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Polycomb repressive complexes in hematological malignancies

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The deregulation of polycomb repressive complexes (PRCs) has been reported in a number of hematological malignancies. These complexes exert oncogenic or tumor-suppressive functions depending on tumor type. These findings have revolutionized our understanding of the pathophysiology of hematological malignancies and the impact of deregulated epigenomes in tumor development and progression. The therapeutic targeting of PRCs is currently attracting increasing attention and being extensively examined in clinical studies, leading to new therapeutic strategies that may improve the outcomes of patients with hematological malignancies. (*Blood*. 2017;130(1):23-29)

Introduction

Epigenetic regulation is important in gene expression because of its modulation of the chromatin structure and function.[1] Recent advances in genomic and epigenetic research have revealed a central role for aberrant epigenetic regulation in the pathogenesis of hematological malignancies.[2,3] Among epigenetic regulators, many of the genes encoding DNA and histone modifiers are targeted by somatic gene mutations and deletions or are involved in chromosomal abnormalities, highlighting their causative role in tumor development. Polycomb group (PcG) genes encoding histone modifier proteins are representative epigenetic genes that are deregulated in many hematological malignancies. In this review, I summarize the current knowledge on polycomb dysfunctions in hematological malignancies as well as therapeutic approaches that target PcG complexes.

Function of PRCs in hematopoiesis

PcG proteins form multiprotein complexes that play an important role in maintaining the transcriptional repression of target genes through chromatin modifications. Canonical polycomb repressive complex 1 (PRC1) and PRC2 have been characterized in detail.[4] PRC1 and PRC2 exhibit catalytic activities that are specific to the monoubiquitination of histone H2A at lysine 119 (H2AK119ub1) and the mono-, di-, and trimethylation of histone H3 at lysine 27 (H3K27me1/2/3), respectively. After the recruitment of PRC2 to chromatin, PRC2 trimethylates H3K27 (H3K27me3), which, in turn, recruits canonical PRC1 via the CBX subunit that binds to H3K27me3. In contrast, variant PRC1 complexes, which have recently been identified, are proposed to catalyze H2AK119ub1 modifications independently of PRC2 activity or the H3K27me3 mark. H2AK119ub1 then promotes the recruitment of PRC2 and potentiates its catalytic activity (Figure 1A).[5] Thus, PRC1 and PRC2 function in concert or independently to establish and maintain gene silencing. However, PRC2 may activate transcription in a specific setting. During erythroid differentiation, EZH1 and SUZ12 have been shown to assemble a noncanonical PRC2 complex independent of EED and positively regulate gene expression.[6] EZH2 also has nonhistone targets and regulates cellular processes such as actin polymerization in the cytoplasm through its methyltransferase activity.[7]

Human additional sex combs like 1 (*ASXL1*) is one of the homologs of the additional sex combs (*Asx*) gene, which encodes a chromatin-binding protein that regulates the balance between trithorax and polycomb functions in *Drosophila*.[8] ASXL1 has been shown to associate with PRC2, but not PRC1, and the loss of ASXL1 results in global reductions in H3K27me3 levels, suggesting its critical function associated with PRC2.[9]

Polycomb complexes function as general regulators of stem cells and maintain the self-renewal capacity and multipotency of hematopoietic stem cells (HSCs).[10] Canonical PRC1 containing BMI1/PCGF4 and PRC2 play central roles in this process and transcriptionally repress the *CDKN2A* locus, the critical target of PcG complexes for maintaining the self-renewal capacity of HSCs.[11-15] They also repress a cohort of developmental regulator genes to maintain the immature state and multipotency of HSPCs.[16] The forced expression of *Bmi1*, *Ezh2*, *Cbx7*, or *Kdm2b/Fbxl10* in mouse HSCs promotes their self-renewal and efficiently preserves HSC potential during serial transplantations.[17-20] In contrast, mice with a heterozygous loss-of-function allele of *Suz12*, *Ezh2*, or *Eed* display enhanced HSC activity,[21,22] suggesting that PRC2 also has a tumor-suppressive function.

Oncogenic function of PRCs

Because of their function in somatic stem cells, PcG genes have been characterized as oncogenes.[10,23] *Bmi1* was initially identified as an oncogene that collaborates with the Eμ-*myc* transgene in murine pre–B-cell lymphomagenesis.[24] Its oncogenic function largely depends on its capacity to transcriptionally repress the *Ink4a* locus encoding p16^Ink4a and p19^Arf.[25] Bmi1 is also essential for maintaining the proliferative activity of leukemic stem cells as well as the leukemic transformation of hematopoietic progenitor cells by the MLL-AF9 fusion gene in mice.[26,27] BMI1 collaborates with BCR-ABL in the leukemic transformation of human CD34^+ cells.[28] The expression of BMI1 has been correlated with disease progression and prognosis of myelodysplastic syndrome (MDS) as well as the prognoses of acute myeloid leukemia (AML) and chronic myeloid leukemia.[29-31] Although *BMI1* gene amplification was previously reported in 4 (11%)

