finger that abrogates H3K4me3 binding also abolishes leukaemic transformation; and the PHD finger can be functionally replaced by other H3K4me3-binding PHD fingers (even one from yeast), but not by those that do not recognize H3K4me3 (REF. 22). Mechanistically, binding of H3K4me3 by the NUP98–PHD finger fusion interferes with the normal differentiation of haematopoietic stem and progenitor cells by preventing the removal of H3K4me3 and inhibiting EZH2-mediated H3K27me3 at developmentally crucial genes that encode transcription factors, such as Hox, *Meis1a*, *Gata3* and *Pbx1* (REFS 22,68). As a result, the master regulator loci of haematopoiesis are locked in an active chromatin state that is characterized by high levels of H3K4me3 and histone acetylation; consequently, the expression of these genes is maintained[22]. Overexpression and activating mutations of these transcription factors are commonly found in human leukaemia and are sufficient

Box 2 | **The plant homeodomain (PHD) finger**

The PHD finger is a zinc finger-like domain, with a signature motif of Cys4-His-Cys3 to coordinate two zinc ions[120]. The folding of this ~60 amino acid-long domain is featured by an interleaved topology of zinc ion-coordinating residues and a couple of anti-parallel β-sheet secondary structures[5,120]. The definition of PHD fingers originates from conserved plant homeodomain proteins, and the classification and distinction of PHD fingers and other similar motifs such as the RING finger are somewhat ambiguous[120]. There are fewer than 20 typical and atypical PHD finger motifs in *Saccharomyces cerevisiae*, around 50 in *Drosophila melanogaster* and up to a couple of hundred in mammals[18,120,121]. Most PHD fingers are found in chromatin-associated factors or nuclear proteins[120,121].

The PHD finger ligand

PHD fingers exhibit diversity and versatility in their interaction partners. Some bind to chromatin modifications, such as highly methylated H3K4 (REF. 5), unmodified H3K4 (REF. 18) and methylated H3K36 (REF. 121), and some serve as a SUMO E3 ligase to interact with the E2-conjugating enzyme[122]. The binding partners and functions of others are still unknown.

The structure of H3K4me3/2-binding PHD fingers

Recent structural analyses of several H3K4me3/2-binding PHD fingers have revealed some commonalities that underlie the specific recognition and binding of H3K4me3, which include a specialized pocket or cleft structure formed by 2–4 aromatic and/or hydrophobic residues to accommodate the H3K4me3 side chain, anti-parallel β-sheet pairing between the histone H3 backbone and a β-sheet of the PHD motif and, in many cases, positioning of H3 arginine 2 (H3R2) in an acidic pocket[5,15–17,20–22]. The structures of H3K4me3-binding PHD fingers from two cancer-associated factors, ING2 and JARID1A, are shown in the figure part **a** and part **b**, respectively (H3 and the H3K4me3 side chain shown in green, PHD finger in lilac, zinc ion in cyan sphere and hydrophobic 'pocket' highlighted in pink; arrows represent β-sheets). With a dissociation constant (K_d) ranging from less than one to several μM, the binding of H3K4me3 by PHD fingers is one of the strongest associations between histone modification and its reading factors[5,15–17,20–22]. The structural illustrations shown are produced using published structural coordinates that have Protein Data Bank IDs of 2G6Q, 3GL6, 2KGG and 2KGI[16,22].

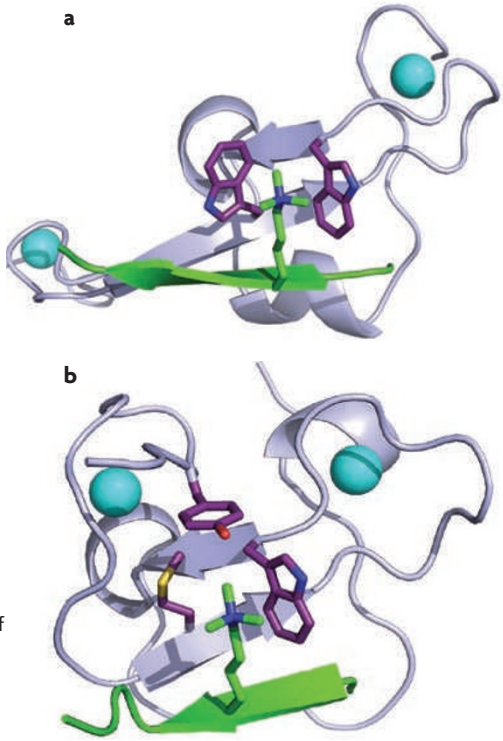

Table 2 | **List of PHD finger-containing proteins that specifically 'read' H3K4me3/2**

