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Distinct functions of H3K4 methyltransferases in normal and malignant hematopoiesis

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Abstract

Purpose of review—Histone H3, lysine 4 methylation (H3K4me) is one chromatin modification that defines distinct regulatory states of euchromatin. Mammals express 6 main histone methyltransferase enzymes (HMTs) that modify H3K4 by mono-, di-, or tri-methylation. Recent studies examine roles of some of these HMTs and their cofactors in hematopoiesis and leukemia. We discuss these emerging studies together with prior embryonic stem (ES) data revealing how these enzymes function.

Recent findings—Murine models have been employed to conditionally or constitutively knock out HMTs (MLL1/KMT2A, MLL2/KMT2B, MLL3/KMT2C, MLL4/KMT2D, SETD1A/KMT2F and SETD1B/KMT2G) as well as specific domains or partners of these enzymes in normal hematopoietic populations and in the context of hematologic malignancies. These studies demonstrate that global or gene-specific changes in H3K4 modification levels can be attributed to particular enzymes in particular tissues.

Summary—Loss-of-function studies indicate largely non-overlapping roles of the six H3K4 HMTs. These roles are not all necessarily due to differences in enzymatic activity and are not always accompanied by large global changes in histone modification. Both gain- and loss-of-function mutations in hematologic malignancy are restricted to MLL1 and MLL3/MLL4, but emerging data indicate that SETD1A/SETD1B and MLL2 can be critical in leukemia as well.

Keywords

Epigenetic regulation; H3K4; HMT; histone methyltransferase; bivalent; poised

Introduction

Hematopoiesis represents an ongoing re-iteration of developmental processes, depending on the ability of stem cells and progenitors to both retain their identity and resist differentiation but also when appropriate, to respond to extracellular cues to change their identity. Identity

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Conflicts of Interest

PE owns Amgen stocks. No other conflicts were declared.

can be defined as the transcriptome status plus the potential to express particular genes. The fidelity of gene expression patterns is critically dependent on chromatin regulation, which can either resist or enable a response to a given developmental cue. Here we focus on a particular group of enzymes that perform H3K4 mono-, di- or tri-methylation, as these modifications are associated with active enhancers, lineage-specific gene expression, or active/poised genes, respectively.

Roles of H3K4 methylation states

Actively transcribed euchromatin is characterized by relative enrichment for certain histone modifications, H3K4 methylation being prominent among these. H3K4 tri-methylation (H3K4me3) typically peaks over transcription start sites (TSS) with a nucleosome-free region centered over the TSS. Recent studies have illustrated correlations between the breadth H3K4me3 enrichment with transcriptional fidelity and enhanced elongation rates [1,2**]. H3K4 di-methylation (H3K4me2) has a more complex distribution, but can be enriched over TSS's, gene bodies and enhancers. Five patterns of enrichment relative to the TSS have been bioinformatically identified [3,4] and the nature of the genes characterized by each of the patterns is very distinct, suggesting a particular tissue-specific role for this mark. Genes enriched for TSS H3K4me2 without concordant H3K4me3 enrichment have also been identified in hematopoietic progenitors as a type of "poised" gene, since this group marks a particular subset of developmental regulators and genes that are upregulated upon erythroid differentiation [4]. Since methylation of H3K4 does not change the structure or charge of the histone octamer, the outcome of these modifications is thought to attract transcriptional effector complexes through subunits that harbor H3K4 binding proteins, or "chromatin reader" domains. These chromatin reader domains can be part of basal transcription factors, repressive, or activating chromatin modifying complexes (reviewed in [5]).

H3K4me3 promoter enrichment is also associated with "bivalent" promoters. In ES cells, bivalent genes were described as harboring both H3K4me3 and H3K27me3 modifications at TSS-proximal histones, the latter mark conferred by repressive Polycomb complexes [6]. Bivalent genes are typically expressed only at low/undetectable levels, but upon induction of ES differentiation to neuronal precursor cells, genes resolve into predominantly H3K27me3- or H3K4me3-enriched and become silenced or more highly expressed, respectively [7]. This concept of developmentally bivalent genes has since been molecularly characterized further and shown to be relevant in a variety of pluripotent cell types, including hematopoietic progenitors [4,8].