Table 1. Mutations in PcG and PcG-associated genes in hematological malignancies

Malignancy	EZH2, %	EED, %	SUZ12, %	ASXL1, %	Reference
MDS	3-13	Rare	Rare	10.6-18.5	50-55
MPNs	3 (PV)	Rare	Rare	1-3 (ET/PV)	51,56-58
	5-13 (PMF)			25 (PMF)	
MDS/MPNs	8-15.6	1	1.4	15.6-43	51,58
AML	Rare	Rare	Rare	9-18	51,60,61
DS-AMKL	32.7	0	2	2	76
T-ALL*	18	ND	7	0	78
ETP-ALL†	12.7	12.7	17.5	0	77
Non-ETP†	3.3-4.8	3.3-7	0-2.4	0	77,79

DS-AMKL, acute megakaryoblastic leukemia associated with Down syndrome; ET, essential thrombocythemia; ETP-ALL, early T-cell precursor acute lymphoblastic leukemia; ND, not determined; PMF, primary myelofibrosis; PV, polycythemia vera.

*Adults

†Children.

of 36 cases of mantle cell lymphoma,[32] alterations in the BMI1 gene on chromosome 10p13 are generally uncommon in hematological malignancies. Somatic BMI1 gene mutations have not yet been reported. The aberrant regulation of PRC2 functions and H3K27me3 modifications has been reported in various cancers.[10,23,33] EZH2 was found to be overexpressed and/or amplified in prostate, breast, bladder, and colon cancers and its expression correlated with metastasis and poor prognosis. We and other groups have shown that PRC2 is required for MLL-AF9 leukemia in mice and demonstrated that Ezh2 inhibits differentiation programs in leukemic stem cells, thereby augmenting their leukemogenic activity.[34,35]

Next-generation sequencing has led to a paradigm shift in our understanding of PcG functions, revealing monoallelic gain-of-function mutations in EZH2 in germinal center (GC) B-cell–type diffuse large B-cell lymphoma and follicular lymphoma.[36] EZH2 is strongly expressed in GC B cells and is required for the formation of GCs. EZH2 represses proliferation checkpoint genes and transcriptional programs required for exiting the GC reaction and terminal differentiation to transiently suppress GC B-cell differentiation.[37] EZH2 cooperates with BCL6 to recruit a noncanonical PRC1-BCOR complex containing CBX8 in a GC B-cell–specific manner to repress differentiation gene expression.[38] The conditional expression of mutant EZH2 (EZH2^{Y641N}) in mice has been shown to induce GC hyperplasia and accelerate lymphomagenesis.[37,39] Mutant EZH2 not only increased the global abundance of H3K27me3 but also caused the widespread redistribution of this repressive mark, suggesting that mutant EZH2 induces lymphoma through a vast reorganization of the chromatin structure, inducing the persistent repression and aberrant activation of EZH2 target genes (Figure 1B).[39]

The silencing of PRC2 targets is also reinforced in advanced stages of multiple myeloma cells.[40,41] Furthermore, deletions and inactivating mutations in KDM6A (also known as UTX), which encodes a demethylating enzyme that removes methyl residues from H3K27me2/3, have been observed in 3.2% to 10% of patients with multiple myeloma and are associated with shorter overall survival.[42,43] The t(4;14) translocation, which involves WHSC1/MMSET encoding an H3K36 methyltransferase, and KDM6A mutations are potentially mutually exclusive.[42] Although activating EZH2 mutations have not yet been identified in multiple myeloma, the inactivation of KDM6A supports an oncogenic role for PRC2 in multiple myeloma. KDM6A is also targeted by loss-of-function mutations in 5% to 15% of T-cell acute lymphoblastic leukemia (T-ALL) and functions as a tumor suppressor by modulating H3K27me3 modifications at the promoters of tumor-suppressor genes.[44,45] Mutations in KDM6A, which is

located on the X chromosome, are exclusively present in male patients with T-ALL, and T-ALL driven by the inactivation of KDM6A exhibits collateral sensitivity to the pharmacological inhibition of H3K27me3.[45] In contrast, another H3K27 demethylase, JMJD3, is essential for the initiation and maintenance of T-ALL, because it controls important oncogenic genes; thus, it is a potential therapeutic target in T-ALL.[44] Of interest, UTX also functions as a pro-oncogenic cofactor of TAL1 in TAL1$^+$ T-ALL and removes H3K27me3 at the TAL1 target gene loci, thereby promoting the establishment of a TAL1-mediated leukemic gene expression program.[46] UTX is required specifically in TAL1$^+$ TALL, whereas UTX overexpression suppresses cell growth in TAL1$^-$ T-ALL,[46] which is consistent with its role as a tumor suppressor in T-ALL described previously in this paragraph.[44,45] The function of PRC2 is also augmented in adult T-cell leukemia cells, in which EZH2 is upregulated by HTLV-1 Tax and an activated NFκB signal.[47]

