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SPOTLIGHT REVIEW

Polycomb-group proteins in hematopoietic stem cell regulation and hematopoietic neoplasms

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The equilibrium between self-renewal and differentiation of hematopoietic stem cells is regulated by epigenetic mechanisms. In particular, Polycomb-group (PcG) proteins have been shown to be involved in this process by repressing genes involved in cell-cycle regulation and differentiation. PcGs are histone modifiers that reside in two multi-protein complexes: Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2). The existence of multiple orthologs for each Polycomb gene allows the formation of a multitude of distinct PRC1 and PRC2 sub-complexes. Changes in the expression of individual PcG genes are likely to cause perturbations in the composition of the PRC, which affect PRC enzymatic activity and target selectivity. An interesting recent development is that aberrant expression of, and mutations in, PcG genes have been shown to occur in hematopoietic neoplasms, where they display both tumor-suppressor and oncogenic activities. We therefore comprehensively reviewed the latest research on the role of PcG genes in normal and malignant blood cell development. We conclude that future research to elucidate the compositional changes of the PRCs and methods to intervene in PRC assembly will be of great therapeutic relevance to combat hematological malignancies.

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INTRODUCTION

The genome of every organism contains a developmental program that results in expression of various collections of genes in various cell types. In most adult tissues and organs, stem cells have been identified that maintain tissue homeostasis, and the quest to identify novel adult stem cell types is ongoing. Adult stem cells have the capacity to self-renew and the ability to differentiate into the mature cell types of the tissue in which they reside. Although adult stem cells and their differentiated progeny contain identical genetic information, their gene expression patterns differ substantially. This is predominantly accomplished by differential accessibility of the DNA for the transcriptional machinery. Along with this, alterations in the epigenetic landscape of the genome affect the transcriptome, the functioning and the behavior of each cell. However, we are only beginning to understand how collections of genes are turned 'on' or 'off' simultaneously, thereby controlling stem cell self-renewal and differentiation.

The hematopoietic system is a particularly well-studied example of a homeostatic tissue. It generates massive numbers of new blood cells during our entire lifespan by means of hematopoietic stem cells (HSCs), which normally reside in the bone marrow. Recent studies of HSCs have advanced our understanding of the epigenetic mechanisms that maintain the balance between self-renewal and differentiation.

One of the main classes of such epigenetic regulators is provided by the Polycomb-group (PcG) proteins. These proteins are histone modifiers, and their activity results in repression of genes involved in cell-cycle regulation and differentiation. Aberrant PcG expression and mutations in PcG genes have been shown to occur frequently in various types of hematopoietic neoplasms. Emerging evidence indicates that leukemic stem cells (LSCs) evolve from HSCs or progenitor cells, which have acquired abnormal expression of genes involved in cell survival and proliferation. This suggests a vital role for PcG-induced epigenetic modifications in malignant transformation.

In our review, we first describe the PcG gene family and how its members have expanded during evolution. We then focus on their molecular function and their importance for normal HSC regulation. Finally, we describe how aberrant Polycomb functions can result in neoplastic transformation of hematopoietic cells.

PCG GENES

PcG genes were first discovered in *Drosophila melanogaster* as key regulators of homeotic (Hox) gene expression.[1] The PcG proteins reside in two main complexes: Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2). In addition, a Pleiohomeotic Repressive Complex (PhoRC) has been identified to be involved in recruitment of PRCs to chromatin.[2–4]

PRC2 consists of three core subunits corresponding to *Drosophila* PcG genes: Enhancer of zeste (E(z)), Suppressor of zeste 12 (Suz12) and Extra sex combs (Esc). In mammals, the PRC2 complex consists of one of the two Ezh orthologs (Ezh1 or Ezh2), Suz12 and one isoform of Eed (Eed1–4).[5–8] The classical model by which PRC2 induces transcriptional repression involves the activities of Suz12 and Eed, which together contribute to binding the complex to nucleosomes[9] and to the histone methyltransferase activity of SET domain-containing Ezh proteins.[5–9] The capacity of Ezh to transfer methyl groups to

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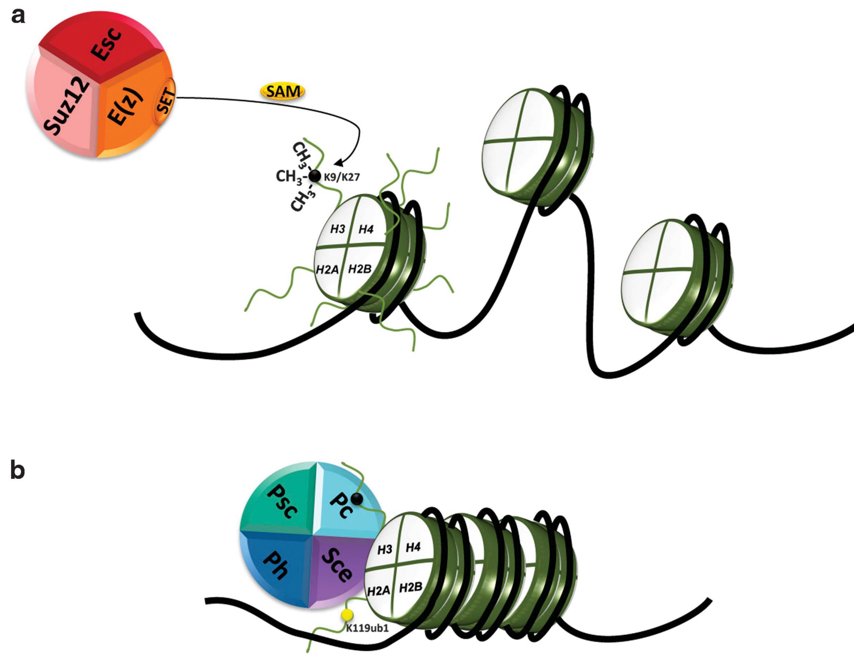

Figure 1. The canonical Polycomb-mediated gene silencing model. (**a**) PRC2 initiates gene repression by methylation of H3 on lysine 9 and 27 (H3K9/H3K27) catalyzed by the histone methyltransferase activity of the SET domain-containing E(z) subunit using the S-adenosyl methionine (SAM) cofactor as a donor for methyl groups. (**b**) The H3K9/27me3 histone marks are specifically recognized and bound by chromodomain-containing Pc subunits of PRC1 complexes. Binding of PRC1 to chromatin can drive further gene repression by mono-ubiquitination of H2A on lysine 119 (H2AK119ub1) by the Sce/Ring subunit.