Use of H3K4 methylation status to categorize enhancers in hematopoietic cells

Enhancer histone composition is so connected to cell identity that H3K4 mono-methylation (H3K4me1) (enriched at enhancers) can accurately cluster hematopoietic stem/progenitor/differentiated populations by cell type [9]. Recent studies provide evidence for both the de novo generation of lineage-specific enhancers as well as the priming-generated acquisition of lineage-specific enhancers, possibly depending on the particular lineage studied [9–11]. H3K4me1 enrichment can be used in combination with other histone modifications to define enhancers of distinct transcriptional outputs and capabilities (Figure 1). Active enhancers are

typically enriched for H3K4me1 and H3K27-acetyl (ac) marks and lack H3K4me3 enrichment (Figure 1A). Primed enhancers are similarly marked, but lack H3K27ac and poised enhancers have been defined as enriched for H3K4me1 but marked with H3K27me3. Therefore these two categories differ in the enzymatic requirements for their activation (reviewed in [12]). An interesting functional capacity defined by H3K4me1 enrichment involves enhancers that became active in macrophages upon treatment with an inflammatory activators or cytokines (Figure 1B). Upon stimulation, these enhancers typically become enriched for H3K4me1 and H3K27ac nucleosomes in the time-frame of ~4 hours in synchrony with induction of expression the associated gene. After 48-hour withdrawal of the inducing signal, these enhancers lost transcription factor occupancy and H3K27ac modification, but approximately 30% of enhancers retained their H3K4me1 enrichment. Remarkably, the kinetics of induction of the genes controlled by the H3K4me1-retaining enhancers was much more rapid upon re-stimulation with the same signal [13]. These data suggest that H3K4me1 at enhancers functions to remember the prior stimulus and more rapidly respond to re-organize the active enhancer. Given these important functional correlations, it is important to determine how these defining histone marks are enzymatically regulated. Building on prior biochemical knowledge, the following loss-of-function studies begin to resolve which enzymes perform which modifications in distinct tissues.

Six major H3K4 methyltransferases in mammals

Mammals have diversified the number and roles of dedicated H3K4 methyltransferases relative to yeast and flies. There are three pairs of SET/MLL paralogs in mammals: SETD1A/SETD1B, MLL1/MLL2, and MLL3/MLL4. We would like to emphasize that there is confusion in the literature in nomenclature for MLL2 and MLL4: here we use MLL2 for the protein encoded by the murine *Wbp7* or *Kmt2b* gene.

All six proteins exhibit H3K4me activity when in complex with their protein partners, which include WDR5, RbBP5, ASH2L and DPY30 (“WRAD”), critical for the enzymatic activity of the Su(var)3–9/Enhacer of Zeste/Trithorax (SET), or catalytic domain of MLL1-4 and the SET proteins [14,15]. In addition to sharing WRAD components, there are protein partners that are specific to the related pairs. For example, Menin and LEDGF/PSIP1 interact exclusively with MLL1 and MLL2, whereas WDR82 and CFP1/CXXC1 interact with the SETD1A/SETD1B complexes [16–18]. In addition to these core component differences, there are differences in transiently interacting proteins among these family members, due to the unique sequences of each of the six enzymes. The expression of MLL1-4 and SET proteins are all fairly ubiquitous, resulting in co-expression of most enzymes in most tissues (http://biogps.org).