Tumor-suppressive function of PRCs

EZH2, located at chromosome 7q36.1, is frequently involved in chromosomal abnormalities such as −7 and 7q−, which are associated with very poor prognosis in hematological malignancies.[48] A functional mapping study using induced pluripotent stem cells derived from a patient with 7q− MDS revealed a causative role for an EZH2 haploinsufficiency in the defective hematopoiesis of 7q− MDS clones, suggesting a tumor-suppressive function for EZH2.[49] Correspondingly, next-generation sequencing also identified deletions and inactivating mutations in EZH2 that abrogate its methyltransferase activity in patients with MDS (3% to ~13%), myeloproliferative neoplasms (MPNs) (3% to ~13%), and MDS/MPN-overlapping disorders (8% to ~15.6%) (Table 1).[2,50-56] Other components of PRC2, EED and SUZ12, are also targeted by somatic-inactivating mutations, although the frequencies of their mutations are markedly lower than those of EZH2 mutations (Table 1).[2,57,58] Collectively, these findings suggest that PRC2 also functions as a tumor suppressor. Loss-of-function mutations have been detected in both monoallelic and biallelic states.[51] Patients with MDS or MDS/MPNs with mutations in EZH2 show significantly poorer outcomes than patients without mutations,[51,52] and survival was found to be slightly shorter in patients with homozygous mutations than in those with heterozygous mutations.[51] EZH2 mutations have also been shown to predict poor survival in patients with primary myelofibrosis regardless of the presence of JAK2 mutations.[59] However, PRC2 mutations are rare in de novo AML, but they are present in secondary AML.[2,60,61] Spliceosomal gene mutations are frequently identified in hematological malignancies, particularly in patients with MDS. Of note, SRSF2 and U2AF1 mutants were shown to cause the missplicing of EZH2.[62,63] SRSF2 mutants promote the inclusion of a cassette exon that results in a premature terminal codon (PTC)–containing isoform of EZH2 messenger RNA. The PTC-containing isoform of EZH2 undergoes degradation by nonsense-mediated decay, resulting in decreased levels of EZH2 messenger RNA. The link between SRSF2 mutations and insufficient EZH2 expression may explain the mutual exclusivity between SRSF2 and EZH2 mutations in patients with MDS.[62] Thus, multiple mechanisms deregulate EZH2 in myeloid malignancies. Indeed, EZH2 expression in CD34$^+$ HSPCs is significantly weaker in patients with MDS, particularly those with chromosome 7 abnormalities, than in healthy individuals.[15,64]

The tumor-suppressive function of EZH2 has been analyzed using Ezh2-deficient mice. We have shown that the hematopoietic cell–specific deletion of Ezh2 results in the development of heterogeneous
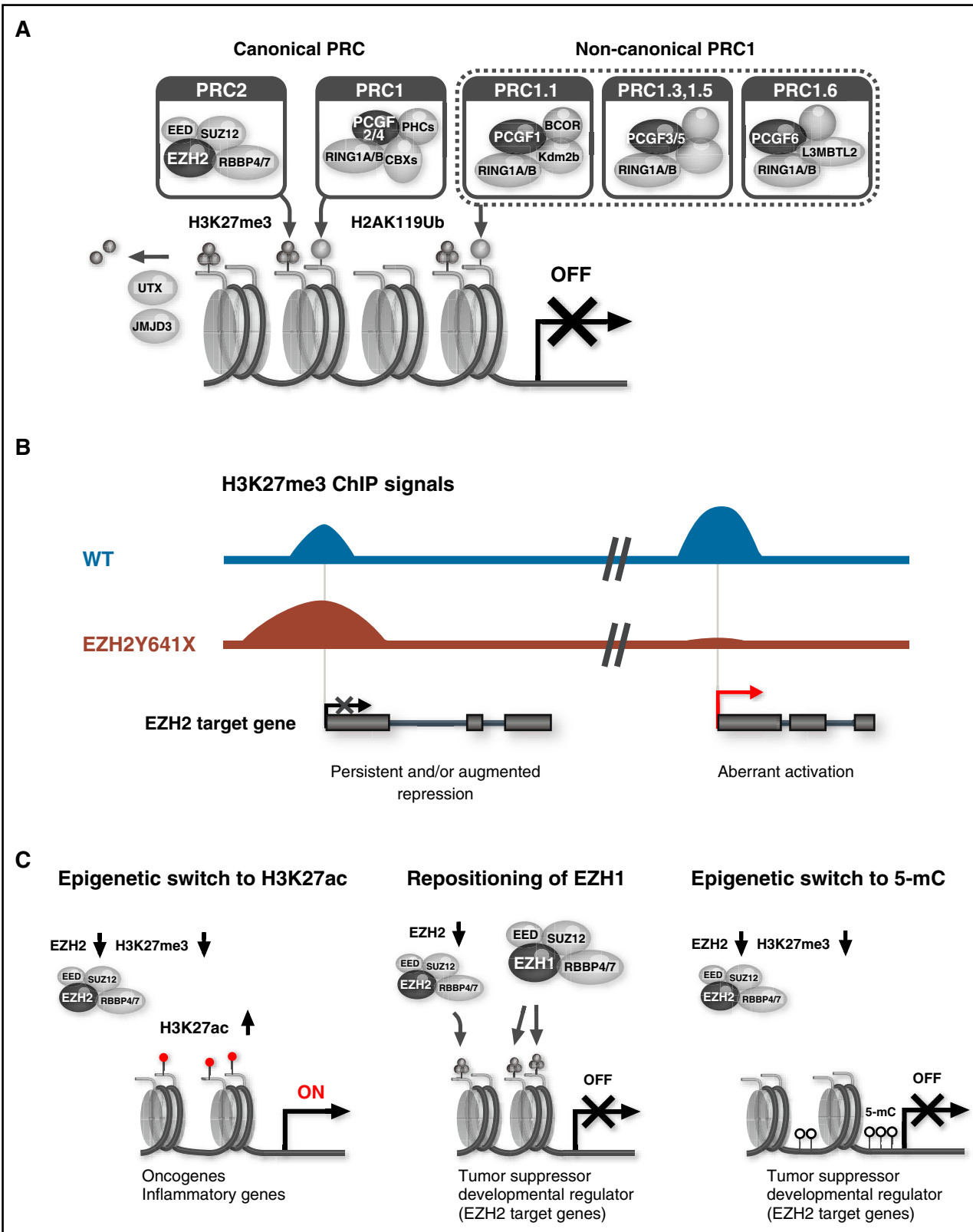
Figure 1. PcG complexes and aberrant polycomb functions in hematological malignancies. (A) Composition of canonical and noncanonical PcG complexes. (B) Oncogenic functions of activating EZH2 mutants. EZH2Y641 mutants (Y641E, Y641F, Y641N, Y641S, and Y641H) increase the global abundance of H3K27me3 and reinforce the repression of EZH2 target genes (left). They also cause the widespread redistribution of H3K27me3, inducing the aberrant activation of EZH2 target genes (right). ChIP, Chromatin ImmunoPrecipitation; WT, wild type. (C) Tumor suppressive function of PRC2. An *EZH2* insufficiency induces the activation of PRC2 target genes via a methylation-to-acetylation switch at H3K27 at promoters, thereby conferring a growth advantage to malignant clones (left). Key tumor suppressor and developmental regulator genes are largely maintained in a transcriptionally repressed state in *EZH2*-insufficient hematopoietic stem and progenitor cells (HSPCs) via the locus-specific repositioning of EZH1 to the EZH2 target loci (middle) and an epigenetic switch from H3K27me3 to DNA methylation at EZH2 target genes (right).