lysine 9 and 27 of histone 3 (H3K9me3, H3K27me3) is stimulated by Eed.[10–12] Furthermore, PRC2 can recruit cofactors such as Aebp2, Mtf2, Pcl3, Jarid2 and Phf1, which can modulate its enzymatic activity and recruitment to target genes.[13–19] The well-documented trimethyl modification of H3K27 (H3K27me3) serves as a docking site for PRC1 assembly and subsequent induction of higher chromatin organization (Figure 1).[5]

PRC1 consists of four core subunits, which are homologous to *Drosophila* Polycomb (Pc), Polyhomeotic (Ph), Posterior sex combs (Psc) and Sex combs extra (Ring/Sce). Each of these four core components has several orthologs in the mammalian genome,[2,20] which compete for incorporation into PRC1.[21] These orthologs are sorted into families of Cbx, Phc, Pcgf and Ring1 genes. The H3K27me3 repressive mark, established by PRC2, induces binding of PRC1 through the chromodomain-containing Cbx subunit.[22,23] Binding of PRC1 to chromatin allows mono-ubiquitination of histone 2A on lysine 119 (H2AK119ub1), which is stimulated by Pcgf and executed by Ring1 proteins.[24] H2A ubiquitination is believed to be the final step in stable gene silencing, as it blocks Pol II transcriptional elongation.[3,25–27]

Polycomb complex evolution

The emergence of multiple Polycomb-encoding genes during evolution coincided with the requirement for compaction of genetic information as genomes and multicellular organisms became more complex.[28,29] PcG genes expanded by multiple gene duplications and subsequent diversification of the ancestral gene.[28,29] In addition, an accumulation of single-nucleotide polymorphisms (SNPs) and the advent of new regulatory elements resulted in small changes in the DNA sequence and the emergence of alternative splice variants.[28,29] For example, in mammals four alternative splice variants exist for the PcG gene Eed,[8] and recently a new Cbx2 isoform has been discovered that lacks a polycomb repressor domain.[30] The expansion and divergence of different PcG genes ultimately resulted in large Polycomb gene families in mammals, such as the Pcgf and Cbx gene families. This resulted in multiple PRC sub-complexes, which can consist of various PcG orthologs.

PRC2 first emerged during early eukaryotic specification. It is possible that this occurred in the last common unicellular ancestor of eukaryotes, since several important protein domains of PRC2 members are conserved between plants and animals. RNAi-mediated silencing of the E(z) homolog in unicellular algae *Chlamydomonas reinhardtii* suggested that one of the primary roles of PRC2 in early eukaryotes was to maintain genome stability by suppressing transposable elements.[31] This suppression might have protected cells against adverse horizontal gene transfer. In multicellular organisms, their function might have been adapted to regulate cell identity. The largest expansion of the PRC2 gene family occurred during plant evolution. Plants have up to eight PcG members, which can form an array of PRC2 sub-complexes.[32,33] In contrast to plants, PRC2 members underwent little duplication in animals, with only four genes encoding for the core subunits (Eed, Ezh1, Ezh2 and Suz12).[31,34]

PRC1 homologs have not been identified in plants. However, PRC1-like complexes (LHP1-AtRING1/LHP1-AtRING2) were shown to be involved in gene repression by binding to H3K27me3. This suggests that PRC1 function might have been conserved as a result of convergent evolution.[32,33] In contrast to PRC2 genes, expansion of PRC1 genes did occur in the animal kingdom, mainly at the invertebrate–vertebrate transition.[28,35] Although teleost radiation is accompanied by divergence of PRC1 members, further evolutionary events resulted in both expansion and loss of individual members. A combination of specific PcG genes might have increased the fitness of its carriers under certain environmental conditions and contributed to the evolution of tetrapods (see Figure 2, Whitcomb *et al.*[28]; and Table 1, Le Faou *et al.*[35]).[29]

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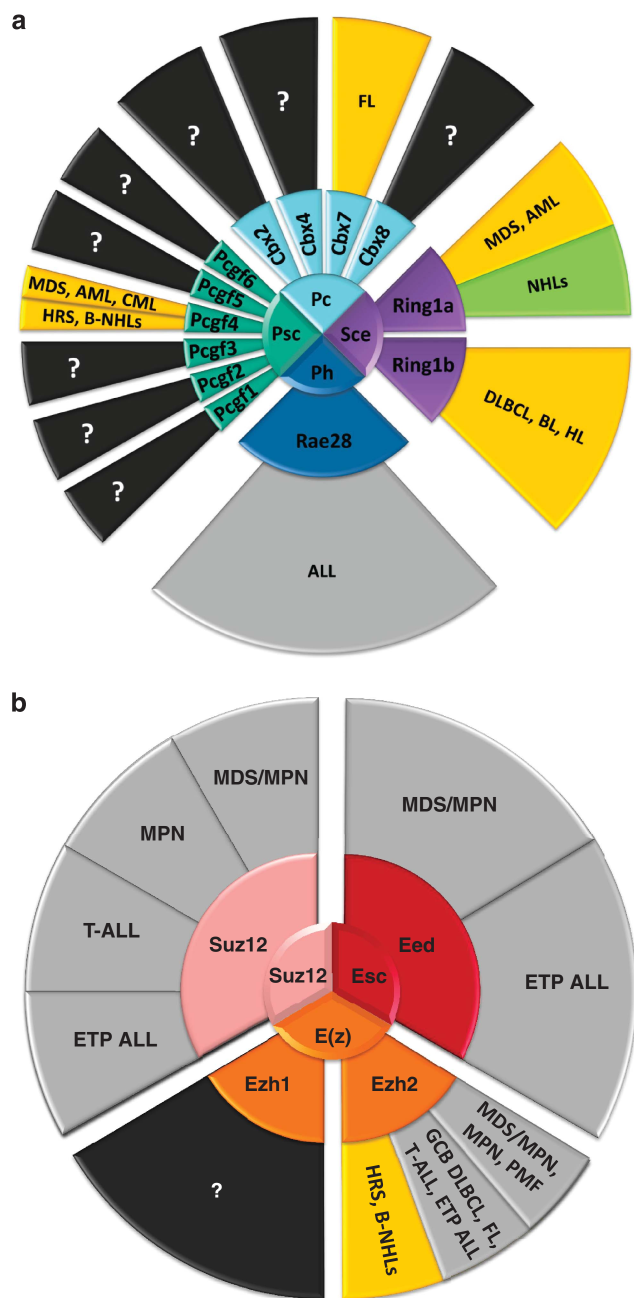

