

## Review Article

# Heterochromatin and Polycomb as regulators of haematopoiesis

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Haematopoiesis is the process by which multipotent haematopoietic stem cells are transformed into each and every type of terminally differentiated blood cell. Epigenetic silencing is critical for this process by regulating the transcription of cell-cycle genes critical for self-renewal and differentiation, as well as restricting alternative fate genes to allow lineage commitment and appropriate differentiation. There are two distinct forms of transcriptionally repressed chromatin: H3K9me<sub>3</sub>-marked heterochromatin and H3K27me<sub>3</sub>/H2AK119ub<sub>1</sub>-marked Polycomb (often referred to as facultative heterochromatin). This review will discuss the role of these distinct epigenetic silencing mechanisms in regulating normal haematopoiesis, how these contribute to age-related haematopoietic dysfunction, and the rationale for therapeutic targeting of these pathways in the treatment of haematological malignancies.

## Introduction

Haematopoiesis involves highly regulated commitment and differentiation processes to transform multipotent haematopoietic stem cells (HSCs) into each and every type of terminally differentiated blood cell [1] (Figure 1). Whether these differentiation processes involves step-wise fate transitions or are part of a continuous differentiation process is an increasingly controversial topic, with the latter view supported by single-cell sequencing analyses revealing unprecedented heterogeneity in both HSCs and their downstream progenitors [2]. Regardless, discrete haematopoietic cell lineages are required to express different sets of genes from within their identical DNA, and are, therefore, dependent on epigenetic mechanisms to co-ordinate these distinct transcriptional programmes [3]. Whilst the co-ordinated expression of key genes such as those encoding lineage-specific transcription factors is obviously necessary, this is not alone sufficient as it is equally critical that lineage-inappropriate genes are efficiently silenced. As such, failure of epigenetic silencing in the haematopoietic compartment is associated with a host of haematopoietic neoplasms [4] as well as impaired immune system responses [5].

## Epigenetic mechanisms of gene silencing

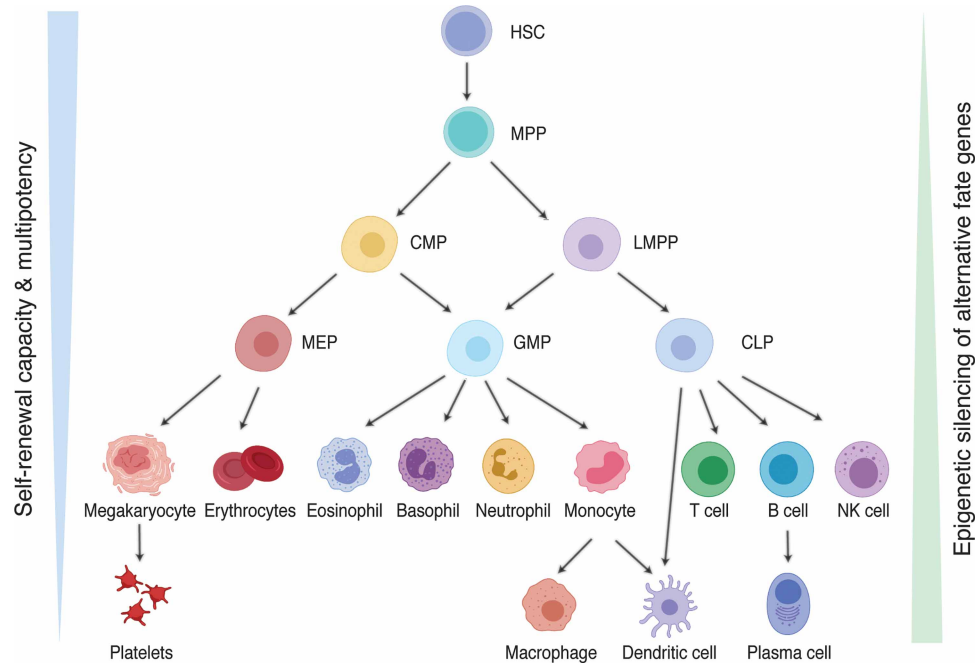
There are two distinct forms of transcriptionally repressed chromatin: classical heterochromatin and Polycomb-associated regions [6] (Figure 2). Classical or constitutive heterochromatin is particularly enriched for trimethylation of lysine 9 of histone H3 (H3K9me<sub>3</sub>), as well as H3K9me<sub>2</sub>, and is associated with repetitive genomic regions such as telomeric and centromeric regions. This heterochromatic state is formed by the recognition of H3K9me<sub>2/3</sub> by heterochromatin protein 1 (HP1) which self-oligomerizes causing compaction of chromatin and rendering it inaccessible to the transcriptional machinery [7]. HP1 in turn recruit SUV4-20H enzymes which deposit another histone mark of heterochromatin H3K20me<sub>3</sub> [8]. Moreover, histone methylation at H3K9 further reinforces transcriptional silencing by promoting DNA methylation and preventing histone acetylation [9]. In contrast, Polycomb-associated regions are marked by trimethylation at lysine 27 of histone H3 (H3K27me<sub>3</sub>) and ubiquitination of lysine 119 of histone H2A (H2AK119ub<sub>1</sub>) and are involved in cell-type-specific

