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Epigenetic abnormalities and therapies for hematological malignancies

Deregulated Polycomb functions in myeloproliferative neoplasms

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Abstract

Polycomb proteins function in the maintenance of gene silencing via post-translational modifications of histones and chromatin compaction. Genetic and biochemical studies have revealed that the repressive function of Polycomb repressive complexes (PRCs) in transcription is counteracted by the activating function of Trithorax-group complexes; this balance fine-tunes the expression of genes critical for development and tissue homeostasis. The function of PRCs is frequently dysregulated in various cancer cells due to altered expression or recurrent somatic mutations in PRC genes. The tumor suppressive functions of EZH2-containing PRC2 and a PRC2-related protein ASXL1 have been investigated extensively in the pathogenesis of hematological malignancies, including myeloproliferative neoplasms (MPN). BCOR, a component of non-canonical PRC1, suppresses various hematological malignancies including MPN. In this review, we focus on recent findings on the role of PRCs in the pathogenesis of MPN and the therapeutic impact of targeting the pathological functions of PRCs in MPN.

Keywords BCOR · EZH2 · ASXL1 · BRD4 inhibition · DNA methylation

Introduction

Polycomb group (PcG) proteins were originally identified in *Drosophila* as regulators of body segmentation through the repression of homeotic genes and were subsequently identified in mammals. PcG proteins function in the maintenance of gene silencing via post-translational modifications of histones and chromatin compaction. In mammals, there are two major complexes of PcG complexes: Polycomb repressive complex 1 (PRC1) and 2 (PRC2), which modify mono-ubiquitination at lysine 119 of histone H2A (H2AK119ub1) and mono-, di-, and tri-methylation at lysine 27 of histone H3 (H3K27me1/me2/me3), respectively [1, 2]. Genetic and biochemical studies have revealed that the repressive function of PcG complexes in transcription is counteracted by the activating function of Trithorax-group (TrxG) complexes, which methylate H3K4. The functional balance between PcG and TrxG complexes fine-tunes expression of critical genes for development, adult tissue homeostasis [3], and stem cells [1]. On the other hand, dysregulated PRC results in a failure to maintain cellular homeostasis. The function of PRCs is frequently dysregulated in various cancer cells because of altered expression or recurrent somatic mutations in PRC genes, which have oncogenic and tumor suppressive roles that depend on cancer types [4, 5]. *EZH2*, which encodes the enzymatic component of PRC2, is one such PcG gene. The pathological role for deregulated *EZH2* has been extensively investigated in various hematological malignancies, including myeloid malignancies, lymphoma, and acute T-cell lymphoblastic leukemia (T-ALL). Gain-of-function mutations in *EZH2* play an oncogenic role in the development of B-cell lymphoma, while EZH2 also has a tumor suppressive role as missense and frame-shift mutations in *EZH2* that abrogate its methyltransferase activity are frequently observed in MDS, MPN, and MDS/MPN overlap disorders [6]. There is also a tumor suppressive function of PRC1.1, a non-conical PRC1, in the pathogenesis of myeloid malignancies [7]. In this review, we focus on recent findings on

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the role of PRCs in the pathogenesis of myeloproliferative neoplasms (MPN) and the therapeutic impacts of targeting the pathological function of PRCs in MPN and related hematological malignancies.

Composition of Polycomb repressive complexes

PRC2 contains three core subunits: SUZ12, EED, and one of the two methyltransferases, EZH1 or EZH2. PRC2 is recruited to non-methylated CpG islands (CGIs) and tri-methylates H3K27 (H3K27me3), which subsequently recruits canonical PRC1 via chromodomain proteins (CBX), a subunit of PRC1 that binds to H3K27me3 [8]. Canonical PRC1 contains four core subunits: PCGF4/BMI1 or PCGF2/MEL18, PHC, CBX and one of the two histone H2A mono-ubiquitylases, RING1A or RING1B, which has E3 ubiquitin ligase activity on histone H2A at lysine 119 (H2AK119ub1) thereby inducing the compaction of chromatin (Fig. 1) [9]. In addition to canonical PRC1, four non-canonical PRC1 variants (PRC1.1, PRC1.3, PRC1.5, and PRC1.6) containing a distinct PCGF subunit have been identified [10]. Non-canonical PRC1 complexes bind to target sites independently of PRC2-mediated H3K27me3 and deposit H2AK119ub1, which results in the subsequent recruitment of PRC2 and deposition of H3K27me3. In the case of PRC1.1, KDM2B binds to non-methylated CGIs through its DNA-binding domain and recruits other components of PRC1.1 (Fig. 1).

