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Covalent histone modifications: miswritten, misinterpreted, and miserased in human cancers

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Abstract

Post-translational modification of histones provides an important regulatory platform for many DNA-templated processes such as gene transcription and DNA damage repair. It has become increasingly apparent that the misregulation of histone modification, caused by deregulation of factors that mediate its installation, removal and/or interpretation, actively contributes to the initiation and progression of human cancer. In this review, we summarize recent advances in understanding the interpretation of certain histone methylation by PHD finger-containing proteins and how misreading, miswriting and miserasing histone methylation marks are associated with oncogenesis. This quickly emerging field not only provides a greater mechanistic understanding of human cancers, but also may help direct novel therapeutic interventions in future.

Introduction

Modulation of chromatin through covalent histone modification represents one fundamental way to regulate DNA accessibility during gene transcription, DNA replication, DNA damage repair, and many other cellular processes. According to the 'histone code hypothesis' (Box 1), the biological outcome of histone modifications is manifested either by direct physical modulation of nucleosomal structure or by providing a signaling platform to recruit downstream 'reader' or 'effector' proteins[1, 2]. A rapidly emerging body of 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 tumor cells to silence tumor 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). Here, we propose that epigenetic alternations involving histone modification lead to misregulation of gene expression and perturbation of the states of cell identities, which, in return, contribute to tumor initiation, progression and metastasis.

Methylation of histones occur at both lysine and arginine resides. Once thought to be very stable, histone methylation is now appreciated as a reversible process. Its homeostasis is mediated by two opposing 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 MLL family of histone methyltransferases (HMTs) (Figure 1A)[5], and removed by the LSD1 and JARID1 family of histone demethylases (HDMs) (Table 1)[6].

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Within histone H3, methylation has been observed at multiple lysine (K) sites including H3K4, K9, K27, K36 and K79, and addition of up to three methyl groups at each lysine produces a total of four methyl states - unmethylated, and mono-, di- or tri-methyl. Each of different histone methylation sites and states exhibits a quite 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 the transcriptional start sites of highly expressed genes, whereas H3K27 trimethylation (H3K27me3) is frequently associated with gene silencing, especially repression of unwanted differentiation programs during lineage specification[7–9]. 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 histone modifications co-exist and co-mark developmentally critical genes, where a monovalent feature, either active or repressive marks, is often kept in differentiated cell lineages[8, 9]. It has been proposed that a bivalent chromatin state serves as a mechanism to retain chromatin plasticity and to keep the cell/chromatin state poised at the early stages of embryogenesis and development[8, 9]. As epigenetics and histone modification are intimately related to cell fate determination, it has been proposed that epigenetic aberration may be involved in early phases of tumor development and establish the state of tumor-initiating cell populations[10]. Indeed, Esteller and colleagues have reported that the global loss of trimethylation at Lys 20 and acetylation at Lys16 of histone H4 is a hallmark of cancer cells[11].

Histone methylation, a component of chromatin indexing mechanisms

One important issue in chromatin biology and epigenetics is to understand how the pattern of a potential ‘histone code’ or ‘epigenetic code’ (Box 1) is translated into the meaningful biological consequence, especially in the context of cell fate determination and gene regulation. Towards this end, identifying factors that specifically recognize or ‘read’ histone modifications has greatly improved our understanding of the interpretation and meanings of these histone marks. A recent breakthrough is the discovery of a specialized group of protein modules termed as plant homeo domain (PHD) finger (Box 2) as the ‘reading’ motif specifically for tri- and di-methylated H3K4 (H3K4me3/2), with H3K4me3 as the preferred ligand[12–15] (Table 2). Despite the fact that a large number of PHD finger motifs are encoded by the human genome, only a subset contain the critical hydrophobic or aromatic residues that enable them to form a specialized structural pocket or channel to accommodate the H3K4me3 side chain[5] (Box 2). We refer readers to several recent reviews that cover the classification and structure of these histone modification-‘reading’ modules in greater details[5, 16, 17]. Here, we only focus on how these histone modification ‘reading’ factors are involved in normal cellular processes such as transcriptional regulation and DNA recombination, as well as in oncogenesis.

To date, about a dozen of PHD finger-containing readers for H3K4me3/2 have been experimentally confirmed (Table 2), which include a RNA polymerase II-associated general transcriptional machinery component TFIID/TAF3, a V(D)J recombinase RAG2, and several critical chromatin modifying or remodeling factors. Conceivably, the H3K4me3 mark serves as a critical chromatin 'index' or 'beacon', allowing specific genomic regions to be readily recognized by their downstream ‘readers’ and/or associated effectors. For example, it has been suggested that the targeting of TFIID/TAF3 to H3K4me3 at promoters helps to anchor and/or recruit TFIID and associated machinery for active transcriptional initiation (Figure 2a)[18, 19]. In addition, recognition of H3K4me3 by the PHD finger of RAG2 at V(D)J gene segments has been proven to be critical for efficient V(D)J recombination during B and T cell development and maturation, and deleterious germ-line mutations that abrogate such recognition of H3K4me3 lead to severe immunodeficiency

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syndromes (Figure 2b)[20]. Now, emerging evidence also reveals that deregulation in the ‘reading’ of H3K4me3 contributes to various aspects of cellular transformation and even leads to cancers in some case, i.e., acute leukemia induced by chromosomal translocation of the H3K4me3-'reading' PHD finger of PHF23 or JARID1A [21] (Table 1). In addition, many enzymes that mediate the ‘writing’ or ‘erasing’ of histone methylation are also strongly associated with oncogenesis (Table 1). It should be noted that certain histone methylation 'writer' or 'eraser' contains the methyl-'reading' module (e.g. MLL or JARID1A, in Table 1 and 2), which indicates coordination between 'reading' and 'writing'/'erasing' steps of histone modification.

(De)Methylation of histones and beyond

A complication of histone modifying enzymes is the potential involvement of non-histone substrates. Although histone modification ‘writers’ or ‘erasers’ were originally identified as enzymes that modify histones, an increasing body of evidence shows that they may also target non-histone proteins. For example, LSD1 (also known as KDM1A) not only targets its canonical substrate, histone H3, but also demethylates the tumor suppressor p53 at lysine 370 and represses p53 activities[22, 23]. Similarly, the histone methyltransferase G9a and SET7/9 induce methylation of a number of non-histone proteins[23, 24]. To our knowledge, none of the histone methyltransferases or demethylases listed in Table 1 has been formally shown to act on non-histone substrates, although it remains an open question and a formal possibility that they target beyond histones. In the following sections, we focus on recent evidence that has linked the mis-writing, mis-interpretation and mis-erasing of the ‘histone code’ to oncogenesis, using mutations affecting H3K4me3-reading PHD finger 'readers' and mutations affecting chemical modification of H3K4me3 and H3K27me3 as instructive examples.

Histone methylation miswritten during oncogenesis

Establishment of an appropriate pattern of histone methylation is not only crucial for normal development and differentiation, but is also intimately associated with tumor initiation and development (Table 1). Soon after their discovery, some histone modifying enzymes have been found to be frequently mutated in human cancer. Conversely, some famed cancer-associated genes turn out to be direct regulators of histone methylation much later on after initial cloning.

MLL gene rearrangement in leukemia

The *Mixed Lineage Leukemia* (*MLL*) (also known as *ALL-1, KMT2A*) was initially identified as the gene involving recurrent translocations of chromosomal band 11q23 in human myeloid and lymphoid leukemias[25], and was later shown to encode a major H3K4-specific HMT enzyme[26, 27]. MLL forms a large macromolecular nuclear complex with the core complex components (WDR5, RBBP5, ASL2), and induces H3K4me3 for efficient transcription[5, 26, 28] (Figure 1a). Other MLL-associated factors, Menin and LEDGF, tether the MLL complex to appropriate targets[29](Figure 1a). Accounting for about 80% of infant leukemia and 5–10% of adult acute myeloid leukemia (AML) or lymphoid leukemia cases, *MLL* gene rearrangements represents one of the most common chromosomal abnormalities found in human leukemia[25, 30].