Figure 2. Schematic overview of PRC1 (**a**) and PRC2 (**b**) complex compositions and aberrations of various PRC members found in human hematological neoplasms. Diseases in the gray fields are caused by mutations, whereas diseases in the yellow fields are caused by overexpression of the corresponding Polycomb gene. Green fields represent SNPs in the corresponding Polycomb gene that correlated with a higher incidence of that particular disease. Black fields with the question mark indicate that no alteration in that particular PcG gene sequence or expression has yet been implicated in human hematological neoplasms.

PcG diversification and functional complexity of PRC1 and PRC2

Functional complexity of PRC1. Due to this evolutionary diversification of PcG genes and proteins, a wide array of combinatorially distinct PRCs can occur in any cell. For example, five Cbx family members exist in mammals. Cbx2, Cbx4, Cbx7 and Cbx8, but not Cbx6, are known to function within the PRC1 complex.[21] Although the N-terminal chromodomain and C-terminal polycomb repressor box of Cbx proteins are highly conserved (typically $>75\%$ amino-acid similarity), their affinity toward histone repressive marks (H3K27me3/H3K9me3) and PRC1 interacting partners varies substantially.[21,23] This distinct affinity might be attributed to high divergence in additional domains and motifs that have been acquired during evolution.[28,29] PRC1 can induce opposing activities, depending on which Cbx protein is present in the complex. For example, Cbx7 sustains pluripotency of embryonic stem cells (ESCs), whereas other Cbx orthologs induce ESC differentiation and are directly repressed by Cbx7.[36,37]

A recent study identified six distinct PRC1 sub-complexes, each containing different Pcgf family members, and showed that these Pcgf sub-complexes bind differentially throughout the genome with little overlap in target genes.[38] This clearly suggests that different PRC1 sub-complexes have different molecular functions. Indeed, two Pcgf family members, Bmi1 and Mel-18, have been shown to have non-redundant functions at the molecular level, even though they share 70% of their protein sequence. Although integration of Bmi1 in PRC1 complexes enhances Ring1b E3 ligase activity, Mel-18 integration does not,[24] indicating that these two orthologs have different molecular functions in chromatin compaction. An opposite effect of Bmi1 and Mel-18 on the cell cycle has also been demonstrated. Using a retroviral insertional mutagenesis approach in predisposed Eμ-Myc transgenic mice, Bmi1 was characterized as a proto-oncogene that collaborates with c-Myc.[39] It induces S-phase entry by inhibiting the function of retinoblastoma protein (Rb) through repression of the Ink4a/Arf locus.[40] In contrast, its ortholog Mel-18 arrests the cell cycle before entry to the S phase by repressing c-Myc, resulting in downregulation of several cyclin-dependent kinases.[41,42] Moreover, Mel-18 could function as a cell-cycle inhibitor by downregulating Bmi1 expression.[42,43]

Accumulating evidence indicates that the activity of PRC1 is highly dependent on its exact molecular composition.[4,37,38,44–46]

Functional complexity of PRC2. Ample evidence also indicates that PRC2 has different functions depending on whether Ezh1 or Ezh2 is present in the complex. The catalytic SET domain of both Ezh proteins is highly conserved[28] and both possess catalytic activity to mono-, di- and tri-methylate H3K27. Ezh1 and Ezh2 compete for binding to Suz12 and Eed[11,47] and are part of distinct PRC2 complexes. These Ezh2- and Ezh1-containing PRC2 complexes appear to have complementary transcriptional repressive functions in ESCs.[11,47] Depletion of Ezh2 or Ezh1 shows differential effects on the level of methylated H3K27 in ESCs. H3K27 methylation was severely impaired upon Ezh2 depletion, whereas knockdown of Ezh1 did not result in global change of H3K27 methylation levels.[11] However, depletion of Ezh1 in Ezh2$^{-/-}$ ESCs abrogated residual methylation on H3K27, resulting in derepression of H3K27me3 target genes.[47] Thus, both Ezh1- and Ezh2-containing PRC2 complexes are necessary for proper H3K27me3-mediated silencing of target genes in ESCs. Functional complementation of Ezh1 and Ezh2 has also been observed in skin stem cells and in adult, but not in fetal liver, HSCs. In skin, ablation of both Ezh1 and Ezh2 resulted in reduction of H3K27me3 levels, arrested skin development and degeneration of hair follicles, while ablation of a single Ezh ortholog had no effect.[48] In fetal liver HSCs, conditional deletion of Ezh2 strongly compromised their function. However, in adult HSCs, Ezh1 is highly expressed and can compensate for the loss of Ezh2 and restore H3K27me3 levels.[49]

Very recently, a novel role of Ezh1 became apparent in the regulation of transcriptional activation. In primitive skeletal muscle cells, Ezh1 can promote RNA polymerase elongation at gene bodies. In these cells, Ezh1-containing PRC2 complexes preferably associate with the active histone mark H3K4me3, while Ezh2-containing PRC2 complexes show canonical H3K27me3 association. Consequently, the overlap between target genes for Ezh2 and Ezh1 is very limited, and Ezh1- and Ezh2-containing PRC2 complexes show highly opposing roles in myoblast regulation.[45]

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Table 1. Common members of the PRCs and their hematopoietic phenotypes