Role of PRC in normal hematopoiesis

The biological function of canonical PRC1 and PRC2 has been characterized in detail in ES cells and hematopoietic stem cells (HSCs). Canonical PRC1 and PRC2 regulate the transcription of critical regulators for self-renewal and multipotency of HSCs to maintain homeostasis in hematopoiesis. The dysfunction of PRCs induced by targeted gene ablation of PRC component genes leads to the exhaustion of HSCs and/or impaired differentiation and production of blood cells [1, 11].

The loss of PRC2 function by the deletion of *Eed* markedly compromises adult hematopoiesis and impairs HSC function, in part, because of aberrant activation of *Cdkn2a*, a major target of PRCs that encodes $p16^{Ink4a}$ and $p19^{Arf}$ genes [12]. Ezh1 prevents premature senescence of HSCs via silencing the expression of *Cdkn2a* [13], while Ezh2 is dispensable for self-renewal of HSCs, at least in part, because of the compensatory function of Ezh1. Ezh1 co-regulates a large number of Ezh2 target genes and redistributes to a significant portion of Ezh2-specific targets in the context of *Ezh2* insufficiency in normal and malignant hematopoietic stem cells [14, 15]. Ezh2-containing PRC2 (Ezh2-PRC2) also regulates the differentiation of B- and T-lymphoid cells and myeloid cells by regulating the expression of lineage-specific transcription factors [16]. These findings indicate that Ezh1-PRC2 and Ezh2-PRC2 coordinately regulate the transcription of PRC2 target genes but also have distinct functions in hematopoiesis.

PRC1.4, one of two canonical PRC1 that contain BMI1/PCGF4, is essential for the self-renewal capacity of HSCs