The partial tandem duplication of *MLL (MLL-PTD)*, the most frequent form of MLL rearrangement in AML[30, 31], contains an in-framed duplication of *MLL* exon 4 to 11 (or exon 4 to 12) and retains the H3K4 HMT activity[25](Figure 1a). Dorrance et al 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 *Hox-A* gene

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cluster[32, 33](Figure 1a). Over-expression of *Hox* genes initiates and/or promotes leukemia induction[34]. Normally, the expression of *Hox-A* genes such as *Hoxa9* is developmentally restricted – they are highly expressed in early hematopoietic precursors and silenced following differentiation[35](Figure 1a). In the *MLL-PTD* knock-in mice, altered histone methylation and/or acetylation correlates with a significant increase in *in vitro* colony formation potentials of erythroid, myeloid, or pluripotent hematopoietic progenitors, as well as a drastic increase in *Hoxa9* expression among terminally differentiated blood cells (by 100~250 fold increase) and unsorted hematopoietic tissues (by 4~150 fold)[32, 33]. However, these *MLL-PTD* knock-in mice fail to develop frank leukemia, indicating that additional alternations are required for malignant transformation.

MLL fusion, a second type of *MLL* gene rearrangement, results in deletion of a large C-terminal fragment, which includes the H3K4 HMT domain, and also acquisition of additional transformation mechanisms provided by MLL fusion partner[25, 36](Figure 1b,c,d). Over fifty different MLL fusion partners have been identified in leukemia[25, 36]. Although leukemogenic mechanisms underlying many rare MLL fusion forms are poorly understood, recent studies have started to unveil a common transformation pathway for the most frequent MLL fusion forms (Figure 1b,c). Okada et al first report that the AF10 portion of MLL-AF10[37] and CALM-AF10[38] fusions directly recruits DOT1L (also known as KMT4), a histone methyltransferase that mediates or 'writes' the methylation of histone H3 lysine 79 (H3K79me)[37](Figure 1b). This scenario can be applied to MLL-ENL, because ENL also directly associates with DOT1L and the interaction surface is retained in MLL-ENL[39](Figure 1b). Aberrant induction of H3K79me was observed at leukemia-promoting oncogenes (such as *Hoxa9,* Figure 1b) in leukemia cells transformed by MLL-AF10[37], MLL-ENL[39, 40], MLL-AF4[41, 42] and MLL-AF9[43], which represent the most common MLL fusion forms. Mutations of MLL-ENL[39] or CALM-AF10[38] that disrupt the interaction with DOT1L abolish leukemia transformation. DOT1L and H3K79me are associated with active transcription[44], especially at MLL fusion target loci[37, 41], thus providing a potential mechanism for aberrant transcriptional activation found in leukemia. DOT1L and by inference H3K79me has been also found involved in cell cycle progression[45], silencing of telomere-proximal genes[46], and regulation of Wnt signaling target genes[47]. Recent biochemical studies have further revealed that DOT1L actually associates with an amazingly long list of factors that are known MLL fusion partners, which include AF10[37, 47–49], ENL[39, 47, 49], AF9[47–50], AF17[47], AF4 (also known as MLLT2 or AFF1)[39, 47, 48], AF5q31 (also known as MCEF or AFF4)[39] and LAF4[39]. AF10, AF17, and ENL (or AF9) were identified as stable components of DOT1L-containing complexes[47]. It remains as an intriguing model that DOT1L is responsible for aberrant transcription in many MLL fusion-induced leukemia, however, it has been complicated by the fact that DOT1L complexes are also linked to transcription elongation. Via a protein-protein interaction network, DOT1L-AF10-ENL/AF9 complexes further associate with a transcription elongation-promoting complex that contains AF5q31/AFF4, AF4, ELL1/2/3 (also known MLL fusion partners), and the Pol II transcription elongation factor b (P-TEFb) kinase (consists of CDK9 and Cyclin T1/2a /2b)[39, 51] (Figure 1b,c). ENL and AF5q31/AFF4 are shared components of these two complexes[39, 47, 49, 51]. In addition, two recent studies further demonstrate that MLL fusions involving component in this elongation complex, including MLL-AF4, MLL-ENL, MLL-AF9, and MLL-ELL1, all interact with AF5q31/AFF4 and recruit p-TEFb transcription elongation complexes to promote the transcription of downstream targets such as *Hox*[51, 52] (Figure 1c). Thus, mechanisms underlying aberrant transactivation in MLL leukemia have been linked to H3K79me and also transcription elongation.

While the activities of P-TEFb complexes during transcription were well established, the role of H3K79 methylation in transcription suffers from lack of mechanistic understandings. Is H3K79me equally important, or does DOT1L merely bridge MLL fusions (such as MLL-

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ENL or MLL-AF10) to P-TEFb elongation complexes (Figure 1b)? Several lines of evidence suggest that H379me is critical in leukemia induction. First, replacing the AF10 fragment of MLL-AF10 with the wildtype but not catalytically inactive form of DOT1L succeeded in leukemia transformation[37]. Second, inhibition of DOT1L by knockdown significantly interferes with MLL-AF4 induced transformation and also the activation of *Hox* genes[41], although MLL-AF4 associates with AF5q31/AFF4 and p-TEFb elongation complexes[51]. In addition, DOT1L also directly interacts with p-TEFb[52]. Further investigation will be needed to examine the role of H3K79me during transcriptional activation or elongation.

Another MLL fusion, MLL-EEN, recruits histone arginine methyltransferase PRMT1, and its methyltransferase activity towards histone H4 arginine 3 has been shown to be critical for leukemia transformation[53] (Figure 1d). Taken together, miswriting of histone methylation marks often correlates with aberrant transcription of oncogenes in leukemia patients harboring *MLL* gene rearrangements.

EZH2 over-expression and mutation in cancers

EZH2, an H3K27-specific methyltransferase or 'writer', provides another connection between miswriting histone methylation marks and oncogenesis. EZH2 is frequently found over-expressed in a wide variety of solid tumors including prostate, breast, colon, skin, and lung cancers[54, 55] (Table 1). Suppression of EZH2 by RNA interference significantly decreased tumor growth in breast and prostate tumor xenograft models[56, 57]. Furthermore, over-expression of EZH2 confers invasiveness to fibroblasts and immortalized benign mammary epithelial cells, and this effect is dependent on the H3K27 HMT activity of EZH2[57–59]. Mechanistically, the oncogenic function of EZH2 polycomb factor has been attributed to the silencing of tumor suppressor genes including *INK4B-ARF-INK4A* [55] (Figure 1e), *E-cadherin*[58, 60], *p57^{KIP2}/CDKN1C*[61], *p27*[62], *BRCA1*[56], and *adrenergic receptor* β2[57]. Contrary to the overwhelming evidence showing EZH2 overexpression in tumors, a recent next-generation sequencing based study of human cancer genomes discovered the recurrent inactivating mutations of *EZH2* in follicular lymphoma and diffuse large B-cell lymphoma[63] (Table 1). The identified *EZH2* mutations specifically target a single tyrosine residue that is required for EZH2-mediated HMT activities towards H3K27me3[63]. It is tempting to speculate that the homeostasis of H3K27me3 mark might be disrupted through deregulation or mutation of EZH2, however, the oncogenic roles of EZH2 over-expression and mutations remain to be validated more rigorously in genetically engineered animal models in future. Ideally, genomic (mis)-localization of EZH2 and its effect on H3K27me3 or transcription need to be examined in matched normal versus tumor samples.