ID	H3K4me3/2 reading motif	Known function and disease relevance	Refs
BPTF	The second PHD finger	Component of a chromatin remodelling complex NURF, which contains a SWI/SNF family helicase and SMARCA1	13,15
ING1	PHD finger	Component of HDAC–Sin3A transcriptional repressive complexes	14,72,75
ING2	PHD finger	Component of HDAC–Sin3A transcriptional repressive complexes	14,72,78
ING3	PHD finger	Forms a transcriptional activation complex with a histone acetyltransferase (HAT) Tip60	72,78
ING4	PHD finger	Forms an HBO-containing transcriptional activation complex	72,76–78
ING5	PHD finger	Component of a transcriptional activation complex that contains a HAT protein, either HBO or MOZ/MORF family	78
JARID1A	The third PHD finger	H3K4me3/2-specific histone demethylase	22
JARID1B	The third PHD finger	H3K4me3/2-specific histone demethylase	19,22*
MLL	The third PHD finger	Histone methyltransferase, specific for H3K4	5*
PHF2	PHD finger	Putative histone demethylase	5*
PHF8	PHD finger	Putative histone demethylase; *PHF8* mutation associates with X-linked mental retardation	5,19*
PHF13	PHD finger	Unknown function	5,19*
PHF23	PHD finger	Unknown function	22
Pygo	PHD finger	PYGO1 and PYGO2 interact with a cofactor BCL9, and are required for Wnt–β-catenin-induced transcriptional activation	89–91
RAG2	PHD finger	A V(D)J recombinase crucial for the development and maturation of B and T cells. Loss-of-function mutations of the RAG2 PHD finger lead to severe combined immunodeficiency and Omenn syndrome	21
TAF3	PHD finger	Component of RNA polymerase II-associated general transcription factor machinery TFIID, which contains TATA-binding protein (TBP) and 12–13 additional TBP-associated factors, TAF1–14	19,20

BPTF, bromodomain PHD finger transcription factor; PHD, plant homeodomain; PHF, PHD finger protein; RAG2, recombination activating gene 2; TAF3, TATA box binding protein (TBP)-associated factor, 140 kDa. * The H3K4me3-binding property was predicted based on domain homology[5].

to block haematopoietic differentiation and induce leukaemia[36,69]. Therefore, the perturbation of histone modification dynamics associated with haematopoiesis, as in the case of NUP98–PHD finger fusion, causes the enforced expression of vital developmental genes and interferes with appropriate transition of cellular states — a crucial step in leukaemia initiation[22].

In many cases, acute leukaemia is a disease of mis-regulated differentiation in haematopoiesis. The key to understanding how NUP98 or MLL translocations interfere with chromatin dynamics in leukaemogenesis is to first understand the mechanisms underlying the chromatin landscape changes during normal haematopoiesis. Conceivably, NUP98–PHD finger fusions might mimic some endogenous chromatin-associated machinery, functioning as a chromatin boundary factor that prevents the intrusion of transcriptional repressive complexes[22]. In this case, NUP98–PHD finger fusions could have a dominant-negative effect on JARID1, blocking the erasure of H3K4me3 (REF. 22); acetylation of H3K27 that is induced by NUP98-associated HATs can also antagonize the methylation of the same lysine by EZH2 complexes[22,70] (FIGS 2c,3b). Gain-of-function mutations in the PHD finger motif — for example, NUP98–PHD finger fusions in leukaemia[22] — and loss-of-function mutations in the PHD finger, as mentioned earlier for RAG2 and immunodeficiency syndromes[21], unveil pathologies underscored by a failure to appropriately interpret histone modification[18].

Somatic mutation of ING PHD fingers in solid tumours. Another family of PHD finger-containing proteins, inhibitor of growth (ING), are putative tumour suppressors. Loss-of-function mutations of INGs (especially *ING1*, *ING3* and *ING4*) by somatic mutation, allelic loss, reduced gene expression and aberrant cytoplasmic sequestration have been reported in various solid tumours[71,72] (TABLE 1). INGs regulate many cellular processes that are associated with tumorigenesis, including cell cycle progression, senescence, apoptosis, DNA repair, cell migration and contact inhibition[71–74]. All INGs have a C-terminal PHD finger that specifically binds H3K4me3 (REFS 14,75–77) (BOX 2). Despite this common feature, different INGs are incorporated into protein complexes with distinct properties in transcriptional regulation (TABLE 2). ING1 and ING2 recruit the mSin3–HDAC transcriptional repressors (FIG. 2d), whereas ING3, ING4 and ING5 recruit HATs to induce gene activation[14,78]. ING3 and ING4 complexes contain a HAT, either TIP60 or HBO, respectively, and ING5 complexes include either HBO or a MOZ/MORF family member as the HAT[76,78]. INGs (ING1, ING4 and ING5) also interact with p53

Chromatin dynamics
A fine-tuning mechanism that introduces meaningful variations to chromatin and/or modulate chromatin structure, which includes DNA methylation, covalent histone modification, ATP-dependent chromatin remodelling and the use of histone variants.

Chromatin boundary factor
A factor that interacts with chromatin boundary elements to ensure the appropriate physical separation of chromatin regions that have distinct properties such as regions of active versus silenced transcription. One example is CTCF, a protein that binds to insulator *cis*-elements.