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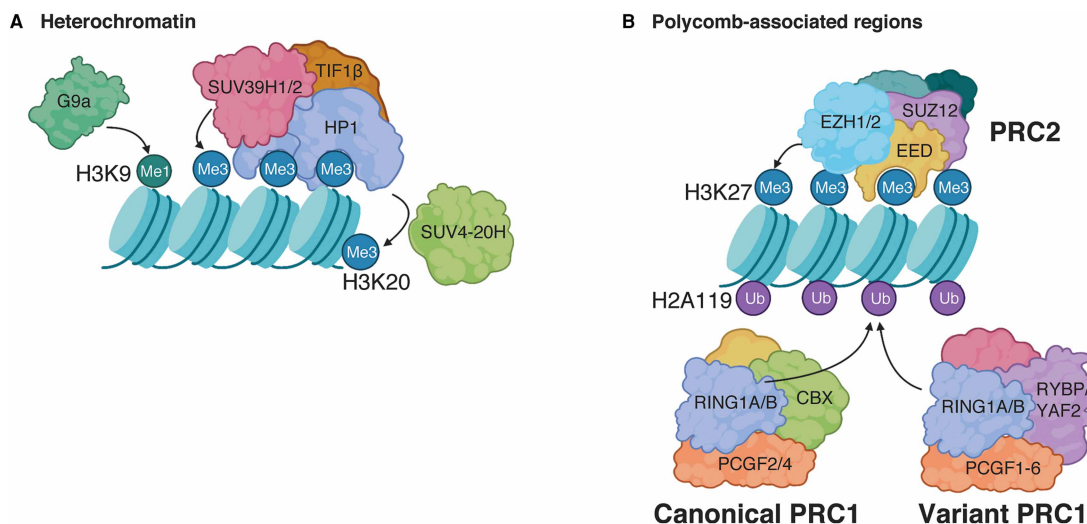
**Figure 1. Haematopoiesis involves the epigenetic silencing of alternate fate genes, reducing multipotency and the self-renewal capacity as cells differentiate.**

Haematopoietic stem cells (HSC) differentiate to multipotent progenitors (MPP) which in turn differentiate to the increasing more committed common myeloid progenitor (CMP) and lymphoid-primed multipotent progenitors (LMPP). These in turn produce the myeloid lineages and lymphoid lineages, respectively, via the megakaryocyte-erythrocyte progenitors (MEP), granulocyte-monocyte progenitors (GMP) and common lymphoid progenitors (LMPP). Whilst it is contentious how step-wise these differentiation events are, discrete cell lineages are required to express different sets of genes from within their identical DNA, and are, therefore, dependent on epigenetic silencing mechanisms to repress alternative fate genes.

silencing of developmentally regulated genes. Polycomb-associated regions are often referred to as ‘facultative heterochromatin’. However, whilst H3K27me3 has been shown to block transcription initiation [10], H3K27me3-marked regions can remain accessible to binding by both transcription factors as well as paused polymerase [11]. Indeed, recent biophysical assays based on the sonication resistance of chromatin regions have found ~30% of H3K27me3 marks to be on lowly transcribed genes in euchromatin, whereas ~97% of H3K9me3 are found in the sonication-resistant fraction [12]. There are, therefore, clear functional and biophysical differences between heterochromatin and Polycomb-associated regions.