MLL1 and MLL2

Due to its discovery in chromosomal translocations of childhood leukemia, *MLL1* was the first of this family to be studied extensively in the hematopoietic system using loss-of-function approaches. Multiple groups demonstrated that *Mll1* knockout was embryonic lethal and affected development of the hematopoietic system [19–24]. Specifically, *Mll1* in germ-line knock-out embryos is required to produce transplantable hematopoietic stem cells (HSCs) from the aorta-gonad-mesonephros region [19]. When specifically deleted in the

hematopoietic lineage, *Mll1* is not required for fetal liver hematopoietic cell expansion [22,25], suggesting that there were cell-extrinsic contributions to the germ-line knock-out phenotype. Nonetheless, *Mll1*−/− fetal liver hematopoietic cells also do not engraft irradiated adult recipients [22,25]. Similarly, adult HSCs (generated through conditional knockout) are severely defective in transplantation assays, likely due to the loss of expression of transcriptional factors including *Mecom*/*Evi-1*, *Hoxa9*, *Prdm16*, *Pbx1* and other important HSC regulators [26]. Interestingly, maintaining the expression of these genes in HSCs did not require the histone methyltransferase (HMT) domain of *Mll1*, rather, correlated with the ability of the MLL1 complex to impart Histone H4, lysine 16 (H4K16) acetylation. This acetylation activity is not encoded by *Mll1* itself but was attributed to recruited acetyltransferases [27].

MLL2 is the paralog of MLL1 and is highly similar in the SET domain and in primary structure [28]. Unlike MLL1, MLL2 is not involved in chromosomal translocations. In fact, the N-terminus of MLL2 cannot replace MLL1 in leukemia oncoproteins, likely due to a lower affinity for CpG sequences [29,30]. Germ-line deletions of *Mll2* result in delayed development early in embryogenesis, neural tube defects and widespread apoptosis [28]. Surprisingly, *Mll2* deletion results in global H3K4me2/3 loss in oocytes [31]. In contrast, complete deletion of *Mll2* mid-gestation had no effect on global H3K4 methylation, hematopoietic or other organ homeostasis [32].

One hematopoietic cell type in which *Mll2* does play a role is macrophages. An inducible Cre system was used to generate *Mll2*−/− macrophages from bone marrow, which appear grossly normal. However, these cells exhibited a specific defect in the induction of NFκB target genes through toll receptor 4 (TLR4) stimulation due to the failure to express MLL2 target genes critical for this signaling pathway. Several direct MLL2 target genes were identified in this study and these all exhibited reduced TSS H3K4me3 peaks with a corresponding increase in H3K27me3, a mark associated with repressed or bivalent genes. This observation suggests that MLL2's role in maintaining expression of these target genes is to maintain H3K4me3 promoter enrichment and resist invading repression complexes. Interestingly, many other genes that were hypo-H3K4 methylated in *Mll2*−/− macrophages exhibited no change in expression level [33]. These data demonstrate that some genes may not be as sensitive to H3K4me3 promoter depletion than others. Nonetheless, at least some MLL2-dependent genes appear to be regulated by promoter-targeted H3K4me3/me2 modification.

Although MLL1 was known to be a proto-oncogene for more than 25 years [34], it was not clear whether it participated in non-MLL-translocation hematologic malignancies. Recently, deletion of the endogenous *Mll1* gene in NUP98-HOXA9 or MN1-driven acute myelogenous leukemia (AML) demonstrated that endogenous MLL1 activity does contribute to leukemia maintenance in each of these distinct cytogenetic AML models [35**,36]. The role of endogenous MLL1 in MLL-driven leukemia is evolving, with some studies suggesting that it is required and some suggesting that it is not [27,37–39]. Furthermore, emerging data suggests that MLL2 may play a more important role in MLL-fusion-driven leukemia (Yufei Chen, University of Colorado, personal communication) as well as a role in solid tumors (reviewed in [40]).

MLL3 and MLL4

MLL3/4 in mammals were identified as HMTs in a co-activator complex associated with nuclear receptors [41–44] and are also recruited by other sequence-specific activators [43,45,46]. Complete knockout of either gene is embryonic or perinatal lethal and there is also evidence for redundancy between these genes, as co-deletion of both MLL3 and MLL4 was required to observe a global decrease in H3K4me1 in adipocytes [43]. MLL3 and MLL4 in vivo predominantly maintain H3K4me1 levels on enhancers [43,47] and recruit CBP/p300 to enhancers [47,48*].

