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REVIEW

Lysine-specific histone demethylases in normal and malignant hematopoiesis

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The epigenetic control of gene expression is central to the development of the hematopoietic system and the execution of lineage-specific transcriptional programs. During the last 10 years, mounting evidence has implicated the family of lysine-specific histone demethylases as critical regulators of normal hematopoiesis, whereas their deregulation is found in a broad spectrum of hematopoietic malignancies. Here, we review recent findings on the role of these enzymes in normal and malignant hematopoiesis and highlight how aberrant epigenetic regulation facilitates hematopoietic cell transformation through subversion of cell fate and lineage commitment programs.

Although histone methylation was originally thought to be an irreversible chromatin modification, biochemical characterization of KDM1A/LSD1, a flavin-dependent amine oxidase, led to the discovery of the first known histone demethylase. KDM1A is a component of the CoREST transcriptional repressor complex and exhibits H3K4me2/me1 demethylase activity [1,2]. Subsequent studies showed that KDM1A also associates with nuclear receptors, where it functions as a coactivator through demethylation of repressive H3K9me2 [3,4]. The KDM1A homolog KDM1B/LSD2 is also an H3K4me2 demethylase [5,6]. However, KDM1A/B cannot remove trimethyl groups from modified lysines, which suggests that alternative enzymatic activities exist. Indeed, several research groups have discovered that the Jumonji C (JmjC) domain family of Fe(II)- and α-ketoglutarate–dependent dioxygenases function as histone demethylases in a mechanism that involves oxidative hydroxylation by radical attack of the methyl group (for review, Kampranis and Tsichlis, 2009 [7] and Kooistra and Helin, 2012 [8]). The human genome encodes approximately 30 JmjC domain histone demethylases that, based on structural homology of the JmjC domain, can be further divided into groups with activities toward H3K4, H3K9, H3K27, and H3K36 modifications. Several of these demethylases have also been shown to act on nonhistone substrates [7,8]. Considering the fast pace of advancements in the field of epigenetics, it is likely that additional enzymatic systems capable of demethylating histones will be described in the future. Mass spectrometry–based studies revealed that lysine-specific histone demethylases participate in multiprotein complexes to regulate gene expression, heterochromatin formation, and genome organization [7,8]. In recent years, several of these enzymes have been implicated in the epigenetic regulation of normal hematopoiesis, whereas their deregulation has been linked to the development of leukemias. Given the reversibility of histone methylation, understanding the context-dependent roles of histone demethylases will be critical for the development of novel therapies.

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Histone demethylases in hematopoiesis

In murine embryogenesis, definitive hematopoiesis commences around embryonic day 10.5 from the hemogenic endothelium (HE) of the aorta–gonad–mesonephros

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(AGM) region. The hematopoietic stem and progenitor cells (HSPCs) that arise from the AGM are the first to harbor multilineage reconstitution capacity [9,10]. These HSPCs subsequently migrate to the fetal liver, where they expand before homing to the bone marrow, the permanent site for hematopoiesis throughout adulthood [9,10]. In adult mice, HSPCs reside in a compartment defined by a lack of lineage-specific markers (Lin^{neg}) and positive for both c-Kit and Sca-1 ($Lin^{neg}KS^{+}$), whereas long-term hematopoietic stem cells (HSCs) are further identified by the presence of CD150 and the absence of CD48 and can rescue myeloablated mice from hematopoietic failure and establish long-term multilineage reconstitution [11].

The first insight into the role of histone demethylation in hematopoietic development began with the finding that deletion of Kdm1a perturbs terminal differentiation of erythroid, megakaryocytic, and granulocytic cells by derepressing Gfi-1/1b-lineage-specific transcriptional programs [12]. Further studies revealed an indispensable role for Kdm1a in the emergence of HSCs from the AGM through silencing the endothelial program within the HE in mice and zebrafish [13,14], suggesting an evolutionarily conserved role in definitive hematopoiesis. Conditional deletion of *Kdm1a* in fetal ($Vav1^{Cre}$) and adult ($Mx1^{Cre}$) HSPCs resulted in severe pancytopenia and compromised terminal differentiation of granulocytic and erythroid lineages [15,16]. At the molecular level, Kdm1a binds enhancers of genes that regulate self-renewal and lineage commitment pathways and demethylates H3K4me1/2 as part of the CoREST repressive complex [12,13,15].