Complex	*Drosophila gene*	*Mammalian gene*	*Alias*	*Expression alteration*	*Mouse phenotype*	*References*
PRC1	Psc	Pcgf1	Nspc1, Rnf3a-2, Rnf68	NA	NA	
		Pcgf2	Mel-18, Rnf110, Zfp144	Knockout/knockdown	3–6 weeks perinatal lethality; hypoplasia of spleen and thymus (defects in B-cell production)	41,63,67,68
		Pcgf3	Rnf3, Rnf3a	NA	NA	
		Pcgf4	Bmi1, Bmi-1	Knockout/knockdown	4–6 weeks perinatal lethality; impaired HSC self-renewal; hypoplasia of spleen and thymus	63–65,71,72
				Overexpression	enhanced HSC activity	63
		Pcgf5	Rnf159	NA	NA	
		Pcgf6	Mblr, Rnf134	NA	NA	
	Pc	Cbx2	M33, Hpc1, Mod2, Cdca6, Srxy5	Knockout/knockdown	5–6 weeks perinatal lethality; maintenance of HSC activity; hypoplasia of spleen and thymus	63,77–79
				Heterozygosity for a null allele/ hypomorphic gene	No effect on HSC function	77
				Overexpression	Reduced HSC activity	63
		Cbx4	MPc2, HPc2	NA	NA	
		Cbx7	—	NA	NA	
				NA	Na	
		Cbx8	Pc3, Hpc3	Knockout/knockdown	No effect on HSC activity	120
	Sce/Ring	Ring1	Ring1a, Rnf1	NA	NA	
		Rnf2	Ring1b, Ding, Ring2, Bap1	Knockout/knockdown	Early embryonic lethal	72,86
	Ph	Phc1	Rae28, Edr, Edr1, Mph1, Hph1	Knockout/knockdown	3–6 weeks perinatal lethality; reduced HSC activity; hypoplasia of spleen and thymus	84,85
		Phc2	Ph2, Edr2, Mph2, Hph2, p36	NA	NA	
		Phc3	Edr3, Hph3	NA	NA	
PhoRC						
	Pho	Yy1	Nf-e1, Ucrbp, Delta, Ino80s	Knockout	Peri-implantation lethality	87,141
				Heterozygosity for a null allele (Yy1 +/−) in Mpl −/− genetic background	Reduced HSC activity	87
				Overexpression	Enhanced HSC activity; impaired lymphoid differentiation	88
	Sfmbt	Sfmbt1	Ru1	NA	NA	
PRC2	E(z)	Ezh1	Enh-2, Kmt6b	NA	NA	
		Ezh2	Enx1, Enx1h, Kmt6, Kmt6a, Wvs2	Knockout/knockdown	Early embryonic lethal; enhanced HSC activity; hypoplastic thymi; impaired lymphoid differentiation	49,92,93
				Overexpression/ knock-in	Maintenance/enhanced HSC activity; myeloproliferative disorder; splenomegaly	90,91
				Heterozygosity for a null allele ($Ezh2^{Del/+}$) in $Mpl^{-/-}$ genetic background	Enhanced HSC activity	87
	Esc	Eed	l(7)5Rn, l7Rn5, Heed, Wait1	Knockout/knockdown	Early embryonic lethal	142
				Heterozygosity for a null allele/ hypomorphic gene	Myelo- and lymphoproliferative defects	94
				Heterozygosity for a null allele ($Eed^{3354/+}$) in $Mpl^{-/-}$ genetic background	Enhanced HSC activity	87

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Table 1. (Continued)

Complex	Drosophila gene	Mammalian gene	Alias	Expression alteration	Mouse phenotype	References
	Suz12	Suz12	Chet9, Jjaz1	Knockout/knockdown	Early embryonic lethal	143
				Heterozygosity for a null allele/ hypomorphic gene	Enhanced HSC activity	95
				Heterozygous for a loss-of-function point mutation ($Suz12^{Plt8/+}$) in $Mpl^{-/-}$ genetic background	Enhanced HSC activity	87

Abbreviations: PRC, Polycomb Repressive Complex; HSC, hematopoietic stem cell; NA, not applicable.

A non-canonical model of PRC1 function. The canonical Polycomb-mediated model of gene silencing implies the hierarchical recruitment of PRC2 and PRC1 complexes at target sites. PRC2 initiates gene repression by methylation of H3K9 or H3K27, after which PRC1 is recruited by binding Cbx protein to this histone modification. PRC1 then drives further gene repression by ubiquitination of H2AK119. However, this model has recently been challenged on two grounds. First, PRC1 and PRC2 gene targets are not completely overlapping.[50,51] Non-overlapping genes targeted by PRC2 therefore lack ubiquitination of H2AK119. Second, disruption of PRC2 (partial or otherwise) does not prevent binding of PRC1 and ubiquitination of H2A on target genes, although the methylated histone mark is lacking.[44,46,52]

A non-canonical PRC1 complex apparently exists, which could explain, at least partially, the functional independence of PRC1 from PRC2 and the H3K27me3 mark. Whereas the canonical PRC1 complex contains a Cbx protein, the non-canonical PRC1 contains either Rybp or Yaf2.[38,46] Cbx proteins (Cbx2, Cbx4, Cbx7 or Cbx8) are mutually exclusive for PRC1,[21] and they specifically assemble in PRC1 complexes containing either Bmi1 or Mel-18, but not in PRC1 complexes containing other Pcgf orthologs.[38] These canonical PRC1 complexes bind H3K9me3 and/or H3K27me3 repressive marks set by PRC2 complexes. Strikingly, both canonical and non-canonical complexes have similar H2A ubiquitinating activity.[46]

Polycomb PhoRC and recruitment of polycomb complexes to chromatin. The diversity of PRC sub-complexes most likely results in targeting different collections of genes. In *Drosophila*, the PRC is recruited to DNA elements called Polycomb Response Elements (PREs) through interaction with the DNA-binding protein Pho.[34,53] Pho forms a stable two-subunit complex with the protein Sfmbt1 (Scm-like with four MbT domain-containing protein 1), which is referred to as the PhoRC.[3,14,54]