Both MLL3 and MLL4 act as tumor supressors in leukemia, and have also been implicated in sold tumors [40]. MLL3 resides in the 7q region deleted in AML and knockdown of *Mll3* collaborates with *Nf1* knockdown in mouse models to produce an aggressive AML [49]. MLL4 is mutated in 30–90% of human diffuse large B-cell lymphomas (DLBCL) and follicular lymphomas (FL) [50, 51**, 52]. Genomic alterations are predicted to encode loss-of-function proteins, with about half mono-allelic and half bi-allelic. The direct role of MLL4 as a tumor suppressor was confirmed in murine leukemia models. Knock-down of *Mll4* in a Vav-Bcl2 transgenic model or deletion of *Mll4* in an activation-induced cytidine deaminase transgenic lymphoma model accelerated lymphomagenesis [53**]. In wild type animals, B cell-specific deletion of *Mll4* increased steady-state transitional B cell numbers, enhanced germinal center formation and enhanced proliferation in response to CD40 stimulation. Loss of MLL4 was also sufficient for lymphoma development with a mean survival of just under 1 year [53**] and to observe a global reduction in H3K4me3/2/1 [51**]. Therefore MLL4 functions in a non-redundant manner to suppress mature B-cell proliferation and act as a tumor suppressor in FL and DLBCL.

The role of the MLL3/MLL4 proteins in normal hematopoiesis appears generally similar as either knockdown of *Mll3* or knockout of *Mll4* in hematopoietic stem/multipotent progenitor cells (HSPCs) results in impaired differentiation of HSPCs and increased HSPC numbers [49,54]. *Mll4*−/− HSPCs exhibit reduced engraftment in secondary recipients, accompanied by increased reactive oxygen species [49,54]. In the case of the *Mll4* knockout, these defects were attributed to reduced expression of MLL4-dependent genes that protect from oxidative stress [54]. In contrast to the lymphoma context described above, deletion of *Mll4* in an MLL-AF9-driven AML model system limited leukemia progression [54]. These distinct tumor-promoting or tumor-suppressor roles of MLL4 most likely reflect the different cellular contexts and target genes in AML versus lymphoma.

SETD1A and SETD1B

These two proteins are highly related overall and in SET domain sequence, but are each independently required for embryogenesis. *Setd1a*-/- blastocysts do not gastrulate and exhibit a defective inner cell mass and ES cells cannot be derived from knockout blastocysts. *Setd1b*-/- embryos exhibit growth defects from embryonic day (E) 7.5 and die before E11.5. In ES cells engineered with inducible (Rosa-ER^{T2}) Cre, SETD1A loss is more severe, with growth arrest and cell death occurring upon protein reduction, as well as a global reduction in H3K4me3/2/1. Parallel experiments to delete *Setd1b* did not result in global H3K4

methylation changes, but it is not yet clear if redundancy between these two SET proteins accounts for this observation [55].

The conditional deletion of *Setd1a* in bone marrow hematopoietic cells revealed a unique role in B cell differentiation from the pro-B to pre-B cell stage. Global H3K4 methylation levels were not reported in these studies, but reduction of H3K4me3 at important B-cell regulators and the immunoglobulin heavy chain locus was suggested to underlie the block in differentiation [56]. Similarly, erythroid-specific *Setd1a* deletion partially blocked erythropoiesis, resulting in mild anemia. Reduced expression of lineage regulators such as *Gata1* and *Tal1* was noted, as was reduced H3K4me3 at the promoters of these genes [57*].