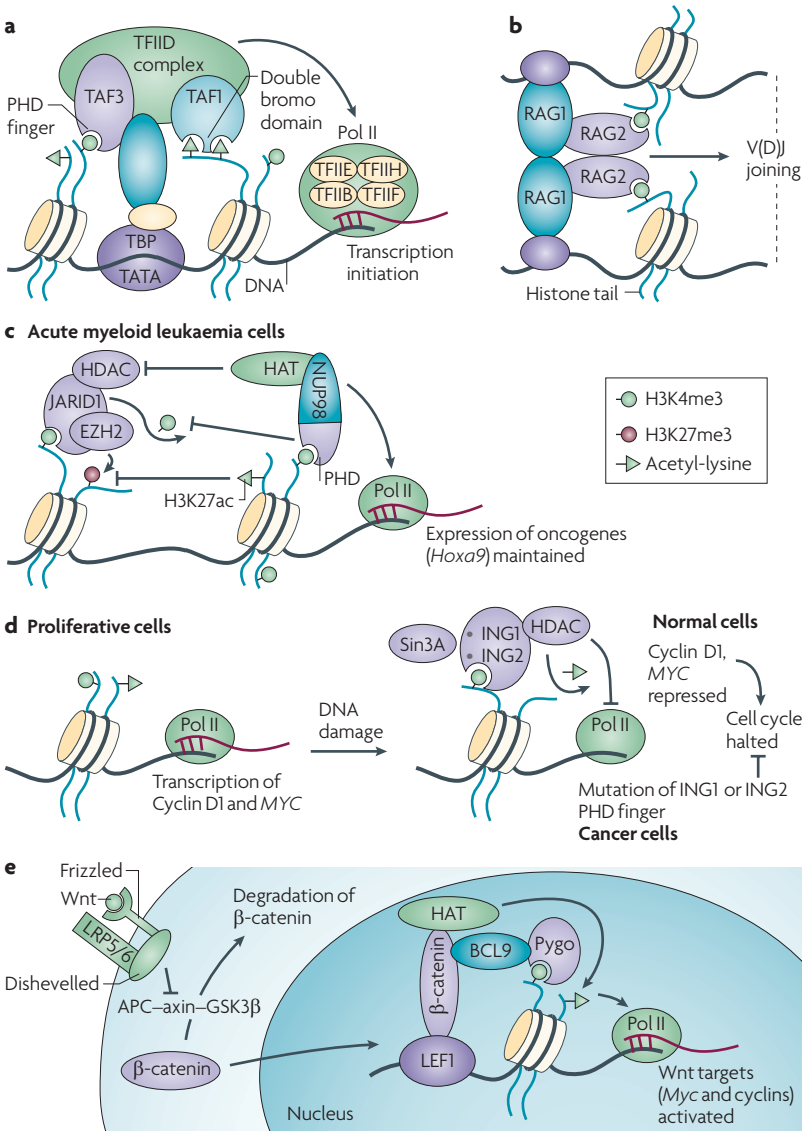

Figure 2 | **Reading or mis-reading the H3K4me3 marks by PHD finger-containing factors in normal cellular processes and during cancer development. a** | Interactions with histone modifications (H3K4 trimethylation (H3K4me3) recognized by the TAF3 plant homeodomain (PHD) finger[19,20] and histone acetylation by the double bromodomain of TAF1 (REF. 17) and binding to specific DNA elements (TATA-binding protein (TBP) to the TATA box) anchor and/or stabilize the TFIID complex to core promoters[19,20]. **b** | Both the recognition of H3K4me3 by the RAG2 PHD finger and binding of RAG1–RAG2 complexes to recombination signal sequences are crucial for recruiting and/or stabilizing the RAG1–RAG2 complex to V(D)J gene segments during B and T cell development[18,21]. **c** | In acute myeloid leukaemia, chromosomal translocation nucleoporin 98 (NUP98)–jumonji AT-rich interactive domain 1A (JARID1A) or NUP98–PHF23 fuses the amino terminal part of a nucleoporin protein, NUP98, to an H3K4me3-binding PHD finger of JARID1A or PHF23 (REF. 22). This prevents the removal of H3K4me3 and addition of H3K27me3 thus enforcing the expression of leukaemia-associated oncogenes such as *Hoxa9* and *Meis1* (REF. 22). **d** | On DNA damage, H3K4me3 is a mark to recruit and/or stabilize the inhibitor of growth 1 (ING1) and ING2 repressive complexes to genes responsible for cell proliferation such as *Myc* and cyclins, leading to gene repression and the halt of cell cycle progression. A subset of cancer-associated somatic mutations of *ING1* specifically interferes with the binding to H3K4me3/2 and proper response to DNA damage[14,18,75]. **e** | Recognition of H3K4me3 by the PHD finger of Pygopus (Pygo) has been suggested to be crucial for efficient activation of the Wnt signalling pathway[89]. APC, adenomatous polyposis coli; EZH2, enhancer of zeste homolog 2; GSK3β, glycogen synthase kinase 3β; HDAC, histone deacetylase. LEF1, lymphoid enhancer-binding factor 1 (also known as TCF).

and modulate its activity[71,79–81]. Here, we focus on recent advances that link ING mutations to the misinterpretation of histone methylation in transformation.