Despite these, blockade of EZH2 has been proposed a therapeutic strategy to inhibit tumorigenesis and initiate tumor regression[54]. Indeed, Fisku et al show that combined usage of inhibitors of EZH2 and HDACs, another type of repressors that physically interact with EZH2 (Figure 1e), de-repress several tumor suppressor genes (*p16, p19* & *p27*), selectively induces apoptosis of leukemia cells, and improves survival of mice bearing xenograft leukemia[64]. Due to the limited space, we also refer readers to recent nice reviews[54, 55], where the involvement of EZH2 in oncogenesis is discussed in greater details.

Histone methylation misinterpreted during oncogenesis

1. Aberrant fusion of PHD finger motifs and mis-interpretation of H3K4me3 in leukemia

Chromosomal translocation of *nucleoporin-98* (*NUP98*), a nuclear pore complex component gene, represents one of the most promiscuous gene rearrangements found in various forms of hematopoietic malignancies[65]. In a subset of AML patients, NUP98 translocation results

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in fusion of the N-terminus of NUP98 to the C-terminal PHD finger motif (and also nuclear localization signals) of PHF23 or JARID1A (also known as KDM5A or RBBP2) (Figure 2c)[21, 65]. Recently, leukemia induced by NUP98-JARID1A or NUP98-PHF23 fusion has been experimentally recapitulated using *in vitro* and *in vivo* leukemia models[21]. The leukemogenic potential of these two fusion oncoproteins relies on the ability of the PHD finger motif to recognize the H3K4me3/2 marks[21] (Box 2). A single point mutation in the PHD finger that abrogates the H3K4me3 binding also abolishes leukemic transformation, and the PHD finger can be functionally replaced by other H3K4me3-binding PHD fingers (even one from yeast), but cannot by those that do not recognize H3K4me3[21]. Mechanistically, binding of H3K4me3 by the NUP98-PHD finger fusion interferes with normal differentiation of hematopoietic stem/progenitor cells by preventing the removal of H3K4me3 and inhibiting EZH2-mediated H3K27me3 at developmentally critical genes *Hox*, *Meis1a, Gata3* and *Pbx1*[21, 66]. As a result, the chromatin state at master regulator loci of hematopoiesis is locked in an active one (marked with high levels of H3K4me3 and histone acetylation), and the expression of these genes is maintained[21]. It has been well documented that over-expression or activating mutation of these transcriptional factors such as *Hoxa9*, *Pbx1*, and *Meis1a* is commonly found with human leukemia, and also sufficient to arrest hematopoietic differentiation and induce leukemia[34, 67]. Thus, perturbation of histone modification dynamics associated with hematopoiesis, as in the case of NUP98-PHD finger fusion, causes enforced expression of critical developmental genes and interferes with appropriate transition of cellular states, which represents a critical step of leukemia initiation[21].

As acute leukemia is a disease of misregulated differentiation, it becomes important to first understand molecular mechanisms that underlie the establishment and transition of the chromatin landscape during hematopoietic development. Understanding this issue will also help us to dissect how leukemia lesions such as NUP98 or MLL translocation interfere with the dynamic regulation of chromatin. Conceivably, NUP98-PHD finger fusions may mimic some endogenous chromatin-associated machinery, acting as a chromatin boundary factor that prevents the intrusion of the repressive complexes that include an H3K27me3 'writer' EZH2 and an H3K4me3 'eraser' JARID1[21, 68] (Figure 2c and Figure 3a). Gain-of-function mutation of the PHD finger motif, as exemplified by NUP98-PHD finger fusions in leukemia[21], and loss-of-function mutation of the PHD finger, as mentioned earlier in the case of RAG2 and immunodeficiency syndromes[20], unveil a type of human pathologies that are underscored by failure in appropriate interpretation of histone modification[17].

2. Somatic mutation of ING PHD fingers in solid tumors

Another family of PHD finger-containing proteins, inhibitor of growth (ING), are putative tumor suppressors. Loss-of-function mutations of *ING*s (especially *ING1, ING3* and *ING4*) via somatic mutation, allelic loss, reduced gene expression, or aberrant cytoplasmic sequestration, were reported in a variety of solid tumors (Table 1)[69, 70]. INGs regulate many cellular processes associated with tumorigenesis, including cell cycle progression, senescence, apoptosis, DNA repair, cell migration and contact inhibition[69–72]. A structural characteristic of all INGs is a PHD finger that locates at C-terminus and binds to H3K4me3 specifically (Box 2)[13, 73–75]. 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 (Figure 2d), whereas INGs3/4/5 recruit histone acetyltransferase (HAT) to induce gene activation[13, 76]. ING3- and ING4-complexes contain a HAT protein, either Tip60 or HBO respectively, and ING5-complexes include either HBO or MOZ/MORF as the HAT[74, 76]. INGs (ING1, ING4 and ING5) also interact with p53, and modulate its activity[69, 77–79]. Here, we focus on recent

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advances that link *ING* mutations to mis-interpretation of histone methylation in cancerous transformation.

ING1 was initially identified in a functional screen as an inhibitor of neoplastic transformation[80], and somatic mutation of *ING1* is later found in breast, gastric, and pancreatic cancers, as well as squamous cell carcinomas[69, 70]. Deletion of *Ing1* in mice only led to a mild phenotype, with a slight increase in incidence of lymphomas, indicating that other mutations may cooperate with *ING1* inactivation in tumorigenesis[77]. A subset of *ING1* somatic mutations found in human tumor samples specifically target its PHD finger motif[17, 69–71]. Some hotspot mutations, C215S and C253stop (amino acid number refers to the $p33^{ING1b}$ isoform), target the critical zinc ion-coordinating cysteines in the PHD finger, which causes a global misfolding[17]. A recent biophysical study demonstrated that several other *ING1* mutations, N216S, V218I and G221V, interfere with either appropriate formation of the structural pocket to accommodate H3K4me3 or appropriate positioning of the histone H3 tail, leading to a decrease in H3K4me3-binding affinities by 10–40 fold[73]. Despite these advances, animal models that establish a direct causal role of *ING1* mutations in tumorigenesis are still lacking. Nonetheless, at the cellular level, it has been shown that the decreased binding of ING1 to H3K4me3 results in an inefficient response to DNA damage or apoptosis[73].

ING2, an *ING1* related member that is also found downregulated in many types of solid tumors[69–71], initiates an acute response aimed at silencing proliferative genes including *Cyclin D1* and *c-MYC* and decelerating the cell cycle upon insults of DNA damage[13] (Figure 2d). This response relies on the ability of ING2 to bind to H3K4me3 associated with proliferative genes, followed by the recruitment and/or stabilization of ING2-associated repressors HDAC1 and HDAC2[13, 76] (Figure 2d). Eventually, histone deacetylation occurs at proliferative genes, their expression is downregulated, and the cell cycle progression is halted[13]. Since the H3K4me3 level at these genes stays the same before and after DNA damage[13], it remains poorly understood what causes the recruitment of ING2 to proliferative genes upon 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 accumulated in the nucleus upon cellular stress[13, 81]. The binding surface for PtdIns(5)P in ING2 is located at the very carboxyl terminus, which include a small portion of the PHD finger and a lysine/arginine-rich polybasic region[82]. This polybasic region is found in ING1/2 only, and not in other ING members[69, 71]. Further investigation is required to dissect how multivalent interactions between INGs, signaling transducers, and histone modifications are coordinated to execute an efficient response to DNA damage and cellular stress.