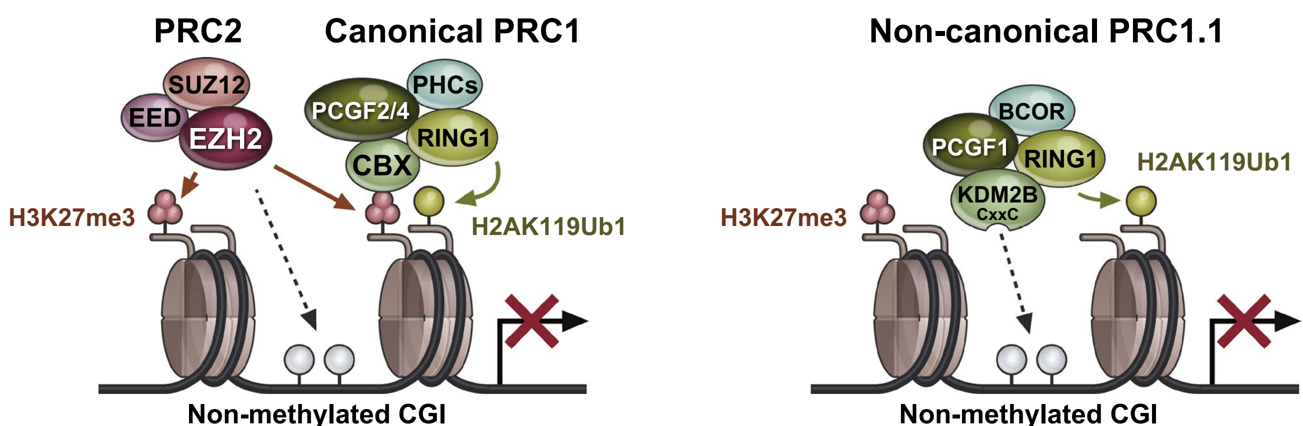

Fig. 1 Canonical PcG complexes and non-canonical PRC1.1. PRC2 is recruited to non-methylated CGIs and tri-methylates H3K27 (H3K27me3), which subsequently recruits canonical PRC1 via chromodomain proteins (CBX), a subunit of PRC1 that binds to H3K27me3. Canonical PRC1 mono-ubiquitylates histone H2A at lysine 119 (H2AK119ub1), thereby inducing the compaction of chromatin (left panel). Non-canonical PRC1 complexes bind to target sites independently of PRC2-mediated H3K27me3 and deposit H2AK119ub1, which results in the subsequent recruitment of PRC2 and deposition of H3K27me3. In the case of PRC1.1, KDM2B binds to non-methylated CGIs through its DNA-binding domain and recruits other components of PRC1.1 (right panel)

via repressing expression of *Cdkn2a* [17]. *Bmi1*-deficient HSCs de-repress the expression of $p16^{Ink4a}$ and $p19^{Arf}$. The deletion of both $p16^{Ink4a}$ and $p19^{Arf}$ largely rescues the self-renewal defect of *Bmi1*-deficient HSCs [18]. Conversely, overexpression of *Bmi1* augments the function of HSCs by enhancing symmetrical cell division [18, 19]. PRC1.4 also restricts the ectopic expression of lineage-specific transcription factors, such as B cell master regulator genes *Ebf1* and *Pax5*, in HSCs and multipotent progenitors (MPPs). *Bmi1*-deficient HSCs and MPPs have enhanced B cell commitment via premature activation of *Ebf1* and *Pax5* in HSCs and MPPs [20].

In contrast, the function of non-canonical PRC1 in hematopoiesis remains unclear. A genome-wide RNA interference screen found that Pcgf1, a component of PRC1.1, functions in concert with Runx1 transcription factor in Runx1-dependent differentiation. The depletion of both *Runx1* and *Pcgf1* resulted in the maintenance of proliferation of hematopoietic progenitor cells and perturbed their differentiation because of increased expression of posterior *Hoxa* cluster genes such as *Hoxa9* [21]. Similarly, myeloid cells in mice lacking *Bcor* exons 9 and 10, which generates a truncated form of Bcor that does not bind Pcgf1, have a higher proliferative capacity, which results in myeloid-biased hematopoiesis [22, 23]. *Bcor*-deficient HSCs have increased expression of *Cebp* family genes in MPPs and sustained expression of posterior *Hoxa* cluster genes in myeloid progenitors [23]. These findings indicate that PCR1.1 negatively regulates critical transcriptional regulator genes, such as *Cebp*, and *Hoxa9*, to restrict the proliferation and differentiation of myeloid progenitor cells.

Additional sex combs like 1 (ASXL1) is a Polycomb-related protein frequently mutated in myeloid malignancies and clonal hematopoiesis in older healthy adults [24, 25]. ASXL1 regulates the function of Ezh2-PRC2 and modifies the function of BAP1, a nuclear deubiquitinase [26]. Hematopoietic-specific deletion of *Asxl1* impairs the self-renewal capacity of HSCs, but results in multi-lineage cytopenia and dysplasia and increased numbers of stem and progenitor cells, which are characteristic features of MDS [27]. Loss of Asxl1 reduces global H3K27me3 levels and activates the expression of posterior *Hoxa* genes, such as *Hoxa9*, because of impaired recruitment of PRC2, which leads to the induction of MDS despite the reduced repopulating capacity of *Asxl1*-deficient HSCs [27–29] (Fig. 2).

Roles of PRC and PRC-related protein in myeloproliferative neoplasms

Classical MPNs include essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). Driver mutations in *JAK2, MPL*, and *CALR* genes occur in all subtypes of MPNs. These mutations are generally considered mutually exclusive, but 10–15% of ET and PMF patients do not carry any of these mutations. In addition to these MPN driver gene mutations, it is important to identify mutation profiles of additional clonal marker genes, such as mutations in *ASXL1, EZH2, TET2, IDH1*/*2*, and spliceosome-related genes, because of their prognostic and therapeutic implications [30]. In addition, mutations in epigenetic modifiers promote the initiation and progression of MPN. We overview the pathogenic function of PRC genes including *EZH2, ASXL1*, and *BCOR* in the development of MPN.