ING1 was initially identified in a functional screen as an inhibitor of neoplastic transformation[82], and somatic mutation of *ING1* was later found in breast, gastric and pancreatic cancer, and squamous cell carcinoma[71,72]. Deletion of *Ing1* in mice led to a mild phenotype only, with a slight increase in the incidence of lymphomas, indicating that other mutations may cooperate with *ING1* inactivation in tumorigenesis[79]. A subset of *ING1* somatic mutations found in human tumour samples specifically target its PHD finger motif[18,71–73]. Some hotspot mutations, such as C215S and C253stop (the amino acid number refers to the p33^{ING1b} isoform), target the crucial zinc ion-coordinating cysteines in the PHD finger, causing global misfolding[18]. A recent biophysical study demonstrated that other *ING1* mutations, N216S, V218I and G221V, interfere with either appropriate folding of the structural pocket to accommodate H3K4me3 or the appropriate positioning of the histone H3 tail, leading to a decrease in H3K4me3-binding affinities by 10–40-fold[75]. Despite these advances, animal models that establish a direct causal role of *ING1* mutations in tumorigenesis are still lacking. Nonetheless, at the cellular level, the decreased binding of ING1 to H3K4me3 has been shown to result in inefficient DNA damage repair and apoptosis[75].

ING2, an *ING1*-related member that is also down-regulated in many types of solid tumours[71–73], initiates an acute response to silence proliferative genes, including cyclin D1 and *MYC*, and decelerates the cell cycle after DNA damage[14] (FIG. 2d). This response relies on the ability of ING2 to bind to H3K4me3 marks that are associated with proliferative genes, followed by the recruitment and/or stabilization of the ING2-associated repressors HDAC1 and HDAC2 (REFS 14,78) (FIG. 2d). Eventually, histone deacetylation occurs at proliferative genes, their expression is downregulated, and cell cycle progression is halted[14]. As the H3K4me3 level at these genes remains the same before and after DNA damage[14], it is still poorly understood what causes the recruitment of ING2 to proliferative genes after DNA damage. Recently, the association of ING2 with chromatin has been linked to phosphatidylinositol-5-phosphate (PtdIns(5)P), a lipid ligand of ING2 that was found to accumulate in the nucleus after cellular stress[14,83]. The binding surface for PtdIns(5)P in ING2 is located at the C terminus, which includes a small portion of the PHD finger and a lysine- and arginine-rich polybasic region[84]. This polybasic region is found only in ING1 and ING2 (REFS 71,73). Further investigation is required to dissect how multivalent interactions between INGs, signalling transducers and histone modifications are coordinated to execute an efficient response to DNA damage and cellular stress.

Downregulation, allelic loss and somatic mutation of ING3 and ING4 has also been found in cancer[71,72]. Recognition of H3K4me3 is crucial for ING4 and ING5–HBO complexes to promote genotoxic stress-induced apoptosis and to inhibit anchorage-independent cell growth[76–78]. Association between the ING4 and ING5 PHD finger and H3K4me3 modulates the substrate

Multiple osteochondroma
A skeletal disease characterized by the development of benign cartilage-capped bone tumours growing outwards from the bone surface, resulting in various orthopaedic deformities.

specificity of the HBO complexes, making H3K4me3-containing nucleosomes a preferred substrate[76,77]. Using genome-wide chromatin immunoprecipitation (ChIP)-chip analyses, Hung *et al.*[77] observed that, after DNA damage, the recruitment of ING4–HBO complexes to the downstream targets is enhanced, followed by increased levels of H3 acetylation and transcriptional activation. Confirmed target loci include many tumour suppressor genes such as *PHD2* (also known as *EGLN1* and *HPH2*) and exostosin 1 (*EXT1*)[76,77]. PHD2 is an inhibitor of hypoxia-inducible factor (HIF) and its downregulation results in increased angiogenesis in tumour tissues and the promotion of tumorigenesis[85]. Mutations in *EXT1* and *EXT2* are responsible for multiple osteochondroma, a skeletal disease that is characterized by benign bone tumours[86]. The misregulation of these potential tumour suppressors needs to be further examined in primary tumour samples and animal models harbouring loss of *ING4*.

Notably, both types of ING complexes (ING1–ING2 and ING4–ING5) seem to engage tumour suppressive activities[74]. Despite having opposite effects on transcriptional regulation[73,78], different INGs can apparently target different sets of genes — both oncogenic and tumour suppressor loci[14,77]. However, this targeting specificity of distinct INGs cannot be determined by their PHD fingers, as they all bind to H3K4me3. Nevertheless, reading of H3K4me3 by the PHD finger is a crucial step for efficient chromatin binding and DNA damage- or stress-induced responses[14,75–77]. Many other questions remain unsolved. For example, do different ING-containing complexes cooperate? And how is the interaction of p53 and other DNA-binding factors involved in these cellular responses[81]? It is also important to appreciate how events such as sensing and signalling of DNA damage, assembly and recruitment of ING complexes, chromatin recognition and modulation, the subsequent transcriptional regulation, DNA repair and chromatin restoration post-repair, are integrated.