An interesting comparison to the *Setd1a/Setd1b* knockouts described above are knockouts of two genes encoding components of the SET complexes, CFP1/CXXC1 and DPY30. CFP1 provides CpG-element and chromatin targeting to the SET complexes. *Cxxc1* germ-line deletion results in a pre-gastrulation phenotype very similar to *Setd1a-/-* embryos [58]. In *Cxxc1−/−* ES cells, about half of all H3K4me3 peaks at promoters are reduced, particularly from the highly expressed genes. In addition, H3K4me3 is ectopically-acquired in new nuclear territories, resulting in ectopic transcription of formerly silenced genes [59,60]. These cells can be maintained in pluripotent conditions, but fail to differentiate. Amazingly, the most highly expressed genes suffered the greatest H3K4me3 loss, yet did not exhibit reduced expression, similar to the *Mll2* observations discussed above [60]. Therefore, CFP1 is critical for directing SET1 protein-mediated H3K4 methylation activity to proper genomic locations. DPY30, in contrast to CFP1, participates in all H3K4 methyltransferase complexes. In ES cells, depletion of *Dpy30* does result in a global reduction in H3K4me3/me2. Similar to *Cxxc1*−/− ES cells, *Dpy30*-depleted ES cells can be maintained as pluripotent cells but fail to differentiate [61]. These ES studies suggested that 1) reduction in H3K4me3/2 level at the TSS does not necessarily result in reduced expression of the corresponding gene, and 2) H3K4me3/2 change is required for differentiation.

Within the hematopoietic system, conditional deletion of *Setd1a*, *Cxxc1*, or *Dpy30* results in the accumulation of short-term HSCs and multi-potent progenitors but these cells do not engraft secondary recipients in the case of *Setd1a* and *Dpy30* knockouts [62**,63]. These data suggest that the loss of *Setd1a*-mediated H3K4 modifications may dominate the phenotype in the *Dpy30* knockout, but more detailed side-by-side comparisons will be required to determine this. Genes deregulated in HSPCs are very distinct between the *Dpy30/Setd1a* as compared to *Mll1* and *Mll2* knockouts, further underscoring the unique functions of each of the HMTs in the hematopoietic system ([62**,63], Waskow C, personal communication, TU Dresden).

Conclusions

Significant progress has occurred in the last several years toward understanding both the roles of H3K4me1/me2/me3 enriched genomic regions and in unraveling which enzymes are responsible for which modifications in specific tissues. Hematopoietic differentiation has provided a very well-characterized biological system for understanding the dynamics of these modifications and their relationship to differentiation. One principle emerging from

murine knockout and human leukemia/lymphoma studies is that despite similarity between paralogs and co-expression in many of the same tissues, each H3K4 HMT has mainly unique, non-redundant functions, although one can find examples of redundancy. The first observation underscores the importance of the molecules that recruit the particular HMT complexes to particular loci in the genome, which is currently poorly understood. Most H3K4 HMTs play multiple and significant roles in the hematopoietic system and likely also play important roles in hematologic malignancies. Determining in what setting and how to target these enzymes with small molecule inhibitors represents an exciting new frontier.

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Key points

- H3K4me1, in combination with other marks, defines several enhancer states
- H3K4me3 enrichment can be uncoupled from active gene expression
- Most H3K4 HMTs play important roles in leukemia/lymphoma and MLL1, MLL3 and MLL4 are mutated in leukemia
- All six mammalian H3K4 HMTs perform unique functions in the hematopoietic system