Loss-of-function mutations in *EZH2* promote the development of MPN

Although gain-of-function mutations in *EZH2* have oncogenic roles in the development of B-cell lymphoma, missense and frame-shift mutations in *EZH2* that abrogate its methyltransferase activity are frequently observed in MDS, MPN, and MDS/MPN overlap disorders (Table 1). These mutations reduce global levels of H3K27me3 and have been shown to increase expression levels of EZH2 target genes including potential oncogenes in tumor cells in patients and murine models, which indicate that EZH2 has a tumor suppressor function [4, 30, 31]. Loss-of-function mutations in *EZH2* significantly predict poor outcomes in MDS, and the survival of patients with homozygous mutations is shorter than those with heterozygous mutations [32, 33]. Primary myelofibrosis (PMF) is a subtype of MPN driven by $JAK2^{V617F}$ activating mutations or *CALR* mutations that constitutively activate JAK2. *EZH2* mutations independently predict poor survival in patients with PMF [34], consistent with the tumor suppressive function of EZH2. Monosomy 7 and deletion of the long arm of chromosome 7 (-7/7q-), characteristic cytogenetic anomalies frequently observed in myeloid malignancies, commonly involve *EZH2* at 7q36 and are associated with the poor prognosis of PMF. A functional mapping study using 7q- MDS patient-derived iPS cells demonstrated that impaired production of hematopoietic cells is rescued by exogenous *EZH2*, which indicates that *EZH2* haploinsufficiency contributes to the pathogenesis of 7q- MDS [35].

We and other groups have examined the impacts of loss-of-function mutations of *EZH2* on the pathogenesis of myeloid malignancies using *Ezh2* conditional knockout mice. Hematopoietic cell-specific deletion of *Ezh2* resulted in the development of myeloid malignancies including MDS, MDS/MPN, and T-ALL, but not AML, after a long latency [14]. *EZH2* mutations frequently occur with loss-of-function mutations in *TET2* and *RUNX1* in patients [36, 37]. Loss of Ezh2 enhances the formation of *RUNX1* mutant-induced MDS, but inhibits leukemic transformation [38], which