Down-regulation, allelic loss, or somatic mutation of ING3 and ING4 was also found in cancers[69, 70]. Recognition of H3K4me3 has shown to be critical for ING4/5-HBO complexes to promote genotoxic stress-induced apoptosis and to inhibit anchorage-independent cell growth[74–76]. Association between the ING4/5 PHD finger and H3K4me3 modulates the substrate specificity of HBO complexes, making H3K4me3-containing nucleosomes a preferred substrate[74, 75]. Using genome-wide ChIP-chip analyses, Hung et al observed that, after DNA damage, the recruitment of ING4-HBO complexes to the downstream targets is enhanced, followed by the subsequent increase in levels of H3 acetylation and transcription[75]. Confirmed target loci include a number of tumor suppressor genes such as *PHD2* (also known as *EGLN1*/*HPH2*) and *Exostosin-1 (EXT1)*[74, 75]. PHD2 is an inhibitor of hypoxia-inducible factor (HIF), and its down-regulation results in increased angiogenesis in tumor tissues and promotion of tumorigenesis[83]. Mutation in *EXT1* or the related gene *EXT2* is responsible for multiple osteochondromas, a skeletal disease characterized by benign bone tumors[84]. The involvement of misregulation of these potential

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tumor suppressors need to be further examined in primary tumor samples or animal models harboring loss of *ING4*.

One interesting issue is that, despite the fact that ING1/2 and ING3/4/5 complexes impart opposite effects on transcriptional regulation[71, 76], both types of ING complexes appear to harbor tumor suppressive activities[72]. Apparently, different INGs target different sets of genes – oncogene vs tumor suppressor loci[13, 75]. 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 critical step for efficient chromatin binding and for execution of DNA damage- or stress- induced responses[13, 73–75]. Many other outstanding questions remain unsolved, for example, do different ING-containing complexes cooperate and how interaction of p53 or other DNA binding factors is involved in these cellular responses[79]? It is also getting important to appreciate how all the following events are integrated: sensing and signaling of DNA damage, assembly and recruitment of ING complexes, chromatin recognition and modulation, the subsequent transcriptional regulation, DNA repair, and chromatin restoration post repair.

3. Pygopus (Pygo), a factor that links the Wnt/β-catenin signaling to H3K4 methylation

Mutations in components of Wnt/β-catenin pathway lead to oncogenesis in several tissue types[85]. Pygopus (Pygo) has recently been identified as a critical factor for efficient Wnt/β-catenin signaling[85, 86]. Pygo interacts with BCL9 (also known as legless in *Drosophila*), an adaptor protein that directly associates with β-catenin (Figure 2e)[87]. The C-terminus of all Pygo homologues (pygopus in *Drosophila*, and Pygo1/2 in mammals) contains a PHD finger, which uses two surfaces to interact with BCL9 and H3K4me2/3 simultaneously (Figure 2e), and the binding of H3K4me2/3 by Pygo is enhanced by its association with BCL9[87]. *Pygo2* was found highly expressed in mammary progenitor cells and up-regulated in breast cancer cells, and the H3K4me2/3-binding property of Pygo2 appears to be critical for cell growth of breast cancer cells[88, 89]. However, a separate study indicates abolition of interaction between H3K4me2/3 and pygopus does not seem to interfere with the activation of Wnt signaling in fruit flies[87, 90]. Further investigation of cross-talk among pygopus, Wnt/β-catenin signaling, and histone modification needs to be performed to address the difference.

Histone methylation miserased during oncogenesis

Histone lysine demethylases (HDM), especially those acting on H3K4me3 and H3K27me3, are found mutated or deregulated in human cancer (Table 1). *JARID1A* was found translocated in myeloid leukemia (Figure 2c). *JARID1B* (also known as *PLU-1/KDM5B*), another H3K4me3/2-specific HDM gene, was found to be over-expressed in advanced stages of breast and prostate cancers[91, 92]. JARID1B facilitates the G1/S transition and attenuates the mitotic spindle checkpoint of cancer cells[91, 93]. Using a syngeneic tumor implantation model, Yamane et al showed that *JARID1B* over-expression promotes the growth of mammary carcinoma[91]. JARID1B represses metallothionein genes and several known tumor suppressor genes (*BRCA1* and *Caveolin-1*) by inducing the erasion of H3K4me3/2[91, 93]. In a recent large-scale next-generation sequencing of primary renal cell carcinomas (RCC) genomes, Dalgliesh et al discovered a number of recurrent mutations that inactivate histone modifying enzymes, including truncating mutations of *JARID1C* (~3% of all RCC samples) and *SETD2* (~3%), a histone H3 lysine 36-specific methyltransferase gene[94]. Inactivation of *JARID1C,* a third member of the H3K4me3/2-specific HDM genes, in RCC tumor samples is correlated with transcriptional alteration of a specific gene signature[94]. The majority of RCCs harboring *JARID1C* mutation also contain a mutation at *von Hippel-Lindau* (*VHL*) gene, a negative regulator of HIF, suggesting that the *JARID1C* and *VHL* mutations may cooperate in driving tumorigenesis of RCC[94]. It is curious that both

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overexpression and loss-of-function mutations of the *JARID1* gene family are suggested to contribute to oncogenesis, although in different cancer types (Table 1).

JHDM1B (also known as *FBXL10/Ndy1/KDM2B*) and *JHDM1A* (also known as *FBXL11/Ndy2/KDM2A*) encode another family of histone demethylases that appear to harbor dual methylation-erasing activities for H3K36me2/1 and H3K4me3[95–97]. In a screen based on retroviral integration-induced T cell lymphomas, Pfau et al found that up-regulation of *JHDM1B/Ndy1* is a common event in T cell lymphomas[98]. JHDM1B/Ndy1 directly represses the tumor suppressor locus, *Ink4b-Arf-Ink4a*, by erasing H3K36me2 and/or H3K4me3[96–98]. Both JHDM1B/Ndy1 and related protein JHDM1A/Ndy2 are shown to inhibit the replicative senescence and oncogene-induced senescence, which represent a critical barrier of oncogenesis[96–98]. *JHDM1B/Ndy1* is down regulated upon senescence induction in normal tissues, while acquired expression of *JHDM1B/Ndy1* in tumors prevents an occurrence of cell senescence, thus facilitating cancerous transformation (Figure 3b)[96, 97].

Sporadic inactivating mutations of *UTX*, an H3K27me3/2-specific HDM gene, have been recently reported in a subset of multiple myeloma, esophageal squamous cell carcinomas, and renal cell carcinomas[99]. Restoration of *UTX* in *UTX*-mutated cancer cells reduced H3K27me3 at tested targets and slowed cell proliferation[99]. *JMJD3*, a related H3K27me3/2-specific HDM gene, was found up regulated during RAS-induced senescence, and opposite to the action of JHDM1 and EZH2, JMJD3 activates the *Ink4a-Arf* locus[100, 101] (Figure 3b). The expression of *JMJD3* has been found down regulated in various cancers including lung and liver cancers[100, 101]. These observations indicate a putative tumor suppressive role of UTX and JMJD3. Despite emerging evidence that links HDMs to cancer, it remains to be investigated whether or not the observed mutation is causal or merely the consequence of tumorigenesis using more rigorous assays.