In *Drosophila* PhoRC has an essential role in PRC recruitment, but no central recruitment mechanism or specific DNA elements have been identified in mammals. A first attempt by Ku *et al.*[51] using ChIP sequencing of PRC1 and PRC2 binding sites in ESs did not result in the detection of specific sequence motifs, although PRC2-targeted sequences were found to be highly enriched in CpG islands. More recently, two studies reported the identification of mammalian PRE-like elements based on PcG recruitment. These PRE-like elements were found only at very specific sites (between the human Hoxd11 and Hoxd12 genes and upstream of the mouse MafB gene), and not throughout the genome.[55] These regions apparently do not qualify as consensus mammalian PREs. Both studies also suggested an important role for the mammalian transcription factor Yy1, the mammalian homolog of *Drosophila* Pho,[22] in PRC recruitment. Multiple reports have now confirmed this hypothesis.[53,56] However, since Yy1 and Polycomb target genes do not show a high degree of overlap,[56] and Yy1 also has many PcG-independent functions in the cell,[57] Yy1-mediated PRC recruitment is most probably not general, and additional mechanisms in mammalian cells are likely to exist. Solid evidence of the interaction of Polycomb complexes with other transcription factors has been lacking, but recent studies have shown that the transcription factors Gata1,[58] Hic1,[59] Rest[60] and the Runx1/CBFβ transcription factor complex[61] can also recruit Polycomb complexes to specific target genes. Besides transcription factors, long non-coding RNAs have also been recognized as important Polycomb recruiters.[3,34]

More work is needed to address several remaining issues of PRC recruitment. For example, distinct Polycomb sub-complexes might utilize different recruiting mechanisms, thus binding different collections of genes; the non-canonical PRC1 complex, but not the canonical Cbx-containing PRC1 complex, may utilize Yy1-mediated recruitment since it contains the Rybp protein that has been shown to physically interact with Yy1.[62]

POLYCOMB PROTEINS AND HSC REGULATION

Although we found no studies on the effects of distinct PRC compositions in the hematopoietic system, hematopoietic phenotypes based on the ablation or overexpression of single PcG genes have been reported frequently (Table 1). Modulating PcG expression, thereby changing its protein abundance, is likely to alter the composition of PRCs, since PcG orthologs compete for incorporation. Therefore, these studies provide insight into the function of distinct PRCs in HSCs.

PRC1 members and HSCs

Bmi1 and Mel-18. Although highly similar in protein structure, the Pcgf family members Bmi1 and Mel-18 have very different effects on the maintenance of self-renewal of HSCs.[63] Despite normal embryonic hematopoiesis, Bmi1-null mutant mice displayed postnatal pancytopenia and died within 2 months after birth.[63–65] Park *et al.*[64] showed that the number of HSCs in $Bmi1^{-/-}$ fetal liver was not affected, whereas the number of HSCs in adult bone marrow (BM) was severely reduced. However, transplantation of $Bmi1^{-/-}$ fetal liver cells resulted in only transient hematopoietic reconstitution. This is compatible with the notion that $Bmi1^{-/-}$ HSCs cannot maintain long-term hematopoiesis, which causes hematopoietic failure soon after birth.[63,64] Indeed, it is now well established that Bmi1 is required for HSC self-renewal, both in mice and in humans (Table 1).[66]

Although $Bmi1^{-/-}$ fetal liver cells are functionally defective,[64] $Mel\text{-}18^{-/-}$ fetal liver cells show almost normal repopulating capacity of the hematopoietic compartment,[63] but a defect in B cells might indicate its potential role in more differentiated cells (Table 1).[41,67] Another study even reported a slight increase in HSC self-renewal capacity of adult Mel-18-deficient BM cells.[68] It seems

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likely that upon depletion of Mel-18 in HSCs, PRC1 complexes exclusively incorporate Bmi1, which then establishes the self-renewal phenotype. The specific function of Bmi1 in stem cells and of Mel-18 in more differentiated cells coincides with their endogenous expression patterns. Whereas Bmi1 is found to be specifically expressed in immature hematopoietic cells, its ortholog Mel-18 becomes upregulated along with differentiation.[69]

Bmi1 is one of the best-studied PcG members and many attempts have been made to clarify how Bmi1 sustains HSC self-renewal. In Bmi1$^{-/-}$ mice, the Ink4a-Arf locus was shown to be partially responsible for the hematopoietic defects; a double deletion of Bmi1 and Ink4a-Arf largely rescued the phenotype.[40] This locus encodes two proteins, p16^{Ink4a} and p19^{Arf}. p16 functions as a cyclin-dependent kinase inhibitor and hampers cell-cycle progression by activating the Rb pathway, while p19 is important for p21/p53-mediated cell-cycle arrest and apoptosis.[70] Ineffective self-renewal in Bmi1-deficient HSCs has been widely attributed to the derepression of the Ink4a-Arf locus and subsequent induction of premature senescence.[63,64,66]

More recently, it was shown that Bmi1 not only protects HSCs against premature cellular senescence, but also safeguards their multipotency. In HSCs, Bmi1 represses lineage specification through reinforcement of bivalent domains (overlapping repressive H3K27me3 and activating H3K4me3 histone marks) at the lymphoid regulator genes Ebf1 and Pax5. Bmi1-deficient HSCs showed loss of H3K27me3 and H2Aub1 repressive marks, resulting in a monovalent active state (H3K4me3) of these key lymphoid loci. Therefore, downregulation of Bmi1 might have promoted early expression of lymphoid genes and accelerated lymphoid specification.[71]

Another mechanism by which Bmi1 can manifest its self-renewal function is its protective effect against oxidative stress and DNA damage. In the absence of Bmi1, reactive oxygen species levels were found to be elevated, resulting in increased apoptosis and reduced HSC numbers and activity.[72,73] Bmi1 also affects DNA repair, which is crucial to maintain genetically stable HSCs. Not only Bmi1, but also other PcG members were found to be recruited to sites with double-stranded break and contributed to the initial steps of double-stranded break repair.[74] Whereas overexpression of Bmi1 protects against the effect of radiation,[75] Bmi1 knockdown increases radiosensitivity and resulted in a severe accumulation of DNA damage.[74] The role of Polycomb proteins in the DNA damage response was recently reviewed by Gieni et al.[76]

Future research should clarify how all these Bmi1-mediated effects on senescence, apoptosis, lineage specification, reactive oxygen species levels and DNA damage are integrated and collectively regulate HSCs functioning.