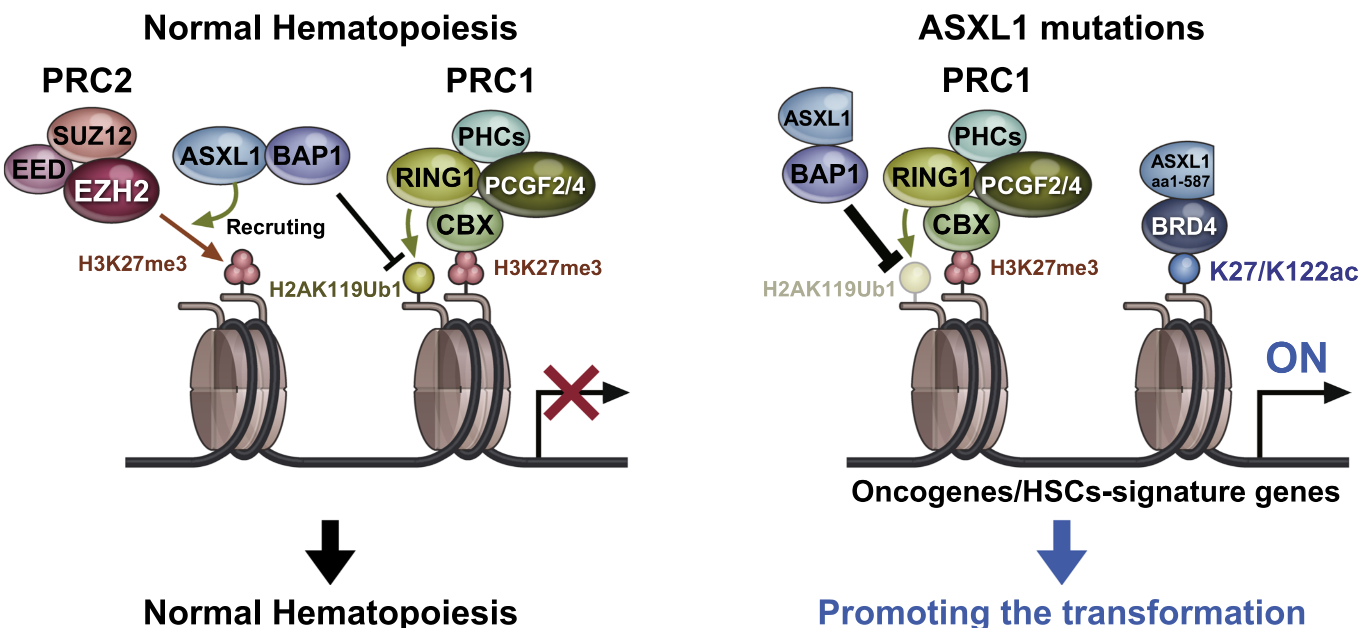

Fig. 2 ASXL1 mutant-induced epigenetic alterations. ASXL1 regulates the function of Ezh2-PRC2 and modifies the function of BAP1 in normal hematopoiesis. Loss of Asxl1 impairs recruitment of PRC2, which reduces H3K27me3 levels. In contrast, ASXL1 mutants enhance the de-ubiquitination activity of BAP1 on H2AK119, which significantly reduces H2AK119ub1 levels without affecting H3K27me3 levels. The ASXL1$^{aa1-587}$ mutant interacts with BRD4, which increases the levels of H3K27ac and H3K122ac

Table 1 Mutations in PcG and PcG-associated genes in myeloid malignancies

	EZH2	*EED*	*SUZ12*	*ASXL1*	*BCOR*
MDS	3–13%	Rare	Rare	10.6–18.5%	4.2%
MPN	3% (PV)	Rare	Rare	1–3% (ET/PV)	1%
	5–13% (PMF)			25% (PMF)	
MDS/MPN	8–15.6%	1%	1.4%	15.6–43%	7.4% (CMML)

is consistent with an oncogenic role of *Ezh2* observed in *MLL-AF9*-induced AML in which Ezh2 reinforces the transcriptional repression of myeloid-differentiation regulator genes [39, 40]. An *Ezh2* deficiency ($Ezh2^{\Delta/\Delta}$) in combination with a *Tet2* hypomorph ($Tet2^{KD/KD}$) in mice accelerates the transformation of HSCs and induces MDS and MDS/MPN [41]. Loss of Ezh2 significantly promotes the development of $JAK2^{V617F}$ mutant-induced myelofibrosis, at least in part, because of enhanced production of megakaryocytes with dysplastic features in BM [42–44]. These findings indicate that EZH2 fine-tunes the commitment and differentiation of HSCs, and *EZH2* insufficiency promotes the transformation of HSCs to MDS and MPN stem cells.