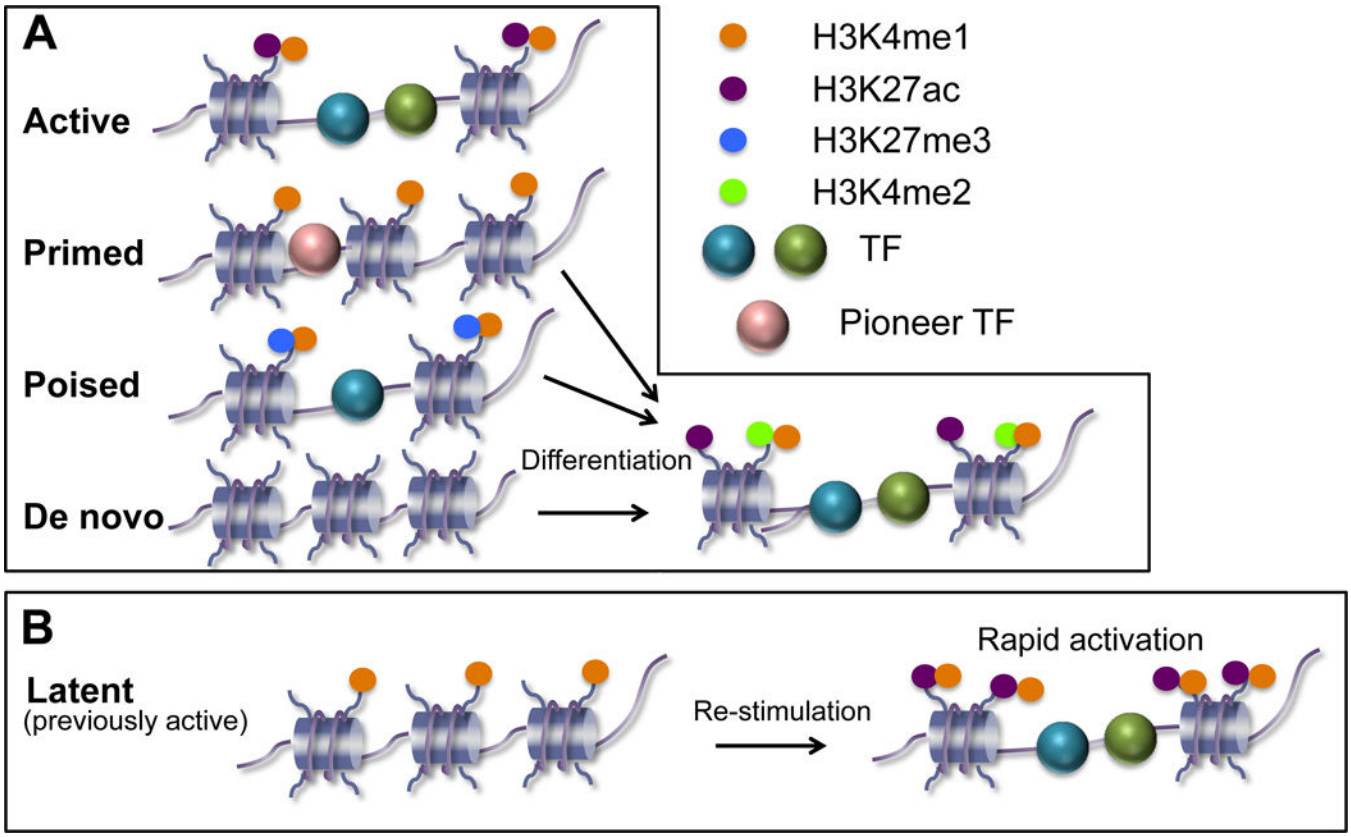

Figure 1. Enhancer categories defined based on enrichment for histone modifications

A) Enhancers and their propensity for becoming active can be defined by combinations of H3K4me1 and other modifications and transcription factor (TF) occupancies. Pioneer TFs are those who may bind to enhancers prior to any histone modifications to recruit subsequent enzymes and remodeling complexes. Active enhancers are open chromatin domains enriched minimally for H3K4me1 and H3K27ac. Primed enhancers are pre-marked by H3K4me1 and can be further activated at upon a differentiation signal. Poised enhancers are enriched for H3K4me1 and repressive H3K27me3, and are thus are “poised” to become active upon removal of the H3K27me3 mark and differentiation [9]. De novo activated enhancers are inferred from the lack of modifications at a prior differentiation stage [10,11]. **B)** In the context of macrophage activation [13], latent enhancers may have been previously activated by transcription factors but retain H3K4me1 as “memory” and are more rapidly activated upon re-stimulation relative to those lacking H3K4me1.