## Classical heterochromatin in haematopoiesis

Canonical heterochromatic H3K9me3 deposition is catalysed by the suppressor of variegation homologue (SUV39H) enzymes SUV39H1 (KMT1A) and SUV39H2 (KMT1B), with the former the predominant of these enzymes in the haematopoietic system (Figure 2A). SUV39H1 is essential for maintaining healthy HSC function [13], as are the HP1 molecules and the accessory protein TIF1β/KAP1 [14]. SUV39H1 is also critical for the downstream differentiation of various haematopoietic lineages such as Th2 cells [15] and CD8<sup>+</sup> effector T cells [16]. In each case, cell differentiation is facilitated by the SUV39H1-mediated silencing of alternative fate genes such as those of the Th1 lineage for Th2 cell differentiation, and stem-cell-related genes for CD8<sup>+</sup> T cell differentiation. Interestingly, transgenic mice overexpressing SUV39H1 show impaired erythroid cell differentiation with immature erythroblasts stuck in a continuous proliferative phenotype [17]. Together, these studies suggest that SUV39H1-dependent heterochromatin plays an important role in governing the balance between proliferation and differentiation potentials of haematopoietic progenitor cells. Moreover, H3K9me3 likely provides a programming barrier reducing the plasticity of cells as they become lineage-committed in



**Figure 2. Heterochromatin and Polycomb-associated regions are distinct forms of epigenetically silenced chromatin.** (A) Heterochromatin is marked by H3K9me3 deposited by the suppressor of variegation (SUV) 39-homologue enzymes (SUV39H1 and SUV39H2). These enzymes preferentially bind already monomethylated substrate (H3K9me1). H3K9me3 is recognised by heterochromatin protein 1 (HP1) which self-oligomerizes causing compaction of chromatin and rendering it inaccessible to the transcriptional machinery. HP1 in turn recruits SUV4-20H enzymes which deposit another histone mark of heterochromatin H3K20me3. (B) Polycomb-associated regions are particularly involved in cell-type-specific silencing of developmentally regulated genes and are marked by H3K27me3 deposited by the Polycomb repressive complex (PRC) 2 and H2A119ub1 deposited by the PRC1 complex. Polycomb-associated regions block transcription initiation but can remain accessible to the binding of transcription factors, unlike heterochromatin.

haematopoiesis. This latter concept fits with studies of somatic cell reprogramming wherein inhibition of SUV39H1 by shRNA strongly enhances the generation of induced pluripotent stem cells (iPSCs) [18].

Heterochromatin stability and the SUV39H enzymes have been linked to ageing-associated dysfunction across a spectrum of cellular systems including the haematopoietic system [13,19,20]. The functional defects of ageing haematopoiesis include reduced HSC potential, myeloid skewing at the expense of lymphoid output [21,22], impaired immunity [23] and an increase in haematological diseases [24]. With age, SUV39H1 expression and H3K9me3 levels in HSCs both reduce in humans and mice [13], causing, or at least contributing to, the dysfunction seen in elderly individuals. Remarkably, Suv39h2 expression can maintain murine HSC function in the absence of Suv39h1, despite H3K9me3 levels being only ~10% that of wildtype levels [20]. This is the first documented role of Suv39h2 in the haematopoietic system although it is also known to be induced in human activated T lymphocytes [25] so may have a role in adaptive immune responses.

A third enzyme SETDB1 (KMT1E) also catalyses H3K9me3 deposition and is also essential for maintaining HSC function by preventing ectopic activation of non-haematopoietic genes [26]. SETDB1, unlike SUV39H1/2, is mainly localised in euchromatin but can in some circumstances be found in heterochromatic regions in a mega-complex also containing SUV39H1 and other factors [27]. Interestingly, the H3K9 methyltransferase that deposits mono and demethylation (H3K9me1/2), G9a (EHMT2), is dispensable for HSC maintenance [28], but is important for the initial differentiation of HSCs from embryonic stem cells (ESCs) [29]. This initial methylation is important in the early establishment of heterochromatin with H3K9me1 the preferred substrate for the SUV39H enzymes [30] (Figure 2A). Together these studies suggest a complex interplay of different H3K9 methyltransferases to guide haematopoietic differentiation and maintain HSC function.

An additional dimension in heterochromatin regulation of haematopoiesis is the biophysical link between heterochromatin and the nuclear lamina which is thought to provide structural support to the nucleus and to protect the genome from damage. Our recent studies suggest the stability of heterochromatin at the nuclear periphery also has an important gene regulatory function supporting gene transcription in euchromatic regions [31], and that this peripheral localisation of heterochromatin may also guide the three-dimensional positioning of chromosomes into multi-lobed neutrophil nuclei [32]. Interestingly, a recent study has shown that the nuclei

of HSCs undergo dramatic morphological changes during myeloid or lymphoid differentiation [33]. Surprisingly, these morphological changes do not seem to be a consequence of a softening of the nuclear lamina, but rather are driven by cytoplasmic microtubules squeezing the nucleus to form large deformations altering chromatin organisation, causing localised loss of H3K9me3 and H3K27me3, and altering gene expression [33]. This new frontier of the mechanobiology will likely reveal further important roles of heterochromatin in haematopoiesis.