Cooperation of ‘writing’, ‘reading’ and ‘erasing’ of histone methylation in oncogenesis

One complication of classification of ‘writing’, ‘reading’ and ‘erasing’ histone modifications is that these processes often act in a concerted way. For example, some histone modification ‘writer’ or ‘eraser’, such as MLL or JARID1A, harbors an intrinsic PHD finger module to ‘read’ H3K4me3 - the enzymatic product or substrate of these enzymes respectively (Table 2). Currently, it is unclear how this ‘reading’ property is involved in the ‘writing’ or ‘erasing’ step of histone methylation. In context of leukemia induction, NUP98-JARID1A (Figure 2c), a translocation form of JARID1A, loses the histone methylation-‘erasing’ activity, and relies on the H3K4me3-‘reading’ PHD finger to initiate leukemogenesis[21]. In addition, histone modifiers often work together to take on different histone modification sites simultaneously and to execute a robust response. For instance, UTX is a stable component of the MLL2/3-containing complexes, which ‘erase’ H3K27me3 and also ‘write’ H3K4me3 at target chromatin [102]. Consistent to the action of UTX, MLL has recently been found to be recruited to the *Ink4a* locus in oncogene-induced checkpoint response, and UTX and MLL may cooperate to promote *p16/INK4a* expression and suppress cancerous transformation[103] (Figure 3b). Similarly, JHDM1B/Ndy1 and JARID1A was reported to interact with EZH2[68, 97], and JMJD3 interacts with MLL[104]. Here, a common theme appears to underlie the repression process of tumor suppressor *INK4B-ARF-INK4A* in cancer cells, that is, deregulated histone demethylases (JARID1B, JHDM1B, UTX, JMJD3) cooperate with EZH2 over-expression or DNA hypermethylation to establish a stable silenced state by elimination of active modifications (H3K4me3) and addition of repressive chromatin modifications (H3K27me3 or DNA methylation) (Figure 3b).

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Conclusions and future directions

In summary, histone modification, as exemplified by H3K4me3 and H3K27me3 in this review, provides a critical regulatory means for gene transcription, DNA recombination, DNA damage repair, and many other DNA-templated processes. A rapidly increasing body of evidence has indicated that 'miswriting', 'misreading', or 'mis-erasing' of histone modifications contributes to the initiation and development of human cancer. However, in terms of mechanistic understandings, the picture is still rather murky, complicated and context-dependent. First, it is unclear how the gene target specificity of many histone modifying or modification-'reading' factors is achieved. For example, recruitment of different sets of INGs to distinct genomic loci cannot be explained by the recognition of H3K4me3. Although Menin and LEDGF were found to be required to tether MLL to its targets[29], it is far from clear how MLL fusions are targeted to their downstream genes (such as *Hox*) in leukemia. Besides histone modification that we focus on, DNA binding factors and their associated co-activators/co-repressors can be equally important in tethering histone modification-associated enzymatic, remodeling and 'reading' factors to appropriate chromatin loci. Due to limitation in space, we refer readers to some comprehensive reviews on this topic[105, 106].

Second, it remains unclear whether many mutations mentioned above are the cause or consequence. More rigorous evidence is generally lacking to establish causality for deregulation that targets the 'writing', 'reading' and 'erasing' of histone modification. Generating animal models with *ING* mutations will not only define their oncogenic roles, but also may serve as a useful tool to understand mechanisms for driving oncogenesis. Similar issues can be applied to the inactivating mutations of *JARID1C* found in renal carcinoma[94] or for *EZH2* in germinal center B-cell lymphoma[63].

Furthermore, the regulatory mechanisms via histone modification can be cell type or context specific. In order to dissect misregulation of histone modifications and epigenetic imbalances in cancer cells, it becomes important to understand how normal cells utilize dynamic chromatin modifications to maintain the appropriate epigenetic balance between crucial oncogenes (e.g. *HOX-A* gene cluster in hematopoietic lineages) or tumor suppressor genes (e.g. *INK4B-ARF-INK4A* cluster) in normal developmental and cellular contexts. For example, although DOT1L-mediated H3K79me and transcriptional elongation has been proposed as mechanism responsible for aberrant transcriptional activation in MLL fusion induced leukemia (Figure 2b,c), they fail to explain why MLL fusions, but not wildtype MLL, are refractory to the silencing mechanism that is able to turn off MLL targets in hematopoiesis. Recently, it has been shown that artificial addition of the PHD fingers of MLL, a portion not retained in MLL fusions, is able to inhibit MLL fusion induced transformation[107, 108]. Loss of such an inhibitory mechanism was proposed to make MLL fusions a constitutive activator[107, 108]. This inhibitory effect appears to be due to recruitment of the repressive proteins cyclophilin-33 (Cyp33) and HDACs by the third PHD finger of MLL[108] or inhibition of MLL fusion targeting by MLL PHD fingers[107]. The third PHD finger of MLL was predicted to bind to H3K4me3/2[5]. Cyp33 as ligand of the PHD finger has only been reported for MLL so far[108], thus this mechanism may be MLL specific. Further efforts need to dissect how the PHD fingers distinguish MLL and their leukemia fusion forms in terms of transcriptional regulation.

Finally, as some histone modifying enzymes also act on non-histone substrates[23, 24, 109], it becomes difficult to ascribe observed results to histone modification alone. Experiments need to be carefully designed, ideally with application of a combination of approaches and methodologies, to dissect the effects that originate from histone modification.

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Is it prime time for therapeutic intervention of epigenetic players that modify or interpret chromatin modifications? The first and foremost goal is to identify the critical 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 treatment of leukemia harboring translocations NUP98-JARID1A or NUP98-PHF23[21]. In addition, many histone modifying enzymes are ideal targets as their enzymatic activity is druggable[54]. However, the enthusiasm of developing such inhibitors can be curbed by a general concern of potential side effect and complications. For example, the H3K4me3-binding 'pockets' of different PHD finger proteins (Table 2) display a high structural similarity[14, 15, 19, 21], and these factors are involved in several critical cellular processes such as general transcription[18]. Yet, we remain confident that further investigation will lead to discovery of relatively specific druggable epigenetic factors that represent the 'Achilles heel' of tumor cells. In support, clinical success of HDAC inhibitors in cutaneous T cell lymphoma and DNA demethylating agents in myelodysplastic syndrome offers a compelling argument[4, 110, 111]. Recently, a genomic study has shown that pharmacological doses of all-trans retinoic acid induces a relative specific effect on histone H3 (de)acetylation in PML-RARα fusion-positive acute promyelocytic leukemia cells (PML), and the change on H3 acetylation underlies differentiation therapy and epigenetic therapy of PML[112]. This study also provides a rational for developing HDAC inhibitors as an alternative therapy for PML patients that are refractory to current standard treatment. 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.

BOX 1. The histone code hypothesis

The histone code hypothesis, initially put forward 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, i.e., the histone code, in a given cellular or developmental context, which is brought about by a series of 'writing' and 'erasing' events performed by histone modifying enzymes. Here, the 'writer' of histone modification refers to an enzyme (for example, a histone methyltranferase) that catalyzes 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 it is transcriptional activation or silencing, or other cellular responses[1, 2, 5]. In addition to such a recruitment or '*trans*' mechanism, the manifestation of histone modification can also achieved by direct physical modulation of chromatin structure or alteration of intra- and inter-nucleosomal contacts via 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], or to fine-tune gene transcriptional regulation at a few gene loci during DNA damage repair[13] or in other DNA-templated contexts.

The histone code hypothesis extended to oncogenesis

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In the contexts of tumorigenesis or cancer epigenetics, we further hypothesize that alteration in the "balance" between epigenetic 'gene-on' versus 'gene-off' chromatin states leads to inappropriate expression or silencing of gene programs that, in turn, alter the states of cellular identity. In certain instances and developmental lineages, these alterations lead to unwanted mistakes made in decisions to proliferate versus to senescence and/or differentiate during tumorigenesis.