Targeting therapy against EZH1 and EZH2 in myeloid malignancies

Because of the bimodal pathogenic functions of *EZH2* as an oncogene and a tumor suppressor gene, pharmacological inhibition of EZH2 and both EZH1 and EZH2 has being tested extensively in pre-clinical and clinical studies in solid tumors, B-cell lymphoma, and AML [45]. An *Ezh2* insufficiency aberrantly activates expression of certain oncogenes because of reduced levels of H3K27me3, but Ezh1-PRC2 partly compensates for Ezh2 loss in the maintenance of transcriptional repression of Ezh2 target genes [46]. In good agreement with these findings, AML cells are efficiently eradicated by the deletion of both *Ezh1* and *Ezh2* and treatment with EZH1/2 dual-inhibitors [47, 48], which indicates that Ezh1 is essential for the self-renewal capacity of leukemic stem cells in *Ezh2*-deficient conditions. These data support therapeutic approaches to target EZH1 and EZH2 using EZH1/2 dual inhibitors. A patient enrolled in a Phase I pediatric study (NCT02601937) of tazemetostat, an EZH2 inhibitor, for relapsed or refractory INI1 (a SWI/SNF component)-negative tumors or synovial sarcoma developed secondary T-cell lymphoblastic lymphoma (T-LBL), which suggests that caution is warranted when inhibiting the function of PRC2 for cancer therapy. However, the anti-tumor efficacies of Ezh2 and Ezh1/2 inhibitors are still encouraging [49]. Further investigation and comprehensive assessment of pre-clinical and clinical studies of Ezh2 and Ezh1/2 inhibitors are now underway.

Dysfunction of PRC2 sensitizes tumor cells to BRD4 inhibition

Because PRC2 represses target genes, loss-of-function mutations in *EZH2* may de-repress expression of potential oncogenes in cancer. Indeed, loss of Ezh2 in $JAK2^{V617F}$ mice promotes an epigenetic switch characterized by reduced H3K27me3 levels followed by elevated H3K27 acetylation (H3K27ac) levels at promoter regions of PRC2 target genes, which results in the activation of potential oncogenes such as *Hmga2* [42–44]. *HMGA2* is significantly upregulated in $CD34^+$ cells in PMF patients with *EZH2* mutations [43, 50], and overexpression of *Hmga2* in $JAK2^{V617F}$ HSCs increases the production of dysplastic megakaryocytes and the development of PMF in mice [42, 51]. Bromodomain inhibitors inhibit the function of enhancers by competitively interfering with the binding of BRD4 to H3K27ac and abrogates the progression of tumors [52]. Loss of PRC2 increases sensitivity to bromodomain inhibition of $JAK2^{V617F}$ myelofibrosis-initiating cells in vitro and in vivo [42]. Furthermore, a combination of bromodomain and JAK kinase inhibition reduces NF-kB-induced inflammation, which completely reverses fibrosis in $JAK2^{V617F}$ model mice [53]. Vulnerability to BRD4 inhibition has also been observed in solid tumors with compromised function of Ezh2-PRC2 by somatic gene mutations in PRC2 genes or in tumors expressing MLL1, which interacts with the p300/CBP complex to cause loss of H3K27me and gain of H3K27ac [54, 55]. Thus, these studies suggest that bromodomain inhibition in combination with JAK kinase inhibition is a novel therapeutic rationale for eradicating tumors and removing fibrosis in MPN patients.

Implication of DNA hypo-methylating therapy for MDS/MPN with *EZH2* mutations

Promoter DNA hyper-methylation silences the expression of tumor suppressor genes, thereby promoting malignant transformation. Because MDS cells have higher levels of DNA hyper-methylation than de novo AML cells [56], hypomethylating agents, such as azacitidine (AZA) and decitabine (DAC), are clinically used to impede the progression of MDS in patients. However, the mutations of epigenetic modifiers and transcription factors, such as *TET2* and *TP53*, do not always predict the response to AZA and DAC in

patients with MDS, MPN, or AML [57–59]. PRC2 target genes defined in ES cells and normal HSPCs preferentially display DNA hyper-methylation in tumor cells, such as colon cancer, AML, and MDS, regardless of genetic mutations in epigenetic modifiers [60, 61]. Loss of Ezh2 significantly promotes the deposition of DNA methylation at promoter regions in many PRC2 target genes including development and differentiation regulator genes during the development of MDS and ETP-ALL in mice [46, 62]. Thus, PRC2 maintains a reversible gene silencing state of target genes by protecting them from aberrant DNA hyper-methylation. After mutation, deletion, or aberrant silencing of *EZH2*, alternative epigenetic machinery, such as promoter-hypermethylation, promotes transformation (Fig. 3).