myeloid malignancies with long latencies, including MDS and MDS/MPNs.[15] *EZH2* mutations were found to co-occur with *TET2*, *RUNX1*, and *ASXL1* mutations.[63] The loss of *Ezh2* cooperated with a *Tet2* hypomorph to induce MDS and MDS/MPNs in mice.[65] The loss of *Ezh2* also enhanced the initiation and progression of *RUNX1* mutant–induced MDS but attenuated the predisposition to leukemic transformation.[66] Furthermore, the loss of *Ezh2* significantly promoted the development of $JAK2^{V617F}$ mutant–induced myelofibrosis, resulting at least in part from the enhancement of aberrant megakaryocytopoiesis.[67-69] These findings clearly indicate that EZH2 plays a tumor-suppressive role in myelodysplastic and myeloproliferative disorders that originate from HSCs. Eμ-*myc* lymphomagenesis was also accelerated by a heterozygous loss-of-function allele of *Suz12* or by short hairpin RNA-mediated knockdown of *Suz12* or *Ezh2*, suggesting that PRC2 restricts the self-renewal of B-lymphoid progenitors.[70]

A series of analyses of these mouse models revealed that an *Ezh2* insufficiency induced the activation of oncogenic genes of direct and indirect polycomb targets (Figure 1C).[65-69,71] These genes include a cohort of fetal-specific genes, such as let-7 microRNA target genes (such as *Lin28*, *Hmga2*, and *Igfbp3*), which are so-called oncofetal genes, suggesting that Ezh2 restricts the transformation of HSPCs by repressing a cohort of oncogenic genes.[67-69,71] *Ezh2* insufficiency also activates the production of inflammatory cytokines and proteins such as interleukin-6, S100a8, and S100a9 from malignant hematopoietic clones, creating an inflammatory bone marrow environment that confers a growth advantage on malignant clones over the residual normal clones while affecting the production of mature progenies.[66,69,71,72]

In contrast, key tumor-suppressor and developmental regulator genes are largely maintained in a transcriptionally repressed state in malignant HSPCs, even in *Ezh2*-insufficient states.[15,65] A Chromatin ImmunoPrecipitation sequence analysis of H3K27me3 revealed a locus-specific compensatory function for Ezh1, another enzymatic component of PRC2, which repositions to the Ezh2 target loci to restrict the activation of tumor-suppressor and developmental regulator genes (Figure 1C).[15] Correspondingly, no recurrent somatic mutations have been reported in *EZH1*. Furthermore, an *Ezh2* insufficiency promoted the propagation of aberrant DNA methylation in a mouse MDS model hypomorphic for *Tet2* combined with the loss of Ezh2.[73] In this model, Ezh2 target genes largely lost the H3K27me3 mark while acquiring a significantly higher level of DNA methylation than Ezh1 target genes that retained the mark. These findings indicate that Ezh2 target genes are the main targets of the epigenetic switch from H3K27me3 to DNA methylation in MDS with an *Ezh2* insufficiency. An *Ezh2* insufficiency also induces a switch from methylation (H3K27me3) to acetylation (H3K27ac) at H3K27 at promoters, which is closely associated with the activation of PRC2 target genes (Figure 1C). The epigenetic switch conferred an oncogenic addiction to the H3K27ac modification, while it sensitized tumor-initiating cells to bromodomain inhibition in a mouse model of myelofibrosis with JAK2V617F combined with the loss of Ezh2.[67] In juvenile myelomonocytic leukemia (JMML), genetic alterations impairing PRC2 functions, such as −7/7q− and deletions and mutations in *EZH2*, *SUZ12*, and *ASXL1*, have been reported in 33% of sporadic JMML patient cases with alterations in the RAS pathway.[74] A cooperative effect of the activation of RAS and impairment of PRC2 has also been reported in NF1-associated cancers, in which the loss of PRC2 augments Ras-regulated transcription by enhancing acetylation at H3K27.[75] A similar molecular mechanism may also be relevant in JMML. *EZH2* has also been targeted with deletions and inactivating somatic mutations in childhood acute megakaryoblastic leukemia associated (33%) or not associated (16%) with Down syndrome and is frequently comutated with genes encoding cohesin components (Table 1).[76]