BOX 2. The plant homeodomain (PHD) finger

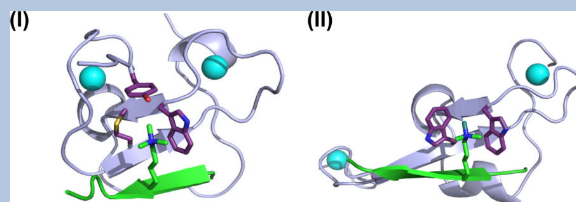
The PHD finger is a zinc finger-like domain, with a signature motif of Cys4-His-Cys3 to coordinate two zinc ions[113]. The folding of this ~60 amino acid-long domain is featured by an interleaved topology of zinc ion-coordinating residues and a couple of anti-parallel β-sheet secondary structures[5, 113]. 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[113]. There are less than twenty typical and atypical PHD finger motifs in *S cerevisiae*, about fifty in *Drosophila*, and up to a couple of hundred in mammals[17, 113, 114]. Most of PHD fingers are found in chromatin-associated factors or nuclear proteins[113, 114].

The PHD finger ligand

PHD fingers exhibit diversity and versatility in terms of their interaction partners. Some binds to chromatin modification such as highly methylated H3K4[5], unmodified H3K4[17] and methylated H3K36[114], some serve as a SUMO E3 ligase to interact with the E2 conjugating enzyme[115], and for others, their binding partner or function is still a mystery.

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, 14–16, 19–21]. The structures of H3K4me3-binding PHD fingers from two cancer-associated factors, ING2 and JARID1A, are shown in panel (I) and (II) respectively (H3 and the H3K4me3 side chain shown in green, PHD finger in lavender, zinc ion in cyan sphere, and hydrophobic 'pocket/cleft' 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 represent one of the strongest associations between histone modification and its 'reading' factors[5, 14–16, 19–21]. The structural illustrations shown are produced using published structural coordinates that have been deposited Protein Data Bank under accessions 2G6Q, 3GL6, 2KGG and 2KGI[15, 21].



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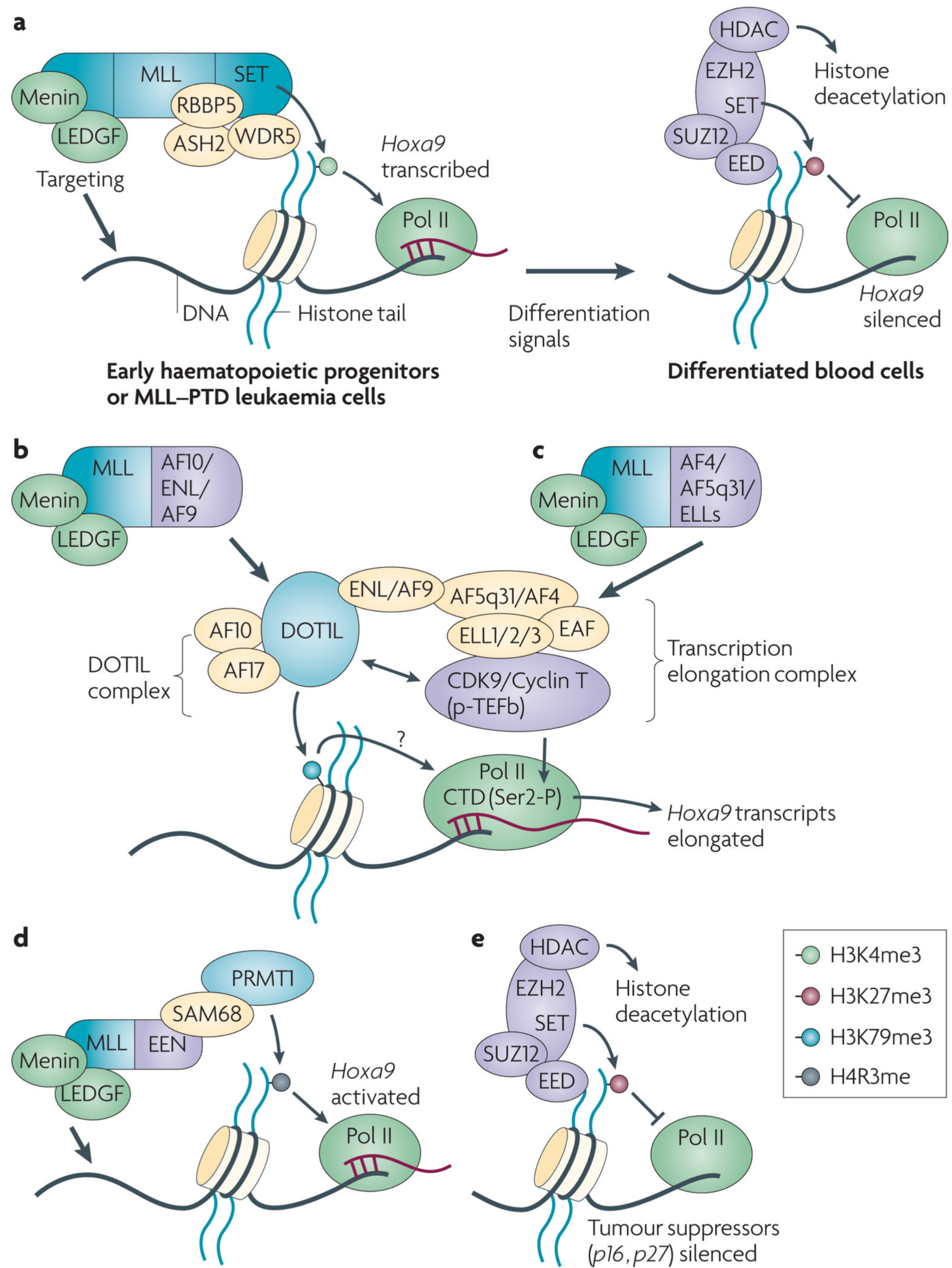

Figure 1. 'Miswriting' of histone methylation is associated with the initiation or progression of human cancer

(a) MLL-containing complexes induce H3K4me3 at *Hox* genes in early hematopoietic progenitor cells. Following terminal differentiation, a transition of chromatin state occurs at *Hox*, which is characterized by loss of H3K4me3 and gain of EZH2-mediated H3K27me3[21, 66]. EZH2 polycomb factors and associated HDACs induce the stable silencing of *Hox*. MLL-PTD, a MLL rearrangement form that harbors a duplication of *MLL* exon 4–12, causes an elevated level of H3K4 methylation.

(b–d) In leukemia, MLL fusion proteins lose a large carboxyl portion that includes the H3K4me3-'writing' SET domain, retain the chromatin targeting factors (Menin and

LEDGF), and also acquire aberrant trans-activation mechanisms through its fusion partner. A subset of MLL fusions, MLL-AF10, MLL-ENL and MLL-AF9, directly interact with DOT1L and induce the methylation of H3K79 at *Hoxa9* (panel b). Some other MLL fusions, MLL-AF4, MLL-AF5q31 and MLL- ELL1, interact with and recruit p-TEFb transcription elongation complexes to *Hoxa9* (panel c). DOT1L-complexes (DOT1L-AF10-AF17-ENL/AF9) associate with p-TEFb complexes via the shared components. Another MLL fusion partner EEN recruits PRMT1 and induce methylation of H4R3 at *Hox* (panel d).
(e) Over-expression of *EZH2* in tumor cells silences the tumor suppressor gene such as *INK4B-ARF-INK4A*. Please note that EZH2 can regulate oncogenes (panel a) or tumor suppressors (panel e) in different cellular contexts.