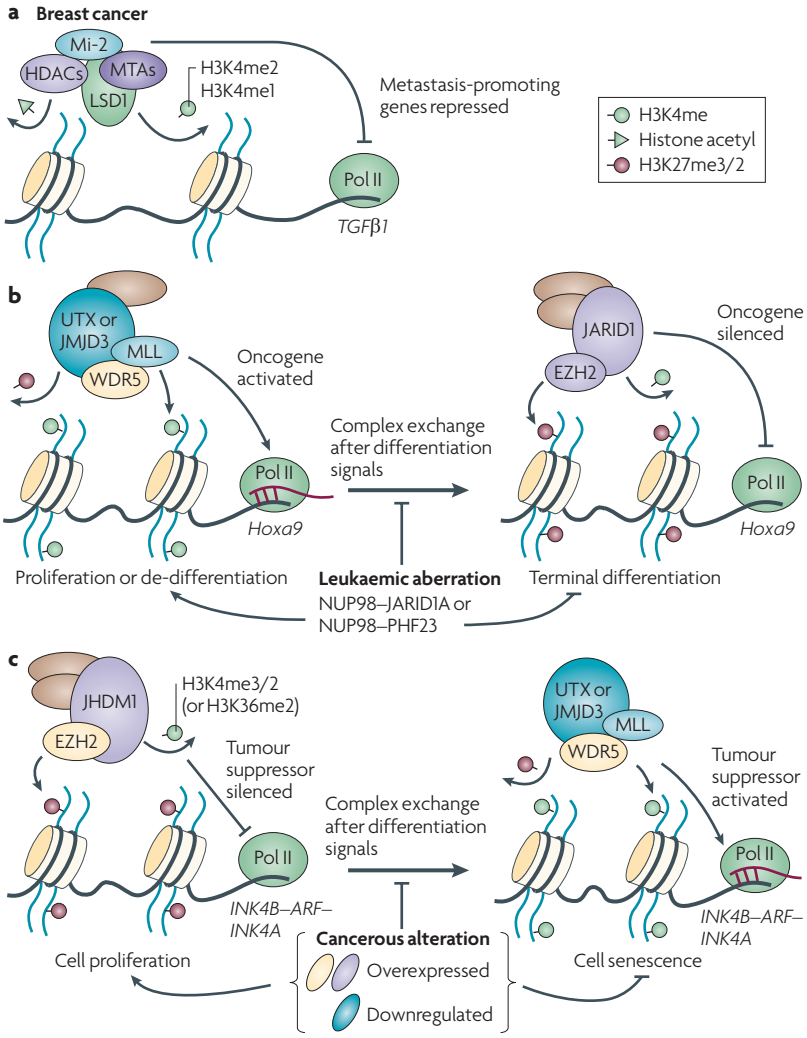

Figure 3 | **Mis-erasing of histone methylation is associated with cancer development. a** | LSD1 is an inhibitor of metastasis in breast cancer. LSD1 removes H3K4 dimethylation or monomethylation (H3K4me2/1) from genes associated with metastasis (such as those involved in transforming growth factor-β (TGFβ) signalling) and represses their expression[94]. **b** | Cooperation between histone methyltransferases and demethylases, exemplified by mixed lineage leukaemia (MLL)–ubiquitously transcribed tetratricopeptide repeat X chromosome (UTX), MLL–JMJD3 interactions and enhancer of zeste homolog 2 (EZH2)–jumonji AT-rich interactive domain 1 (JARID1) interactions, underlies changes in H3K4me3 and H3K27me3 at oncogenes such as *Hoxa9*, a process that is perturbed by leukaemia oncoproteins, such as NUP98–JARID1A (FIG. 2c) or MLL fusions. **c** | On RAS signalling-induced stress, a complex switch in histone methyltransferases and demethylases underlies activation of the tumour suppressor locus *INK4B–ARF–INK4A* and the subsequent induction of senescence[101–103,105,106,123]. In cancer cells, overexpression of EZH2 and JHDM1, or downregulation of JMJD3 interferes with such a switch in the chromatin state and therefore the appropriate senescence response[105,106,124]. HDAC, histone deacetylase; MTAs, metastasis-associated proteins; Pol II, RNA polymerase II.