Loss-of-function mutations in *EZH2* and *SUZ12* have been detected in ETP (42.2%) and non-ETP (11.9%) pediatric T-ALL and in 25% of adult T-ALLs (Table 1).[77-79] Moreover, in several mouse models, *Ezh2*-deficient hematopoietic cells have been reported to induce T-ALL.[15,80,81] In a mouse model and human T-ALL cells, oncogenic *NOTCH1* mutations specifically induced the loss of H3K27me3 modifications by antagonizing the functions of PRC2, leading to the activation of the NOTCH1 transcriptional program.[78] These findings indicate that PRC2 functions as a tumor suppressor in T-ALL. Activating *NOTCH* mutations are much less common in ETP-ALL than in regular T-ALL. In contrast, RAS pathway mutations are common in ETP-ALL. Given the high frequencies of inactivating mutations in PRC2 genes in ETP-ALL, a cooperative effect of Ras activation and PRC2 dysfunction may contribute to the pathogenesis of ETP-ALL as in JMML. PRC2 inactivation also caused derepression of *Il6ra*, resulting in the activation of JAK/STAT signaling in a mouse model of ETP-ALL.[81]

ASXL1, the loss of which causes reductions in H3K27me3 levels, is also targeted by deletions and somatic mutations in patients with MDS, MPNs, and MDS/MPNs and also in those with de novo AML (Table 1).[2,82] The loss of ASXL1 was shown to activate the expression of posterior *HOXA* genes, and the conditional deletion of *Asxl1* in mouse hematopoietic cells resulted in a myelodysplastic phenotype.[9,83] These findings suggest that ASXL1 functions as a tumor suppressor in myeloid malignancies. However, the molecular mechanisms responsible for the pathological impact of ASXL1 mutations in hematological malignancies as well as functional differences from PRC2 component mutations have not yet been elucidated in detail.

Recurrent inactivating mutations have been identified in various hematological malignancies in the X-linked *BCOR* gene encoding the BCL6 corepressor (BCOR) and its closely related homolog, BCOR-like 1 (*BCORL1*).[84] Although BCOR and BCORL1 have recently been identified as components of the noncanonical PRC1.1, limited information is available on their role in hematological malignancies, and it currently remains unclear whether BCOR and BCORL1 mutants are relevant to the functions of PRC1.1.[85]

Therapeutic targeting of PRCs

EZH2 inhibitors that target the oncogenic function of EZH2 have been developed, and preclinical and clinical trials are now under way.[86,87] The efficacy of EZH2 inhibitors was initially demonstrated in lymphoma,[88] and lymphoma and multiple myeloma are now the major targets in clinical trials.[87] Of interest, cancers with mutations in SWI/SNF subunits, such as *ARID1A*, *PBRM1*, and *SMARCA4*, are dependent on EZH2 activity.[89] A synthetic lethality between *ARID1A* mutation and EZH2 inhibition has been reported in *ARID1A*-mutated ovarian cancer cells,[90] and EZH2 inhibition sensitizes *SMARCA4*-mutant lung tumors to topoisomerase II inhibitors.[91] However, SWI/SNF-mutant cancer cells are primarily dependent on a noncatalytic role of EZH2 and are only partially dependent on EZH2 histone methyltransferase activity, raising a concern about the efficacy of EZH2 enzymatic inhibitors in these cancers, including leukemia.[89] In addition to the EZH2-specific inhibitors, UNC1999, a dual EZH1 and EZH2 inhibitor, has been developed. Because EZH1 complements EZH2 functions, targeting EZH2 and EZH1 may represent a promising approach.[92] Furthermore, PRC2 inhibitors have the potential to be used in combination with other agents to enhance their therapeutic value. The leukemic stem cells of chronic myeloid leukemia were shown to be sensitive to the combined effects of an EZH2 inhibitor and tyrosine

kinase inhibitor.[93] Many agents are now being evaluated to obtain synergistic effects in combination with EZH2 inhibitors.