## Polycomb-associated regions in haematopoiesis

There are two molecularly distinct Polycomb repressive complexes (PRC) formed by so-called Polycomb group (PcG) proteins (Figure 2B). PRC1 complexes contain E3 ubiquitin ligases that catalyse the ubiquitination of lysine 119 of histone H2A (H2AK119u1), whereas PRC2 complexes catalyse H3K27me3 [34]. Whilst there is some diversity in PRC2 with two main sub-types described [35,36], all variant complexes contain three core components unique to PRC2: the enzymatic component EZH2, or in some cases the less active paralogue EZH1 [37], as well as the scaffold protein SUZ12, and EED which binds H3K27me3 allowing spreading of the modification to neighbouring nucleosomes [38]. Other PRC2 components such as RBBP-4 and -7 are found in other chromatin complexes, and these additional proteins may mediate distinct modes of recruitment to target genes [36]. The composition of PRC1 is considerably more varied and is broadly categorised into canonical and variant forms based on whether chromobox (CBX) proteins are in the complex. All forms of PRC1 are comprised of a catalytic core formed by a really interesting new gene (RING) E3 ubiquitin ligase RING1A or RING1B and one of six Polycomb group ring finger (PCGF) proteins, with canonical PRC1 containing PCGF2/4 and CBX subunits, and variant forms containing any of the six PCGF proteins and RYBP or YAF2 in place of CBX [39] (Figure 2B). Interestingly, canonical PRC1 has lower catalytic activity than variant PRC1 but has important roles in shaping three-dimensional genome organisation and chromosomal architecture [40]. There is also a complex reciprocal relationship between PRC1 and PRC2 complexes, with each deposited histone mark facilitating binding of the other complex, and they often unsurprisingly co-occupy genomic loci [41].

Given Polycomb-associated regions predominantly mark developmentally regulated genes, it is no surprise that both PRC1 and PRC2 are essential regulators of haematopoiesis. Mechanistically, both PRC1 and PRC2 control haematopoiesis through regulation of cell-cycle genes required for both self-renewal and differentiation as well as regulation of transcriptional programmes controlling differentiation pathways and restriction of alternative fate genes. Loss of PRC1 function [42] and loss of PRC2 function through EZH1/2 [43], SUZ12 [44], or EED [45] deficiency each strongly impairs HSC function and HSC self-renewal, whereas HSCs overexpressing EZH2 show enhanced self-renewal [46]. Interestingly, initial studies using EZH2 deficiency did not show such a dramatic phenotype [47], likely due to compensation by EZH1, and gene dosage appears to play a role with heterozygous mice displaying enhanced HSC and progenitor activity [42]. Mechanistically, PRC1 and PRC2 both promote self-renewal of HSCs through repression of the cell-cycle inhibitor *CDKN2A* (p16<sup>INK4A</sup>) [48]. Of note, EZH2 has several described non-canonical functions beyond Polycomb [49–53] so attributing phenotypes from EZH2 deficiency to loss of Polycomb-mediated repression really requires alternate validation such as through deletion of SUZ12 or EED. Critically, PRC1 and PRC2 both also have important and complex roles in many latter lineage differentiation decisions that are beyond the scope of this brief review (recently reviewed in [38,54]).

The molecular composition of the PRC1 complex appears to govern the self-renewal and differentiation decision of HSCs by altering the loci targeted for silencing [55]. It is currently not thought that variant PRC2 sub-complexes similarly target distinct sets of genes, with ChIP-seq studies of non-core subunits showing largely overlapping targets [56,57]. The milder phenotypes produced from deletion of non-core components, as compared with deletion of core components, further suggests overlap in function between variant PRC complexes [58]. Interestingly, RNA binds both major sub-types of PRC2, and is thought to facilitate PRC2 recruitment to target genes, so as to retain it in a poised state at lowly expressed genes, and remove it from more highly expressed genes [59]. Further studies are required to fully understand the evolutionary reason for, or utility of, the variant PRC2 complexes.