Other PRC1 members in HSCs. Besides Bmi1, other PRC1 components have also been studied for their role in HSCs, although less intensively (Table 1). Most understanding of the role of various orthologs from the Cbx family originates from knockout mice. Studies of Cbx2 and Cbx8-deficient mice showed that neither gene is required for normal hematopoietic stem and progenitor function, although *in vitro* data suggested a role for Cbx2 in lymphopoiesis (Table 1).[63,77–79] Also, in mice deficient for Cbx7, no effect on hematopoiesis was reported.[80] In contrast, overexpression of Cbx7 in the lymphoid compartment showed enhanced lymphomagenesis, which appeared to be dependent on c-Myc.[81] To our knowledge, Cbx4-deficient mice have not yet been generated. Although Cbx-deficient animals do not show overt hematopoietic abnormalities, caution is warranted with respect to the interpretation of these studies. It is likely that the absence of a single Cbx protein can be compensated by incorporation of another Cbx ortholog into PRC1 complexes during development. Functional redundancy between Cbx orthologs may therefore obscure the interpretation of PcG function in these studies.

Rae28-deficient mice exhibited perinatal lethality,[82] and heterozygous Rae28$^{+/-}$ mice showed a severe delay in B-cell development.[83] In addition, fetal liver Rae28$^{-/-}$ HSCs were unable to functionally reconstitute the hematopoietic compartment of lethally irradiated mice.[84,85] Together, this indicates that Rae28$^{-/-}$ HSCs are unable to sustain hematopoiesis during embryonic development. However, the function of Rae28 in adult HSCs has not been investigated. Ring1b exhibited a dual function in hematopoietic cells depending on their stage of differentiation. It restricted progenitor cell proliferation and stimulated maturation of their progeny via regulation of p16Ink4a and cyclin D2 expression.[86]

Since Yy1 is the only—non-canonical—PcG member with DNA binding capacity, it can target PRC1 and PRC2 complexes directly to chromatin. Majewski et al.[87] included this protein in a sensitized *in vivo* screen in which they systematically tested whether individual PcG genes are required for HSCs and progenitor activity. They showed that defects in canonical PcG genes (Bmi1, Mel-18, Ring1a, Ring1b, Phc1/Rae28, Phc2 or Cbx2), and a defect in the non-canonical Yy1 were both associated with HSC/progenitor cell defects. Therefore, it appears that all core PRC1 components and Yy1 enhance HSC activity. The positive effect of Yy1 on HSCs was confirmed in a recent study, which showed that overexpression of Yy1 resulted in accumulation of HSCs.[88]

PRC2 members in HSCs

Orthologous components of PRC2 have different expression patterns and functions in HSCs. While Ezh2 was found to be ubiquitously expressed in human and mouse BM and fetal liver cells,[49,69] its ortholog Ezh1 showed specific expression in adult mouse HSCs (Lin$^-$ Sca$^+$ cKit$^+$) and was decreased upon differentiation.[49]

Although only few studies have explored the role of Ezh1, Ezh2 has been widely studied in HSCs using both gain- and loss-of-function approaches. Using a 'genetical genomics' approach[89] followed by functional studies, Ezh2 was identified as an important HSC regulator.[90] Repeated serial transplantations greatly impair the potential of HSCs to reconstitute the hematopoietic system of recipient mice, but overexpression of Ezh2 completely prevents the exhaustion of HSCs during serial transplantations (Table 1). An inducible Ezh2 knock-in mouse was recently generated, which confirmed that elevated Ezh2 expression indeed increases the self-renewal capacity of HSCs.[91]

However, loss-of-function studies did not support a positive role for Ezh2 in HSCs, but instead suggested an effect in early lymphoid development. Ezh2-deficient mice had no obvious HSC phenotype,[92] although B- and T-cell development and VDJ rearrangement was affected.[93] Ezh2$^{-/-}$ LSK fetal liver cells transplanted into irradiated recipient mice showed high engraftment ability, and a severe impairment of lymphoid lineage differentiation, but the myeloid lineage was not affected.[49] In experiments that tested the effect of Ezh2 in a sensitized Mpl$^{-/-}$ background, not only Ezh2, but also Eed and Suz12 were found to restrict HSC activity. Although other studies also documented inhibitory effects of Eed and Suz12 on HSCs and progenitors,[94,95] this is difficult to reconcile with the studies of Kamminga et al.[90] and Herrera-Merchan et al.[91] We hypothesized that Ezh2 gene dosage and/or the balance between PRC2 complexes, including either Ezh2 or its homolog Ezh1, are important for proper HSC functioning. In addition, upon Ezh2 depletion, Ezh1 might complement Ezh2 in PRC2 integration and contribute to the functional activity of adult HSCs. Indeed, a recent study by Iwama et al. showed that Ezh1 can compensate Ezh2 loss in adult HSCs, but not in fetal liver HSCs.[49]

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Abundant evidence now shows that PcG genes must be correctly expressed in HSCs. We hypothesized that this correct expression ensures the multipotency of HSCs. A seemingly moderate imbalance, for example caused by gain or loss of function of individual PcG components, results in pronounced HSC phenotypes.

The diversity of PRC sub-complexes may regulate the dynamic equilibrium between HSC self-renewal and differentiation, but it is unclear how PRCs composed of different subunits actually affect HSCs. Ideally, proteomic analysis of PRC compositions should be conducted after PcG gene expression modification.

POLYCOMB PROTEINS IN HEMATOPOIETIC NEOPLASMS

Paradigmatically, leukemia is considered to be the consequence of a multistep process ultimately leading to unrestrained cell proliferation. Aberrant activity of genes involved in cell-cycle regulation is one of the prerequisites for malignant transformation of cells; many malignant cells have lost their cell-cycle checkpoint control mechanisms. Both proto-oncogenes and tumor-suppressor genes (for example, Ink4a-Arf) are often deregulated in cancer cells, and both classes of genes have been found to be under the control of PcG proteins.[63,96,97] Interestingly, the aberrant expression of PcG genes has frequently been detected in various types of cancer, including hematological neoplasms (Figure 2).[98,99] In addition, mutations in PRC2-encoding genes have recently been suggested as a causative factor in several types of leukemia. So far, PRC1 mutations have not been described in patients with leukemia. Strikingly, some PcG components, such as Bmi1 and Ezh2, have been reported to have both oncogenic and tumor-suppressor activity. Below we describe the functional involvement of PcG proteins in various types of hematological neoplasms.