Gain-of-function mutations in *ASXL1* in the pathogenesis of myeloid malignancies

Somatic mutations in *ASXL1* frequently mark hematological malignancies, such as AML, MDS, MPN, and CMML, and are associated with advanced age and poor clinical outcomes (Table 1) [32, 63]. Similar *ASXL1* mutations are also found in clonal hematopoiesis in healthy older adults. Deletion of *Asxl1* activates the expression of posterior *Hoxa* genes, such as *Hoxa9*, because of impaired recruitment of PRC2, which induces MDS despite the reduced repopulating capacity of *Asxl1*-deficient HSCs [27–29]. Nonsense or frameshift *ASXL1* mutations generate truncated proteins that lack the C-terminus region [64] and are always heterozygous. These findings imply that *ASXL1* mutations are gain-of-function or dominant-negative. BAP1, a deubiquitinase of H2AK119, assembles complexes with ASXL1, ASXL2, or ASXL3. BAP1 promotes mono-ubiquitination of ASXL1 mutants, which in turn enhance the deubiquitinase activity of BAP1 on H2AK119 and activates the expression of posterior *Hoxa* genes (Fig. 2) [65–67]. Expression of C-terminus-truncated ASXL1 mutants in mice induces myeloid malignancies, such as AML, MDS, MPN, and MDS/MPN, and promotes myeloid transformation in concert with additional mutations [68, 69]. In contrast to the reduced self-renewal capacity of *Asxl1*-deficient HSCs, HSCs with *ASXLl1* mutants have sustained or enhanced competitive repopulating capacity in mice, and are accompanied by altered expression of genes critical for HSCs self-renewal and differentiation. Loss of Asxl1 reduces H3K27me3 levels but not H2AK119ub1 levels, while Asxl1 mutants significantly reduce H2AK119ub1 levels without affecting H3K27me3 levels (Fig. 2) [68]. Furthermore, a liquid chromatography-tandem mass spectrometry experiment revealed that an ASXL1$^{aa1-587}$ mutant protein interacts with BRD4. Correspondingly, bone marrow hematopoietic cells expressing Asxl1^{Y588X} have elevated levels of H3K27ac and H3K122ac and higher sensitivity to BRD4 inhibitors (Fig. 2) [69]. These findings suggest that ASXL1