Pygopus, a factor that links Wnt–β-catenin signalling to H3K4 methylation. Mutations in components of the Wnt–β-catenin pathway lead to oncogenesis in several tissue types[87]. Pygopus (Pygo) has recently been identified as a crucial factor for efficient Wnt–β-catenin signalling[87,88]. Pygo interacts with BCL9, an adaptor protein that directly associates with β-catenin[89] (FIG. 2e). The C terminus of all Pygo homologues (pygopus in *Drosophila melanogaster*, and PYGO1 and PYGO2 in mammals) contains a PHD finger, which uses two surfaces to interact with BCL9 and H3K4me2/3 simultaneously (FIG. 2e); the binding of H3K4me2/3 by Pygo is enhanced by its association with BCL9 (REF. 89). *Pygo2* was found highly expressed in mammary progenitor cells and upregulated in breast cancer cells, and the H3K4me2/3-binding property of PYGO2 seems to be crucial for the cell growth of breast cancer cells[90,91]. However, a separate study indicates that the abolition of interaction between H3K4me2/3 and pygopus does not interfere with the activation of Wnt signalling in *D. melanogaster*[89,92]. Further investigation of cross-talk among pygopus, Wnt–β-catenin signalling and histone modifications needs to be carried out to address these differences.

Histone methylation is mis-erased in cancer

Histone lysine demethylases (HDMs), especially those that remove methylation on H3K4 and H3K27, are found mutated or deregulated in human cancer (TABLE 1).

Misregulation of H3K4-specific demethylases in cancers. LSD1 is the first histone demethylase (HDM) that was isolated by Shi and colleagues, and this nuclear amine oxidase can convert dimethyl or monomethyl H3K4 to the unmodified state[93]. LSD1 was purified as a stable component of several HDAC-containing transcriptional repressor complexes[93]. Recently, one such repressive complex, LSD1–Mi-2–NuRD–HDACs (FIG. 3a), has been shown to inhibit metastasis in breast cancer[94]. LSD1 removes H3K4me2/1 from several downstream targets that include the pathways involving transforming growth factor-β (TGFβ) signalling, cell growth, and migration and invasion[94]. As a result, the expression of these targets is repressed in breast cancer cells[94]. TGFβ1, a key regulator of epithelial-to-mesenchymal transitions and tumour metastasis, is a crucial downstream effector that is inhibited by LSD1–Mi-2–NuRD complexes[94]. Knocking down LSD1 increases the invasive and metastatic potential of breast cancer cells, whereas overexpression of LSD1 suppresses the invasiveness of breast cancer cells[94]. *LSD1* was found downregulated in breast carcinoma tissues[94]. In contrast to its canonical functions, LSD1 also interacts with androgen receptor (AR). This LSD1–AR complex erases methylation of H3K9, a repressive marker, thus leading to the activation of AR signalling in prostate cancer cells[95]. In addition, LSD1 also targets non-histone substrates such as p53. Lysine-specific demethylation of p53 by LSD1 represses p53-induced transcriptional activation and apoptosis[23]. These observations show that LSD1 has both tumour suppressive and oncogenic functions, and these activities are dependent on cellular contexts and substrate differences.

The understanding of histone demethylation quickly proceeded with the identification of a larger and more versatile family of HDMs — the jumonji family of lysine demethylases[6]. The jumonji family of HDMs differ from LSD1 in that these hydroxylases can remove lysine trimethylation[6]. *JARID1A*, a member of H3K4me3/2-specific jumonji demethylases, was found translocated in myeloid leukaemia (FIG. 2c). *JARID1B* (also known as *PLU-1* and *KDM5B*), a related H3K4me3/2 HDM gene, was found overexpressed in advanced stages of breast and prostate cancer[96,97]. JARID1B facilitates the G1/S transition in the cell cycle and attenuates the mitotic spindle checkpoint of cancer cells[96,98]. Using a syngeneic tumour transplantation model, Yamane *et al.*[96] showed that *JARID1B* overexpression promotes the growth of mammary carcinoma. JARID1B represses metallothionein genes and several known tumour suppressor genes (*BRCA1* and caveolin 1) by inducing the erasure of H3K4me3/2 (REFS 96,98). In a recent large-scale next-generation sequencing of primary renal cell carcinoma (RCC) genomes, Dalgliesh *et al.*[99] discovered several recurrent mutations that inactivate histone-modifying enzymes, including truncating mutations of *JARID1C* (~3% of all RCC samples) and *SETD2* (~3%), which is a histone H3 lysine 36-specific methyltransferase gene[99]. Inactivation of *JARID1C*, the third member of the H3K4me3/2-specific HDM genes, is correlated with the transcriptional alteration of a specific gene signature in RCC tumour samples[99]. Most RCCs harbouring *JARID1C* mutation also contain a mutation at von Hippel–Lindau (*VHL*), a negative regulator of HIF, suggesting that the *JARID1C* and *VHL* mutations may cooperate in driving the tumorigenesis of RCC[99]. It is curious that both overexpression and loss-of-function mutations of the JARID1 gene family have been suggested to contribute to oncogenesis, although in different cancer types (TABLE 1).