In contrast, therapeutic approaches that target the tumor-suppressive functions of PRC2 are currently limited. As described earlier in this article, PRC2 insufficiencies induce a methylation-to-acetylation switch at H3K27, thereby sensitizing tumor cells to bromodomain inhibitors.[67,75] JAK/STAT inhibition is a potential therapeutic strategy for hematological malignancies with PRC2 insufficiency such as ETP-ALL.[81] In addition, the ablation of the residual PRC2 activity may be an alternative approach to eradicate tumor cells with PRC2 insufficiency.[94] Therapeutic breakthroughs are needed to treat PRC2-insufficient tumors. PTC596, a small compound that induces the degradation of the BMI1 protein, has entered a phase 1 clinical trial for patients with advanced solid tumors (www.clinicaltrials.gov #NCT02404480).[95]

Finally, recent analyses have linked PRC2 insufficiency to drug resistance in leukemia. Low levels of EZH2 protein induce resistance to multiple drugs in AML partly because of the derepression of *HOX* genes, representative EZH2 targets.[96] Inactivating PRC2 mutations also induce resistance to conventional chemotherapy by inhibiting mitochondrial apoptosis in T-ALL.[97] These findings may represent complex functions of PRC2 in hematological malignancies and underscore the importance of PRC2 status in the treatment of hematological malignancies.

Conclusions and perspectives

The dysregulation of PcG proteins has been implicated in various aspects of the ontogeny of hematological malignancies. Although a lot of detail is known about PcG proteins, we may not yet have the full picture because of their complex functions and broad targets. Noncanonical PRC1 may also be involved in the pathogenesis of hematological malignancies; however, its role remains largely uncharacterized. Recent advances in epigenetic research are contributing to a new stage of translation, and clinical trials on polycomb-targeting agents, which may have both beneficial and adverse effects in patients, are providing important information. Continuous and seamless efforts from basic to clinical research will undoubtedly promote advances in epigenetic therapy.

Authorship

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References

1. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. *Nat Rev Genet*. 2016;17(8): 487-500.

2. Shih AH, Abdel-Wahab O, Patel JP, Levine RL. The role of mutations in epigenetic regulators in myeloid malignancies. *Nat Rev Cancer*. 2012; 12(9):599-612.

3. Hu D, Shilatifard A. Epigenetics of hematopoiesis and hematological malignancies. *Genes Dev*. 2016;30(18):2021-2041.

4. Blackledge NP, Rose NR, Klose RJ. Targeting polycomb systems to regulate gene expression: modifications to a complex story. *Nat Rev Mol Cell Biol*. 2015;16(11):643-649.

5. Comet I, Helin K. Revolution in the polycomb hierarchy. *Nat Struct Mol Biol*. 2014;21(7): 573-575.

6. Xu J, Shao Z, Li D, et al. Developmental control of polycomb subunit composition by GATA factors mediates a switch to non-canonical functions. *Mol Cell*. 2015;57(2):304-316.

7. Su IH, Dobenecker MW, Dickinson E, et al. Polycomb group protein ezh2 controls actin polymerization and cell signaling. *Cell*. 2005; 121(3):425-436.

8. Sinclair DA, Milne TA, Hodgson JW, et al. The additional sex combs gene of Drosophila encodes a chromatin protein that binds to shared and unique polycomb group sites on polytene chromosomes. *Development*. 1998;125(7): 1207-1216.

9. Abdel-Wahab O, Adli M, LaFave LM, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. *Cancer Cell*. 2012;22(2):180-193.

10. Sauvageau M, Sauvageau G. Polycomb group proteins: multi-faceted regulators of somatic stem cells and cancer. *Cell Stem Cell*. 2010;7(3): 299-313.

11. Park IK, Qian D, Kiel M, et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature*. 2003; 423(6937):302-305.

12. Oguro H, Iwama A, Morita Y, Kamijo T, van Lohuizen M, Nakauchi H. Differential impact of Ink4a and Arf on hematopoietic stem cells and their bone marrow microenvironment in Bmi1-deficient mice. *J Exp Med*. 2006;203(10): 2247-2253.

13. Hidalgo I, Herrera-Merchan A, Ligos JM, et al. Ezh1 is required for hematopoietic stem cell maintenance and prevents senescence-like cell cycle arrest. *Cell Stem Cell*. 2012;11(5):649-662.

14. Xie H, Xu J, Hsu JH, et al. Polycomb repressive complex 2 regulates normal hematopoietic stem cell function in a developmental-stage-specific manner. *Cell Stem Cell*. 2014;14(1):68-80.

15. Mochizuki-Kashio M, Aoyama K, Sashida G, et al. Ezh2 loss in hematopoietic stem cells predisposes mice to develop heterogeneous malignancies in an Ezh1-dependent manner. *Blood*. 2015;126(10):1172-1183.

16. Oguro H, Yuan J, Ichikawa H, et al. Poised lineage specification in multipotential hematopoietic stem and progenitor cells by the polycomb protein Bmi1. *Cell Stem Cell*. 2010;6(3): 279-286.

17. Iwama A, Oguro H, Negishi M, et al. Enhanced self-renewal of hematopoietic stem cells mediated by the polycomb gene product Bmi-1. *Immunity*. 2004;21(6):843-851.

18. Kamminga LM, Bystrykh LV, de Boer A, et al. The polycomb group gene Ezh2 prevents hematopoietic stem cell exhaustion. *Blood*. 2006;107(5):2170-2179.

19. Klauke K, Radulović V, Broekhuis M, et al. Polycomb Cbx family members mediate the balance between haematopoietic stem cell self-renewal and differentiation. *Nat Cell Biol*. 2013; 15(4):353-362.