With age, the location of H3K27me3 in the genome of HSCs is largely unchanged, but higher and broader peaks are found [60], although the significance of this is not obvious. Maintenance of H3K27me3 and loss of the active histone mark H3K4me3 at bivalent promoters has been reported in ageing [61] altering the potential for these genes to be activated, a phenomenon also seen during malignant transformation. Interestingly, many PRC2 target genes have been shown to be hypermethylated in aged HSCs [62,63]. Given many PRC2 complex subunits themselves seem to be down-regulated with age [60], this DNA methylation may be a compensatory

mechanism to maintain gene silencing despite lower PRC2 levels. Less is known about the regulation of PRC1 in haematopoietic ageing, although it almost certainly plays a role. A recent study has shown that ectopic expression of PCGF4 (BMI1) target genes is observed in HSC ageing [64], although PCGF4 expression does not change with age so the mechanism by which this repression is lost is not known.

## Histone demethylation in haematopoiesis

Histone modifications were long thought to be permanent additions to chromatin, however, it is now known these modifications are dynamic and actively removed by histone demethylation enzymes [65]. Many histone demethylase enzymes are more promiscuous than histone methyltransferases enzymes showing demethylation activity at multiple residues, unlike the highly site-selective methyltransferases. Three classes of mammalian histone demethylases can remove H3K9 methylation with the KDM4 (JHDM3/JMJD2) family responsible for H3K9me3 demethylation (but can also demethylate H3K9me2, H3K36me2, and H3K36me3) [66–68], whereas KDM3 (JHDM2) and KDM7 (PHF8/KIAA1718) families are responsible for H3K9me1/2 demethylation [67,69] (but some members of these families can also demethylate H3K27me1/2 and H4K20me1). KDM4 demethylation is essential for HSC maintenance [70] suggesting H3K9me3 levels must be finely tuned to regulate the transcriptional programmes of self-renewal and differentiation. In contrast, the KDM3 family member JMJD1C is largely dispensable for normal haematopoiesis [71], but promotes haematopoietic neoplasms [72].

H3K27 demethylation is predominantly catalysed by the KDM6 family made up of 3 members KDM6A (UTX), KDM6B (JMJD3) and the male-specific KDM6C (UTY) encoded on the Y chromosome. KDM6A (UTX) has been shown to be critical for haematopoietic stem cell migration and normal haematopoiesis in female mice, but not in male mice, suggesting that KDM6C can compensate for KDM6A loss [73]. KDM6B is critical for HSC self-renewal [74] and KDM6B over-expression leads to activation of innate immune signalling genes and altered haematopoiesis [75]. Both KDM6A and KDM6B are redundantly important for terminal T cell differentiation and function [76,77] and KDM6 family members are also important for B cell differentiation [78], and iNKT differentiation [79,80]. Interestingly, a recent study exploring the mechanism of KDM6A in suppressing myeloid leukemogenesis found it does this by modulating transcriptional programmes of the oncogenic ETS and tumour-suppressive GATA transcription factors, but importantly that this is through non-catalytic functions of KDM6A with minimal effects on H3K27 methylation levels observed [81]. Together these studies suggest that KDM6 family members exhibit some redundancy and some functions beyond H3K27 demethylation, yet are important regulators of haematopoiesis.

## Targeted epigenetic silencing for the treatment of haematological malignancies

One of the key roles of H3K9me3-marked heterochromatin is to prevent genome instability [82]. Unsurprisingly, SUV39H1 and SUV39H2 expression is often reduced in haematological malignancies such as chronic lymphocytic leukaemia (CLL) [83]. Moreover, H3K9me3 levels decrease at core promoter regions in acute myeloid leukaemia (AML), and are associated with clinical outcome [84]. Conversely, loss of H3K9me3 methyltransferases is protective in some models of leukaemia [85], likely due to a loss of silencing of tumour suppressor genes. SUV39H1 has, therefore, been proposed as a therapeutic target in a host of haematological malignancies. However, the lack of a specific inhibitor has precluded this approach from being progressed into the clinic. Indeed, one study has shown that SUV39H1 inhibition is able to reactivate some tumour suppressor genes [86], however, the inhibitor chaetocin has since been shown to be a pan-methyltransferase inhibitor and not selective for SUV39H1 [87]. Interestingly, this inhibitor is also able to kill primary cells from AML patients [88]. Whilst SUV39H1 is the major heterochromatic H3K9me3 methyltransferase in the haematopoietic compartment, SUV39H2 has been shown to be up-regulated in acute lymphoblastic leukaemia (ALL) [89], therefore, may also be a useful therapeutic target for this disease.