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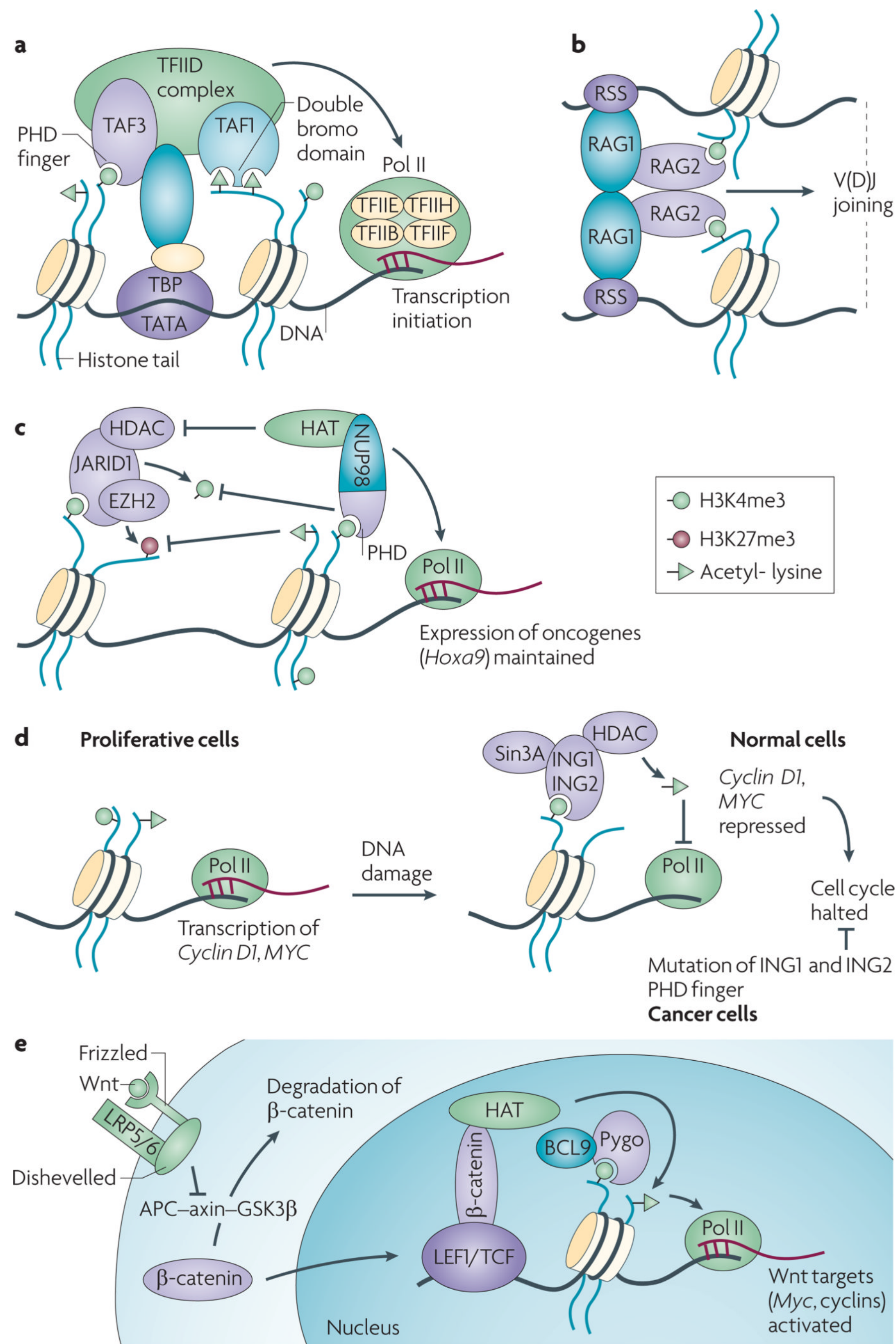

Figure 2. 'Reading' or 'mis-reading' the H3K4me3 marks by the PHD finger-containing factors in normal cellular processes and during cancer development

(a) Interaction with histone modification (H3K4me3 recognized by the TAF3 PHD finger[18, 19] and histone acetylation by the double bromo domain of TAF1[16]) and the DNA binding (TBP to the TATA box sequences) serve to anchor and/or stabilize the TFIID complex to core promoters, a critical step of the assembly of general transcription initiation machineries for active gene transcription[18, 19].

(b) Both recognition of H3K4me3 by the RAG2 PHD finger and binding to the recombination signal sequences (RSS) by RAG1-RAG2 complexes are critical for recruiting

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and/or stabilizing the RAG1/2 complexes at V(D)J gene segments to be recombined during B and T cell development[17, 20].

(c) Chromosomal translocation NUP98-JARID1A or NUP98-PHF23 fuses the N-terminal part of a nucleoporin protein, NUP98, to an H3K4me3-binding PHD finger of JARID1A or PHF23 (left panel)[21]. Such a NUP98-PHD finger fusion oncoprotein prevents the removal of H3K4me3 presumably mediated by the JARID1 histone demethylase and associated repressive factors (right panel) and enforces the expression of leukemia oncogenes such as *HOX* and *MEIS1* [21]. Arrows at the bottom indicate the effect of each complex on transcription.

(d) Upon insult of DNA damage, H3K4me3 serves as a mechanism to recruit and/or stabilize the ING protein complexes to genes involved in the regulation of cell proliferation or apoptosis, which is then followed by their repression (in case of ING1/2-HDAC complexes) or activation (in case of ING4/5-HAT complexes)[13, 74, 75]. A subset of cancer-associated somatic mutations of *ING1* specifically interfere with the binding to H3K4me3/2 marks[13, 17, 73].

(e) Recognition of H3K4me3 by the PHD finger of Pygopus (Pygo), an interacting cofactor of BCL9 and β-catenin, has been suggested to be critical for efficient activation of Wnt signaling pathway[87].

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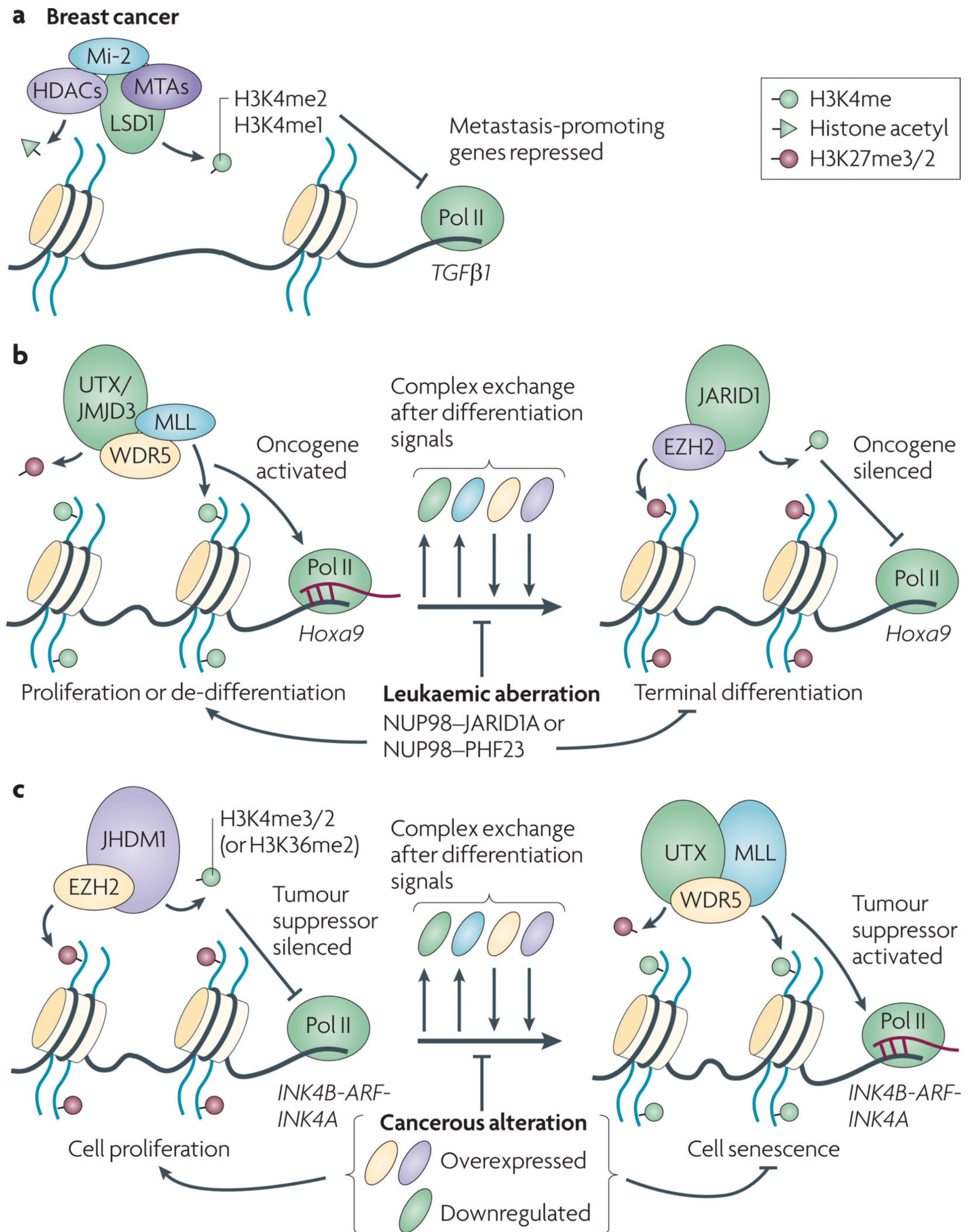