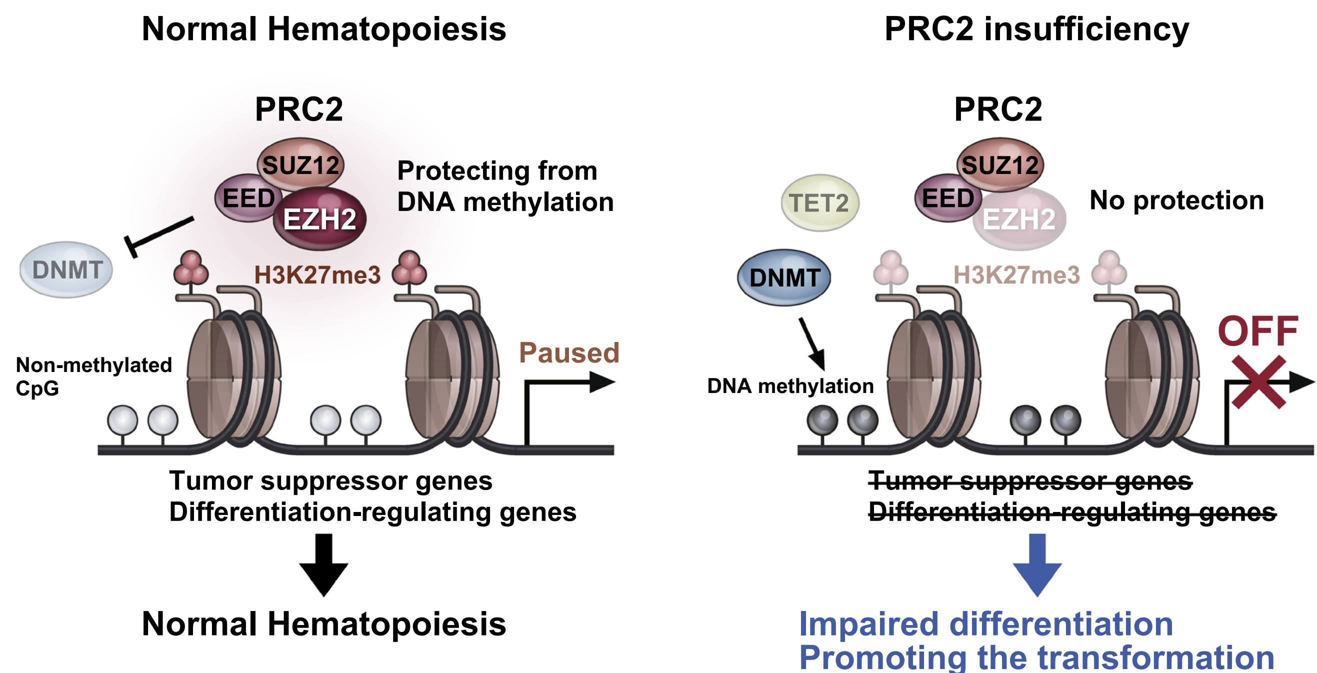

Fig. 3 EZH2 mutant-induced epigenetic alterations. PRC2 maintains a reversible gene silencing state of target genes by protecting them from aberrant DNA hyper-methylation. After mutation, deletion, or aberrant silencing of *EZH2*, alternative epigenetic machinery, such as promoter-hypermethylation, takes place and promotes transformation

mutants have a gain-of-function role in the pathogenesis of myeloid malignancies and provide a novel therapeutic strategy for ASXL1 mutant-induced malignancies.

Role of non-canonical PRC1.1 in the development of MPN

Among non-canonical PRC1 genes, loss-of-function mutations in BCOR occur in various hematological malignancies, such as AML, MDS, CMML, and lymphoid tumors (Table 1), indicating that BCOR functions as a tumor suppressor in these tumors [70–72] Mice deficient for *Bcor* exon 4 ($Bcor^{\Delta E4/y}$), which generate a truncated protein that cannot bind Bcl6, develop Notch-dependent T-ALL [73]. Mice lacking *Bcor* exon 9 and exon 10 ($Bcor^{\Delta E9-10/y}$), which cannot bind Pcgf1, develop T-ALL at a similar latency to that of $Bcor^{\Delta E4/y}$ mice and have myeloid-biased hematopoiesis. $Bcor^{\Delta E9-10/y}$ mice in combination with a *Tet2* hypomorph develop lethal MDS, which transforms into MDS/MPN after a secondary transplantation. $Bcor^{\Delta E9-10/y}$ multipotent and myeloid progenitors have enhanced expression of *Cebp* family genes and posterior *Hoxa* cluster genes, respectively, because of reductions in H2AK119ub1 levels at the promoters of myeloid-regulator genes [23]. In addition, deletion of *Kdm2b* accelerates oncogenic KRAS-mediated myeloid transformation, and ectopic expression of *Kdm2b* suppresses the progression of KRAS-induced myeloid malignancies [74]. These findings suggest that PRC1.1 functions as a tumor suppressor in myeloid malignancies, such as MPN, in concert with other driver mutations. In contrast, several PRC1.1 component genes are overexpressed in AML cells in patients, and their knockdown significantly reduces the proliferative capacity of AML cells [75, 76], which suggests that PRC1.1 has opposing roles in tumor progression in a context-dependent manner.

Conclusion

PcG complexes have multiple epigenetic activities that differ depending on cell context, coexisting mutations, and tumor type. The impact of PcG complexes on transcription is complicated; they primarily function in transcriptional repression, but also activate transcription with non-PcG proteins. Therefore, it is important to determine how PRC dysfunction deregulates the expression of target genes and contributes to transformation. It is also important to understand alternative epigenetic pathways activated by PRC dysfunction such as epigenetic alteration of histone modifications and DNA methylation. A detailed understanding of PRC dysfunction will enable the development of new therapeutic approaches for individual cancer types by targeting novel therapeutic molecules or epigenetic pathways.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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