In contrast, PRC2 inhibitors have been extensively trialled as cancer therapeutics with the first of these, tazemetostat, approved by the FDA (U.S.A.) for the treatment of a rare sarcoma in 2020. These PRC2 inhibitors are predominantly SAM-competitive EZH2 inhibitors, but several allosteric EED inhibitors have also been developed [90]. Most recently, PROTACs targeting EED have also been reported which appear to degrade the whole PRC2 complex including SUZ12 and EZH2 [91,92]. EZH2 in particular is commonly deregulated in haematopoietic malignancies, through a range of over-expression, gain- and loss-of-function mutations [93]. There is a strong rationale for inhibiting EZH2 catalytic activity, or PRC2 function more generally, in multiple

myeloma and B cell lymphomas with EZH2 gain-of-function mutations found in up to 30% of cases [94–96]. Whilst PRC2 inhibition has shown some success in clinical trials, the efficacy and safety profile of these inhibitors have thus far been broadly disappointing for these cancers. Importantly, PRC2 can also act as a tumour suppressor with loss-of-function mutations of component members found in myelodysplastic syndrome, chronic myeloproliferative neoplasms, and T cell acute lymphoblastic leukaemia [97,98]. The context-dependent function of PRC2 and its pro- and anti-tumour effects underscore the need to fully understand the reliance of different tumour sub-types on PRC2 function in order to select patients groups most likely to respond to PRC2 inhibition.

Thus far, given the importance of PRC2 in haematopoiesis and haematological malignancies, the efficacy and safety profile of EZH2 inhibitors have been broadly disappointing for blood cancers and none are yet approved. Interestingly, PRC2 is also implicated in resistance to immunotherapy and is the major mechanism by which the antigen processing and presentation pathway is epigenetically silenced [99,100]. Inhibition of PRC2 may, therefore, hold great promise to increase the efficacy of immunotherapy. It should be noted that PRC1 is also implicated in haematological malignancies but therapeutic targeting of PRC1 is far less advanced compared with PRC2, likely due to the huge molecular complexity and context-dependence of PRC1. Some selective BMI-1 inhibitors have been developed and have shown some promising preclinical efficacy [101] but are yet to enter clinical testing for any blood cancers.

## Conclusions

Heterochromatin and Polycomb are key epigenetic silencing mechanisms that regulate the complex process of haematopoiesis. As such, therapeutic modulation of these pathways presents as an attractive means to correct aberrant haematopoiesis such as occurs in ageing and blood cancers. Whilst candidate molecules, particularly targeting PRC2, have been developed to inhibit gain-of-function mutations in cancer, therapeutic strategies to restore loss of function of these pathways still elude us. Moreover, modulation of these pathways may also yield therapeutic benefit in immune-mediated disease by altering cell lineage fates or by interfering with the function of particular effector cells. As these therapeutics are developed and progress through clinical trials, the imperative for a detailed molecular understanding of these pathways increases. Given the context-specific nature of these pathways, we now look to single-cell technologies to reveal this next layer of insight in the quest to unravel the epigenetic wiring of haematopoiesis.

## Perspectives

- Heterochromatin and Polycomb are both critical for silencing cell-cycle genes, differentiation programmes, and restricting alternative fate genes in normal haematopoiesis, and for silencing tumour suppressor genes in haematological cancers.
- Many epigenetic silencing molecules are now attractive therapeutic targets.
- The major next frontier is the application of single-cell technologies to reveal the true epigenetic wiring of haematopoiesis.

## Competing Interests

The author declares that there are no competing interests associated with this manuscript.

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C.R.K. wrote the article and generated the figures.

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## Abbreviations

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CBX, chromobox; CLL, chronic lymphocytic leukaemia; EED, embryonic ectoderm development; ESC, embryonic stem cell; EZH, enhancer of zeste homologue; H2AK119u1, histone H2A Lysine 119 monoubiquitination; H3K27, histone H3 Lysine 27; H3K9, histone H3 Lysine 9; HP1, heterochromatin protein 1; HSC, haematopoietic stem cell; iPSCs, induced pluripotent stem cells; KDM, lysine demethylase; PcG, polycomb group proteins; PCGF, polycomb group ring finger; PRC, polycomb repressive complex; RBBP, retinoblastoma binding protein; RING, really interesting new gene; SUV39H, suppressor of variegation 39-homologue; SUZ12, suppressor of zeste homologue 12; Th, T helper.

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