20. Konuma T, Nakamura S, Miyagi S, et al. Forced expression of the histone demethylase Fbxl10 maintains self-renewing hematopoietic stem cells. *Exp Hematol*. 2011;39(6):697-709.e5.

21. Majewski IJ, Blewitt ME, de Graaf CA, et al. Polycomb repressive complex 2 (PRC2) restricts hematopoietic stem cell activity. *PLoS Biol*. 2008; 6(4):e93.

22. Majewski IJ, Ritchie ME, Phipson B, et al. Opposing roles of polycomb repressive complexes in hematopoietic stem and progenitor cells. *Blood*. 2010;116(5):731-739.

23. Bracken AP, Helin K. Polycomb group proteins: navigators of lineage pathways led astray in cancer. *Nat Rev Cancer*. 2009;9(11):773-784.

24. van Lohuizen M, Verbeek S, Scheijen B, Wientjens E, van der Gulden H, Berns A. Identification of cooperating oncogenes in E mu-myc transgenic mice by provirus tagging. *Cell*. 1991;65(5):737-752.

25. Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature*. 1999;397(6715):164-168.

26. Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature*. 2003;423(6937):255-260.

27. Yuan J, Takeuchi M, Negishi M, Oguro H, Ichikawa H, Iwama A. Bmi1 is essential for leukemic reprogramming of myeloid progenitor cells. *Leukemia*. 2011;25(8):1335-1343.

28. Rizo A, Horton SJ, Olthof S, et al. BMI1 collaborates with BCR-ABL in leukemic transformation of human CD34+ cells. *Blood*. 2010;116(22):4621-4630.

29. Mihara K, Chowdhury M, Nakaju N, et al. Bmi-1 is useful as a novel molecular marker for predicting progression of myelodysplastic syndrome and patient prognosis. *Blood*. 2006;107(1):305-308.

30. Chowdhury M, Mihara K, Yasunaga S, Ohtaki M, Takihara Y, Kimura A; Expression of Polycomb-group. Expression of polycomb-group (PcG) protein BMI-1 predicts prognosis in patients with acute myeloid leukemia. *Leukemia*. 2007;21(5):1116-1122.

31. Mohty M, Yong AS, Szydlo RM, Apperley JF, Melo JV. The polycomb group BMI1 gene is a molecular marker for predicting prognosis of chronic myeloid leukemia. *Blood*. 2007;110(1):380-383.

32. Beà S, Tort F, Pinyol M, et al. BMI-1 gene amplification and overexpression in hematological malignancies occur mainly in mantle cell lymphomas. *Cancer Res*. 2001;61(6):2409-2412.

33. Comet I, Riising EM, Leblanc B, Helin K. Maintaining cell identity: PRC2-mediated regulation of transcription and cancer. *Nat Rev Cancer*. 2016;16(12):803-810.

34. Tanaka S, Miyagi S, Sashida G, et al. Ezh2 augments leukemogenicity by reinforcing differentiation blockage in acute myeloid leukemia. *Blood*. 2012;120(5):1107-1117.

35. Neff T, Sinha AU, Kluk MJ, et al. Polycomb repressive complex 2 is required for MLL-AF9 leukemia. *Proc Natl Acad Sci USA*. 2012;109(13):5028-5033.

36. Morin RD, Johnson NA, Severson TM, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet*. 2010;42(2):181-185.

37. Béguelin W, Popovic R, Teater M, et al. EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. *Cancer Cell*. 2013;23(5):677-692.

38. Béguelin W, Teater M, Gearhart MD, et al. EZH2 and BCL6 cooperate to assemble CBX8-BCOR complex to repress bivalent promoters, mediate germinal center formation and lymphomagenesis. *Cancer Cell*. 2016;30(2):197-213.

39. Souroullas GP, Jeck WR, Parker JS, et al. An oncogenic Ezh2 mutation induces tumors through global redistribution of histone 3 lysine 27 trimethylation. *Nat Med*. 2016;22(6):632-640.

40. Agarwal P, Alzrigat M, Párraga AA, et al. Genome-wide profiling of histone H3 lysine 27 and lysine 4 trimethylation in multiple myeloma reveals the importance of polycomb gene targeting and highlights EZH2 as a potential therapeutic target. *Oncotarget*. 2016;7(6):6809-6823.

41. Pawlyn C, Bright MD, Buros AF, et al. Overexpression of EZH2 in multiple myeloma is associated with poor prognosis and dysregulation of cell cycle control. *Blood Cancer J*. 2017;7(3):e549.

42. van Haaften G, Dalgliesh GL, Davies H, et al. Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. *Nat Genet*. 2009;41(5):521-523.

43. Pawlyn C, Kaiser MF, Heuck C, et al. The spectrum and clinical impact of epigenetic modifier mutations in myeloma. *Clin Cancer Res*. 2016;22(23):5783-5794.

44. Ntziachristos P, Tsirigos A, Welstead GG, et al. Contrasting roles of histone 3 lysine 27 demethylases in acute lymphoblastic leukaemia. *Nature*. 2014;514(7523):513-517.