JHDM1B (also known as *FBXL10*, *NDY1* and *KDM2B*) and *JHDM1A* (also known as *FBXL11*, *NDY2* and *KDM2A*) encode another family of histone demethylases that may harbour dual methylation-erasing activities for H3K36me2/1 and H3K4me3 (REFS 100–102). In a screen that was based on retroviral integration-induced T cell lymphomas, Pfau *et al.*[103] found that upregulation of *JHDM1B* is a common event in T cell lymphomas. JHDM1B directly represses the tumour suppressor locus *Ink4b–Arf–Ink4a* by erasing H3K36me2 and/or H3K4me3 (REFS 101–103). Both JHDM1B and the related JHDM1A inhibit replicative senescence and oncogene-induced senescence, which are a crucial barrier of oncogenesis[101–103]. *JHDM1B* is downregulated after senescence induction in normal tissues, and acquired expression of *JHDM1B* in tumours prevents cell senescence, thus facilitating cancerous transformation[101,102] (FIG. 3c).

Misregulation of H3K27-specific demethylases in cancers. Sporadic inactivating mutations of ubiquitously transcribed tetratricopeptide repeat X chromosome (*UTX*), an H3K27me3/2-specific HDM gene, have recently been reported in a subset of multiple myeloma, oesophageal squamous cell carcinoma and renal cell carcinoma[104]. Restoration of *UTX* in *UTX*-mutated cancer cells reduced H3K27me3 at tested targets and slowed cell proliferation[104]. *JMJD3*, a related H3K27me3/2-specific HDM gene, was found upregulated during RAS-induced senescence, and opposite to the action of JHDM1 and EZH2, JMJD3 activates the *Ink4a–Arf* locus[105,106] (FIG. 3c). The expression of *JMJD3* has been found downregulated in various cancers, including lung and liver cancer[105,106]. These observations indicate putative tumour suppressive roles for UTX and JMJD3. Despite emerging evidence that links HDMs to cancer, it remains to be investigated using more rigorous assays whether the observed mutation is causal or merely the consequence of tumorigenesis.

Cooperation of histone modifiers

One complication of classifying writing, reading and erasing histone modifications is that these processes often function in a concerted way. For example, some histone modification writers or erasers, such as MLL or JARID1A, harbour an intrinsic PHD finger module to read H3K4me3 (TABLE 2). Currently, it is unclear how this reading property is involved in the writing and erasing steps of histone methylation. In the context of leukaemia induction, NUP98–JARID1A (FIG. 2c), a translocation form of JARID1A, loses its histone methylation erasing activity and relies on the H3K4me3-reading PHD finger to initiate leukaemogenesis[22]. In addition, histone modifiers often work together on different histone modification sites simultaneously to coordinate a robust response. For instance, UTX is a stable component of the

Syngeneic tumour transplantation model
A model of tumorigenesis produced by inoculating mouse or rat tumour cells into a corresponding immunocompetent mouse or rat. Syngeneic tumour models have a normal host immune response and tumour microenvironment, and therefore more closely resemble a biologically relevant situation than xenograft models that use immunodeficient mice.

Next-generation sequencing
A still-developing technology to sequence DNA in a massively parallel fashion, therefore sequencing is achieved at a much faster speed and lower cost than traditional gel-based methods. This technology makes direct sequencing of large numbers of human patient samples possible, including those from cancers.

MLL2- and MLL3-containing complexes, which erase H3K27me3 and also write H3K4me3 at target chromatin[107]. Consistent with the action of UTX, MLL is recruited to the *Ink4a* locus in oncogene-induced checkpoint responses, and UTX and MLL may cooperate to promote *INK4a* expression and suppress cancerous transformation[108] (FIG. 3c). Similarly, JHDM1B and JARID1A were reported to interact with EZH2 (REFS 102,109), and JMJD3 interacts with MLL[110]. In these scenarios, there is a common theme centred on the epigenetic repression process of the tumour suppressor *INK4B–ARF–INK4A* locus in cancer cells. This features the elimination of active modifications (H3K4me3) and the addition of repressive chromatin modifications (H3K27me3 or DNA methylation), which is accomplished by cooperation between histone demethylase deregulation (JARID1B, JHDM1B, UTX and JMJD3), histone methyltransferases (EZH2 overexpression) and/or DNA hypermethylation to establish a stable silenced state of the INK4B–ARF–INK4A locus (FIG. 3c).

Conclusions and future directions

Histone modification, as exemplified by H3K4me3 and H3K27me3 in this Review, provides a crucial regulatory measure for defining cellular context and governing gene transcription, V(D)J DNA recombination, DNA damage repair and many other DNA-templated processes. Rapidly increasing evidence has indicated that miswriting, misreading, and/or mis-erasing of histone modifications contributes to the initiation and development of human cancers. However, the underlying mechanisms of chromatin regulation in oncogenesis are complex and remain to be delineated in a cellular context-dependent manner.

First, it remains unclear whether many mutations and deregulations of epigenetic players are the 'drivers' or 'passengers' of oncogenesis. More rigorous evidence is generally lacking to establish the causality of deregulation of the writing, reading and erasing of histone modification events in oncogenesis. Generating animal models with ING mutations will be needed for defining their oncogenic roles, and as useful tools for understanding the mechanisms driving oncogenesis. Similar issues can be applied to the inactivating mutations of *JARID1C* found in renal carcinoma[99] or for *EZH2* in germinal centre diffuse large B cell lymphoma[65].