PRC1 members in hematopoietic neoplasms

Bmi1. Bmi1 has been linked to leukemogenesis ever since it was identified as a cooperating partner of c-Myc in the induction of B-cell lymphomas.[100]

Overexpression of Bmi1 is commonly found in patients with myelodysplastic syndromes (MDS),[101,102] acute myeloid leukemia (AML),[103] chronic myeloid leukemia (CML)[104,105] and various types of lymphoma.[106,107] In addition, the expression of Bmi1 has been shown to correlate with disease progression (Table 2).[106,107] No evidence has been reported indicating that overexpression of Bmi1 is sufficient to induce leukemia. However, studies in various leukemic mouse models suggested that Bmi1 might be an important collaborating factor in leukemic transformation. For example, Sall4, an oncogene implicated in AML,[108] was found to bind directly to the Bmi1 promoter and induce its expression.[109] Moreover, mice that constitutively overexpress Sall4 developed leukemia and displayed upregulated Bmi1 expression, particularly after disease progression to AML.[109] In a HoxA9-Meis1 leukemia mouse model, Bmi1 was shown to be essential for maintenance of LSCs. Hoxa9 and Meis1 are oncogenes that were shown to induce transformation of murine BM cells.[110] $Bmi1^{-/-}$ fetal liver cells transduced with Hoxa9-Meis1 were able to induce AML when transplanted into irradiated recipient mice, but failed to sustain leukemia in secondary recipients.[111] In an MLL-AF9 mouse model, Bmi1 was shown to have an essential role in malignant transformation of myeloid progenitors into LSCs.[112] The fusion gene MLL-AF9 was found to cause immortalization of granulocyte macrophage progenitors *in vitro* and development of AML upon transplantation into irradiated recipient mice.[113] However, granulocyte macrophage progenitors derived from Bmi1-deficient mice and transduced with MLL-AF9 displayed reduced proliferative and clonogenic capacity *in vitro* and absence of leukemia development *in vivo*.

Several studies have shown that Bmi1 can have a role in the progression of CML toward acute blast crisis. The BCR-ABL oncoprotein is necessary and sufficient to initiate CML,[114] but overexpression of Bmi1 was required to provoke the progression to a more advanced stage of the disease.[104] $CD34^+$ cells from CML patients transduced with Bmi1 showed enhanced proliferative capacity and self-renewal properties *in vitro,* and resulted in a transplantable leukemia *in vivo*.[115] Synergistic effects of Bmi1 and BCR-ABL have also been shown to trigger B-cell acute lymphoid leukemia (B-ALL) development in mouse models.[116]

The above results indicate that Bmi1 cooperates with oncogenic fusion proteins in leukemic transformation. In human leukemias, Bmi1 protects LSCs from senescence and apoptosis by inhibiting p16 and p19 expression,[96,111] but enhanced expression of Bmi1 may also result in protection of LSCs from oxidative stress.[66] Pharmacological downregulation of Bmi1 was therefore predicted to reduce the proliferative capacity of AML cells. This hypothesis was supported in an experimental study where downregulation of Bmi1 in AML $CD34^+$ cells reduced their proliferative capacity and stem/progenitor cell frequency by inducing reactive oxygen species accumulation and apoptosis.[66]

In contrast to the role of Bmi1 in promoting self-renewal of normal BM cells and its oncogenic role in several types of leukemia, a recent study by Oguro *et al.*[97] demonstrated a potential tumor-suppressor function of Bmi1 (Table 2). Irradiated recipient mice, repopulated by $Bmi1^{-/-}$ $Ink4a\text{-}Arf^{-/-}$ BM cells, developed lethal myelofibrosis (MF). These mice showed an expansion of LSK and myeloid progenitor cells, along with abnormal megakaryocytopoiesis in the BM.

Other PRC1 members. Although a role for Bmi1 in leukemogenesis appears to be well established, less is known about other Pcgf family members. Several studies, based on immunohistochemical analyses of leukemic tissues, proposed that aberrant expression of PcG genes results in abnormal formation of PRC1 complexes and suggested that this might have contributed to the development of hematological neoplasms.[117,118] In a study where the expression of multiple Polycomb family members was assessed in 126 AML patients by real-time qPCR, Bmi1 was found to be overexpressed, whereas Mel-18 was one of the few tested PcG genes that showed no overexpression.[119]

Little is known about the contribution of Cbx family members to the development of hematopoietic malignancies. Cbx8 was shown to be essential in MLL-AF9 induced leukemogenesis, even though this function was found to be PRC1 independent (Table 2).[120] Cbx8 was also shown to be required for leukemic transformation of other MLL fusion proteins.[121] One study showed that Cbx7 is often overexpressed in human follicular lymphomas (FLs)[81] and that Cbx7 overexpression in the mouse lymphoid compartment results in T-cell and B-cell lymphomas upon transplantation (Table 2).[81] Cbx2 and Cbx4 have not been associated with leukemia development.

The PRC1 member Ring1a is commonly overexpressed in MDS and AML and correlates with poor prognosis.[102] In addition, SNPs in Ring1a were found to be associated with non-Hodgkin lymphoma.[122] Enhanced expression of its ortholog Ring1b has been detected in lymphomas, such as diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma and Hodgkin's lymphomas (Table 2).[123]

The Rae28 locus was found to be disrupted in patients with hematological malignancies.[83,124–126] Furthermore, it was demonstrated that inactivation of Rae28 is implicated in severe B-cell maturation arrest (Table 2).[83]

PRC2 members in hematopoietic neoplasms

Similarly to PRC1 components, aberrant expression of PRC2 genes, most notably Ezh2, has been associated with the development of

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Table 2. Common members of the PRCs and their alterations in different hematological neoplasms in mouse and human