Figure 3. Physical interaction between histone methylation ‘writers’ and ‘erasers’ ensures a robust response during the transition of chromatin states, and such cooperation is also observed during cancerous transformation

(a) Cooperation between histone methyltransferases and demethylases, exemplified by MLL-JMJD3 interaction[104] and EZH2-JARID1 interaction[68], underlies a dynamic change in H3K4me3 and H3K27me3 at leukemia-associated oncogenes such as *HOX*, a process that is perturbed by leukemia oncoproteins such as NUP98-JARID1A or MLL-fusion in leukemia[21, 25].

(b) Upon RAS signaling-induced oncogenic stress or the replicative stress, switch of histone methyltransferases and demethylases underlies activation of the tumor suppressor locus

INK4B-ARF-INK4A and induction of senescence, a mechanism to prevent cancerous transformation[96–98, 100, 101, 116]. In cancer cells, over-expression of *EZH2* and *JHDM1,* or down-regulation of *JMJD3,* interferes with such a switch of chromatin state and thus senescence response[100, 101, 117].

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Table 1

Deregulation of the 'writing', 'reading', or 'erasing' of histone methylation H3K4me3 and H3K27me3 is associated with the development of human cancers.

Specificity	Category	Gene ID	Deregulation in human cancer	Reference
H3K4me3/2	writer	*MLL*	Rearrangement of *MLL* commonly found in myeloid and lymphoblastic leukemia.	25
		MLL2	Somatic mutation of *MLL2* found in renal cell carcinoma	94
	reader	*ING1/ ING2/ ING3/ ING4/ ING5*	Loss-of-function mutation of putative tumor suppressor gene *ING1-5*, in form of either somatic mutation, allelic loss, downregulation of expression, or aberrant cytoplasmic sequestration, associates with a variety of solid tumors. A subset of *ING2* somatic mutations interferes with the binding to H3K4me3 specifically.	69–71
		PHF23	Due to chromosomal translocation, the H3K4me3-binding PHD finger of PHF23 is fused to NUP98 in myeloid leukemia. It has been shown that the H3K4me3 binding is critical for leukemogenesis induced by NUP98-PHF23 oncoproteins.	21
		Pygo2	Pygo2, component of β-catenin signaling pathway, is critical for self-renewal of mammary progenitor cells. Its protein level is high in malignant breast tumors and low in non-malignant breast cells.	88, 89
	eraser	*JARID1A*	Similar to PHF23, the PHD finger of JARID1A is fused to NUP98 in a subset of myeloid leukemia, forming an oncoprotein NUP98-JARID1A. The H3K4me3 binding by the JARID1A PHD finger is critical for leukemogenesis.	21
		JARID1B	Overexpression of *JARID1B* was found in advanced breast and prostate cancers.	91, 93
		JARID1C	Recurrent inactivating mutation of *JARID1C* was detected in about 3% of renal carcinoma.	94
		JHDM1B[*]	Up-regulation of *JHDM1B* represents a common event in a screen for oncogenes that induce retrovirus-induced T cell lymphomas.	96–98
H3K27me3/2	writer	*EZH2*	Over-expression of *EZH2* is frequently found in a variety of solid tumors including prostate, breast, colon, skin, and lung cancers; On the other hand, recurrent inactivating mutations or haploinsufficiency of EZH2 is detected in about 10% of follicular lymphoma and 20% of diffuse large B-cell lymphoma of germinal center origin.	54, 63
	eraser	*JMJD3*	Downregulation of *JMJD3* was found in lung and liver cancers.	100, 101
		UTX	Sporadic inactivating mutations of *UTX* was reported in a subset of multiple myeloma, esophageal squamous cell carcinomas, renal cell carcinomas and other tumors.	99

[†]Gene full name shown as follows: MLL, mixed lineage leukemia; ING, inhibitor of growth; PHF23, PHD finger protein 23; Pygo, pygopus; JARID1, jumonji AT-rich interactive domain 1; JHDM1B, jumonji C domain-containing histone demethylase 1B; EZH2, enhancer of zeste (*Drosophila*) homolog 2; JMJD3, jumonji domain containing 3; UTX, ubiquitously transcribed tetratricopeptide repeat X chromosome.

[*]JHDM1 factors exhibit dual demethylating activities towards H3K4me3 and H3K36me2 [96–98].

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Table 2

List of PHD finger-containing proteins that specifically 'read' H3K4me3/2.

Gene ID†	H3K4me3/2 reading motif	Known function and disease relevance	Ref.
BPTF	The second PHD finger	Component of a chromatin remodeling complex NURF, which contains a SWI/SNF family helicase/ATPase SMARCA1	12, 14
ING1	PHD finger	Component of HDAC-Sin3A transcriptional repressive complexes	13, 70, 73
ING2	PHD finger	Component of HDAC-Sin3A transcriptional repressive complexes	13, 70, 76
ING3	PHD finger	Form a transcriptional activation complex with a histone acetyltransferase (HAT) Tip60	70, 76
ING4	PHD finger	Form an HBO-containing transcriptional activation complex	70, 74–76
ING5	PHD finger	Component of a transcriptional activation complex that contains a HAT protein, either HBO or MOZ/MORF	76
JARID1A	The third PHD finger	H3K4me3/2-specific histone demethylase	21
JARID1B	The third PHD finger	H3K4me3/2-specific histone demethylase	18, 21*
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, 18*
PHF13	PHD finger	Unknown function	5, 18*
PHF23	PHD finger	Unknown function	21
Pygo	PHD finger	Pygo1/2 interacts with a cofactor BCL9, and is required for Wnt/β-catenin induced transcriptional activation.	87–89
RAG2	PHD finger	A V(D)J recombinase critical 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.	20
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.	18, 19

†Gene full name shown as follows: BPTF, bromodomain PHD finger transcription factor; PHF, PHD finger protein; RAG2, recombination activating gene 2; TAF3, TATA box binding protein (TBP)-associated factor, 140kDa.

*The H3K4me3-binding property was predicted based on domain homology[5].