In addition, the regulatory mechanisms through histone modification can be cell type or context specific. To dissect the misregulation of histone modifications and epigenetic imbalances in cancer cells, it is important to understand how normal cells use dynamic chromatin modifications to establish the appropriate epigenetic homeostasis and induce state transitions at crucial loci encoding oncogenes (for example, the HOX gene cluster in haematopoietic lineages (FIG. 3b)) or tumour suppressor genes (for example, the *INK4B–ARF–INK4A* cluster (FIG. 3c)) in normal developmental and cellular contexts. For example, although DOT1L-mediated H3K79me and transcription elongation have been proposed as mechanisms responsible for aberrant gene activation that are hijacked by MLL fusions in leukaemia cells (FIG. 2b,c), they fail to explain why the MLL fusion, but not wild-type MLL, is refractory to the silencing mechanism that can turn off MLL targets in haematopoiesis. Recently, it has been shown that the artificial addition of the MLL PHD fingers, a portion not retained in MLL fusions, can inhibit MLL fusion-induced transformation[111,112]. These studies shed light on the dynamic regulation of wild-type MLL complexes and the loss of such an inhibitory mechanism may contribute to the conversion of the MLL fusion to a constitutive activator[111,112]. This inhibitory effect seems to be due to the recruitment of the repressive factors cyclophilin 33 (CYP33) and HDACs by the third PHD finger of MLL[112] and/or the inhibition of MLL targeting[111]. The third PHD finger of MLL has been predicted to bind to H3K4me3/2 (REF. 5). So far, CYP33 has only been reported as a PHD finger ligand for MLL[112], thus this mechanism may be MLL specific. Further efforts are needed to understand how the PHD fingers distinguish normal MLL complexes and their leukaemia fusion forms in terms of transcriptional regulation.

Furthermore, it is unclear how the gene-target specificity, if any, of many histone-modifying or modification-reading factors is achieved. For example, the recruitment of different sets of INGs to distinct genomic loci cannot be explained by H3K4me3 recognition. Although menin and LEDGF were found to be required to tether MLL to its target gene loci[31], it is far from clear how MLL fusions are targeted to their downstream genes in leukaemia. Besides the intrinsic motifs that are associated with chromatin and histone modification[17], histone modification reading and enzymatic factors can be recruited to their targets by DNA-binding factors, co-activators, co-repressors, and Pol II-associated mediators and machineries. For example, nuclear receptors assemble different sets of cofactors and histone-modifying enzymes after the activation or depletion of hormone signalling[113], and we refer readers to some comprehensive reviews on this topic[113–115].

Finally, as some histone-modifying enzymes also function on non-histone substrates[24,25,116], it becomes difficult to ascribe observed results to histone modification alone. Experiments need to be carefully designed, ideally by applying a combination of approaches and methodologies to dissect the effects that originate from histone modification.

Is it time for the therapeutic intervention of epigenetic players that modify or interpret chromatin modifications? The first goal is to identify the crucial epigenetic factors that have well-defined roles in the initiation or development of cancers. For example, the H3K4me3-binding pocket is a potential therapeutic target for the treatment of the leukaemia-harbouring translocations NUP98–JARID1A and NUP98–PHF23 (REF. 22). In addition, many histone-modifying enzymes are ideal targets as their enzymatic activity is druggable[56]. However, the enthusiasm of developing such inhibitors can be curbed by a general concern of potential side effects and complications. For example, the H3K4me3-binding pockets of different PHD finger proteins (TABLE 2) have a high structural similarity[15,16,20,22], and these factors are involved in several important cellular processes, such as transcription[19]. However, we remain confident that further investigation will lead to the discovery of relatively specific druggable epigenetic factors that represent the Achilles heel of tumour cells.

In support, the clinical success of HDAC inhibitors in cutaneous T cell lymphoma and DNA demethylating agents in myelodysplastic syndrome offers a compelling argument[4,117,118]. Recently, a genomic study has shown that pharmacological doses of all-*trans* retinoic acid induces a specific effect on histone H3 deacetylation in PML–RARα fusion-positive acute promyelocytic leukaemia cells (PMLs), and the change in H3 acetylation underlies differentiation therapy and epigenetic therapy of PML[119]. This study also provides a rationale for developing HDAC inhibitors as an alternative therapy for patients with PML that are refractory to current standard treatments. With the rapidly growing attention and new discoveries of epigenetic factors that function to govern a steady-state balance or the output of histone modifications, there is considerable promise and excitement on the horizon.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
Hoxa9 | *ING1* | *ING3* | *ING4* | *JARID1B* | *JARID1C* | *NUP98* | *UTX*
UniProtKB: http://www.uniprot.org
DOT1L | ENL | EZH2 | GCN5 | ING2 | ING5 JARID1A | LEDGF | menin | MLL | RAG2

FURTHER INFORMATION
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