Complex	Gene	Aberration	Hematopoietic phenotype	Species	References
PRC1	Bmi1	Overexpression	MDS, AML, CML	Human	101–105
		Overexpression	HRS, B-NHLs	Human	106,107,117,144
		Overexpression	B-ALL	BCR-ABL mouse model	116
		Knockout	MPN (MF)	Mouse (Ink4a-Arf$^{-/-}$)	97
	Cbx7	Overexpression	FL	Human	81
		Overexpression	T and B lymphomas	Mouse	81
	Cbx8	Knockout/knockdown	Loss of AML phenotype	MLL-AF9 mouse model	120
	Ring1a	Overexpression	MDS, AML	Human	102
		SNPs (rs2855429, rs213213)	NHLs	Human	122
	Ring1b	Overexpression	DLBCL, BL, HL	Human	123
	Rae28	Loss of heterozygosity	ALL	Human	83,124–126
		Loss-of-function	B-ALL	Mouse	83
PRC2	Ezh2	Overexpression	HRS, B-NHLs	Human	106,107,117,144
		Loss-of-function/deletion	MDS/MPN (CML, CMML), MPN (MF), PMF	Human	133–135
		Gain-of-function (y641 mutation)	GCB DLBCL, FL	Human	127,128
		Loss-of-function/deletion	T-ALL	Human	131
		Loss-of-function/deletion	ETP ALL	Human	132
		Knockout	Lower AML incidence	MLL-AF9 mouse model	139
	Eed	Loss-of-function (point mut (g255d))	MDS/MPN (CML)	Human	136
		Loss-of-function/deletion	ETP ALL	Human	132
		Knockout	Loss of AML phenotype	MLL-AF9 mouse model	139
		Heterozygosity for a null allele/ hypomorphic allele	Thymic lymphoma	Mouse (Eed$^{3354/+}$,Eed$^{1989/+}$,Eed$^{1989/1989}$)	145,146
	Suz12	Loss-of-function (aupd 17q; point mut (e610g) del(17q11.2))	MDS/MPN (CMML)	Human	136
		Loss-of-function/deletion	T-ALL	Human	131
		Loss-of-function/deletion	ETP ALL	Human	132

Abbreviations: AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; aUPD, acquired Uniparental Disomy; B-ALL, B-cell acute lymphoid leukemia; B-NHLs, B-cell non-Hodgkin lymphomas; BL, Burkitt's lymphoma; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; ETP-ALL, early T-cell precursor acute lymphoblastic leukemia; FL, follicular lymphoma; GCB DLBCL, germinal center B subtype of diffuse large B-cell lymphoma; HL, Hodgkin's lymphomas; HRS, Reed-Sternberg cells of Hodgkin's disease; MDS, myelodysplastic syndromes; MF, myelofibrosis; MPN, myeloproliferative neoplasms; NHLs, non-Hodgkin lymphomas; PMF, primary myelofibrosis; PRC, Polycomb Repressive Complex; T-ALL, T-cell acute lymphoid leukemia.

hematopoietic neoplasms (Table 2). In 2000, it was first reported that increased Ezh2 expression might promote lymphoma development.[106,107,117] More recently, recurrent heterozygous Tyr641 mutations that occur in the catalytic SET domain of Ezh2 were discovered in two types of lymphomas that arise from germinal center B cells. These mutations were found in ~20% of the patients with a germinal center B subtype of diffuse large DLBCL and in 7% of FL patients.[127,128] While the recurrent heterozygous Tyr641 mutation of the Ezh2 allele resulted in a reduced ability to trimethylate H3K27,[127] co-occurrence with the wild-type Ezh2 allele induced increased H3K27me3 levels. Heterozygous Tyr641 mutations therefore effectively represent a gain-of-function mutation.[129] This supports the hypotheses that Ezh2, which is often overexpressed in lymphomas, can contribute to the disease.[107] Very recently, pharmacological inhibition of the methyltransferase activity of Ezh2 by GSK126 was shown to effectively inhibit the proliferation of Ezh2 Tyr641 cell lines and DLBCL xenografts in mice.[130] This drug might therefore be a promising treatment for patients with activating mutations in Ezh2.

However, caution is warranted when administering such inhibitory drugs to patients, since inactivating mutations and deletions of Ezh2 and other PRC2 components have also been discovered recently. In a study of 68 adult T-ALL cases, loss-of-function mutations and deletions of Ezh2 (18%) or Suz12 (7%) were found.[131] In a separate study, the incidence of inactivating PRC2 mutations was found to be even higher in a pediatric subtype of T-ALL; 40% of ETP ALL patients showed a deletion or sequence mutation in Eed, Ezh2 or Suz12.[132] Various inactivating homozygous and heterozygous Ezh2 mutations were also discovered in patients with myelodysplastic and myeloproliferative neoplasms (MPN).[133,134] Ezh2 mutations, which are associated with poor prognosis, were shown to coexist with the well-known Jak2V617F mutation in primary MF.[135] Mutations in other PRC2 components (Eed and Suz12) have also been detected in MDS/MPN patients, albeit with lower frequencies (Table 2).[136]

The above findings indicate that loss or gain of Ezh2 activity (either by altered expression or by mutations) can both contribute to leukemogenesis in patients. These 'two faces of Ezh2 in cancer' have been well described in a recent review by Hock.[137] The function of Ezh2 as both a tumor suppressor and an oncogene has also been described in various mouse models.[138,139] We suggest that a single PcG can display either tumor-suppressor or oncogenic functions depending on the cell context and its interaction partners. Prescreening to determine whether mutations cause either a loss or gain of function is therefore crucial before starting PRC2-targeted therapy in patients.

CONCLUDING REMARKS

It has now become evident that PcG proteins have an important role in both normal hematopoiesis and various hematological malignancies. Depending on the stage of hematopoietic cell differentiation when neoplastic transformation first occurs and which PcG gene is involved, different types of leukemia may arise. In addition, depending on the cell context and interacting partners, individual PcG genes may display either tumor-suppressor or oncogenic functions. Compositional rearrangements of PRCs may provide true oncogenic events, instead of aberrant PcG functions caused by mutations or misexpression. However, the fact that leukemic cells are genetically unstable, and are susceptible to additional genetic abnormalities, make it

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difficult to determine whether PcG aberrations are a cause or a consequence of malignant transformation.

It should be noted that besides PcG proteins, other epigenetic mechanisms, such as DNA methylation and ncRNA interference, are also employed in regulation of gene expression.[140] Understanding the interplay between these distinct epigenetic mechanisms in the maintenance of a cell type-specific epigenome will be important for leukemia research.

The assessment of the epigenetic signatures of normal and LSCs is of great importance for future cancer research since compositional changes of PRCs may present useful prognostic markers. Pharmacological targeting of PRC compositions may therefore be an effective anti-leukemia strategy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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