More recently, the JmjC domain histone H3K36 didemethylase KDM2B/FBXL10 has also been shown to play an important role in definitive hematopoiesis [17]. Kdm2b is highly expressed in the HE, and its deletion ($Tie2^{Cre}$) caused embryonic lethality due to a precipitous drop in the number of hemogenic endothelial cells within the AGM. Conversely, $Vav1^{Cre}$;$Kdm2b^{fl/fl}$ mice were viable, but displayed a dramatic reduction in the number of long-term HSCs and defective lymphopoiesis, which was accompanied by a concomitant upregulation of myeloid differentiation [17]. A similar phenotype was also observed in $Mx1^{Cre}$;$Kdm2b^{fl/fl}$ mice upon *pIpC* administration, suggesting an important role for Kdm2b in the maintenance of HSPCs and the regulation of lymphopoiesis, the latter in a JmjC domain–dependent manner [17]. Gene expression and chromatin immunoprecipitation studies coupled with next-generation sequencing showed that KDM2B associates, in a mutually exclusive manner, with Trithorax (TrxG)-active and Polycomb (PcG)-repressed chromatin to regulate quiescence, cell fate, and lineage commitment. In mammalian cells, the TrxG proteins MLL1–4 reside in a complex that includes WDR5, RBBP5, ASH2L, and DPY30. This complex methylates histone H3K4 to activate transcription. Although it does not interact physically, KDM2B co-binds with TrxG proteins on gene promoters to enhance NOTCH1 signaling and promote T-cell commitment. Conversely, PcG proteins reside in two complexes, Polycomb Repressive Complex 1 (PRC1) and 2 (PRC2), which function as global transcriptional repressors through H2AK119 ubiquitination and H3K27 trimethylation, respectively. KDM2B is an integral component of a noncanonical PRC1 and cross-talks with PRC2 to repress transcriptional programs of myeloid differentiation. Therefore, these distinct functions of KDM2B ensure the faithful execution of transcriptional programs for the initiation of lymphoid and the repression of myeloid commitment [17].

Hematopoietic cell migration is critical for normal hematopoiesis [9,10]. KDM6A/UTX, an H3K27me3 demethylase encoded by the X chromosome, has been shown to play an important role in the migration of HSCs in response to SDF-1/CXCR4 signaling, although the exact molecular mechanism(s) remains elusive [18]. Female *Kdm6a*-null mice displayed hematopoietic phenotypes, such as myelodysplasia and suppressed erythro-megakaryocytopoiesis, whereas their male counterparts showed no phenotype. Given that UTY, encoded by the Y chromosome and exhibiting $>80\%$ similarity to KDM6A, does not demethylate H3K27me3 due to mutations in the JmjC domain [19,20], this suggests that KDM6A regulates these responses in a demethylase-independent manner. KDM6A is an integral component of MLL3/4 H3K4 methyltransferase complexes [21,22] and likely functions as a scaffold to facilitate the recruitment or the activity of this complex toward its substrate. Interestingly, although KDM6A demethylase activity is dispensable in definitive hematopoiesis, H3K27me3 demethylation by either KDM6A or KDM6B/JMJD3 (encoded by an autosomal gene) is crucial for the terminal steps of T-cell differentiation [23]. Another study showed that T-cell–specific ablation of KDM6B promoted Th2 and Th17 and inhibited Th1 and Treg differentiation under different cytokine-induced conditions [24]. The latter was also dependent on active demethylation and changes in the expression of key genes such as *Tbx21, Gata3*, and *Foxp3*. The preferential impact of KDM6A/B on late T-cell maturation may be linked to the enzymes' role in integrating extracellular cues and cytokine signals to regulate differentiation [23,24]. Further studies using *Cre* strains at different stages of hematopoietic development and the generation of demethylation-deficient mutants of KDM6A and KDM6B through CRISPR-mediated genome editing will be useful in determining the demethylase-dependent and -independent functions of these enzymes in hematopoiesis.

Histone demethylases in hematopoietic malignancies

Although epigenetic mechanisms are implicated in the pathogenesis of hematopoietic malignancies, little is known about the role of lysine-specific histone demethylases and