45. Van der Meulen J, Sanghvi V, Mavrakis K, et al. The H3K27me3 demethylase UTX is a gender-specific tumor suppressor in T-cell acute lymphoblastic leukemia. *Blood*. 2015;125(1):13-21.

46. Benyoucef A, Palii CG, Wang C, et al. UTX inhibition as selective epigenetic therapy against TAL1-driven T-cell acute lymphoblastic leukemia. *Genes Dev*. 2016;30(5):508-521.

47. Watanabe T. Adult T-cell leukemia: molecular basis for clonal expansion and transformation of HTLV-1-infected T cells. *Blood*. 2017;129(9):1071-1081.

48. Honda H, Nagamachi A, Inaba T. -7/7q-syndrome in myeloid-lineage hematopoietic malignancies: attempts to understand this complex disease entity. *Oncogene*. 2015;34(19):2413-2425.

49. Kotini AG, Chang CJ, Boussaad I, et al. Functional analysis of a chromosomal deletion associated with myelodysplastic syndromes using isogenic human induced pluripotent stem cells. *Nat Biotechnol*. 2015;33(6):646-655.

50. Nikoloski G, Langemeijer SM, Kuiper RP, et al. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat Genet*. 2010;42(8):665-667.

51. Ernst T, Chase AJ, Score J, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet*. 2010;42(8):722-726.

52. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med*. 2011;364(26):2496-2506.

53. Papaemmanuil E, Gerstung M, Malcovati L, et al; Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013;122(22):3616-3627, quiz 3699.

54. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia*. 2014;28(2):241-247.

55. Makishima H, Yoshizato T, Yoshida K, et al. Dynamics of clonal evolution in myelodysplastic syndromes. *Nat Genet*. 2017;49(2):204-212.

56. Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. *Blood*. 2017;129(6):667-679.

57. Brecqueville M, Cervera N, Adélaïde J, et al. Mutations and deletions of the SUZ12 polycomb gene in myeloproliferative neoplasms. *Blood Cancer J*. 2011;1(8):e33.

58. Score J, Hidalgo-Curtis C, Jones AV, et al. Inactivation of polycomb repressive complex 2 components in myeloproliferative and myelodysplastic/myeloproliferative neoplasms. *Blood*. 2012;119(5):1208-1213.

59. Guglielmelli P, Biamonte F, Score J, et al. EZH2 mutational status predicts poor survival in myelofibrosis. *Blood*. 2011;118(19):5227-5234.

60. Ley TJ, Miller C, Ding L, et al; Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368(22):2059-2074.

61. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367-1376.

62. Kim E, Ilagan JO, Liang Y, et al. SRSF2 mutations contribute to myelodysplasia by mutant-specific effects on exon recognition. *Cancer Cell*. 2015;27(5):617-630.

63. Khan SN, Jankowska AM, Mahfouz R, et al. Multiple mechanisms deregulate EZH2 and histone H3 lysine 27 epigenetic changes in myeloid malignancies. *Leukemia*. 2013;27(6):1301-1309.

64. Pellagatti A, Cazzola M, Giagounidis A, et al. Deregulated gene expression pathways in myelodysplastic syndrome hematopoietic stem cells. *Leukemia*. 2010;24(4):756-764.

65. Muto T, Sashida G, Oshima M, et al. Concurrent loss of Ezh2 and Tet2 cooperates in the pathogenesis of myelodysplastic disorders. *J Exp Med*. 2013;210(12):2627-2639.

66. Sashida G, Harada H, Matsui H, et al. Ezh2 loss promotes development of myelodysplastic syndrome but attenuates its predisposition to leukaemic transformation. *Nat Commun*. 2014;5:4177.

67. Sashida G, Wang C, Tomioka T, et al. The loss of Ezh2 drives the pathogenesis of myelofibrosis and sensitizes tumor-initiating cells to bromodomain inhibition. *J Exp Med*. 2016;213(8):1459-1477.

68. Shimizu T, Kubovcakova L, Nienhold R, et al. Loss of Ezh2 synergizes with JAK2-V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. *J Exp Med*. 2016;213(8):1479-1496.

69. Yang Y, Akada H, Nath D, Hutchison RE, Mohi G. Loss of Ezh2 cooperates with Jak2V617F in the development of myelofibrosis in a mouse model of myeloproliferative neoplasm. *Blood*. 2016;127(26):3410-3423.

70. Lee SC, Phipson B, Hyland CD, et al. Polycomb repressive complex 2 (PRC2) suppresses Eμ-myc lymphoma. *Blood*. 2013;122(15):2654-2663.

71. Oshima M, Hasegawa N, Mochizuki-Kashio M, et al. Ezh2 regulates the Lin28/let-7 pathway to restrict activation of fetal gene signature in adult hematopoietic stem cells. *Exp Hematol*. 2016;44(4):282-96.e3.

72. Schneider RK, Schenone M, Ferreira MV, et al. Rps14 haploinsufficiency causes a block in erythroid differentiation mediated by S100A8 and S100A9. *Nat Med*. 2016;22(3):288-297.

73. Hasegawa N, Oshima M, Sashida G, et al. Impact of combinatorial dysfunctions of Tet2 and Ezh2 on the epigenome in the pathogenesis of myelodysplastic syndrome. *Leukemia*. 2017;31(4):861-871.

74. Caye A, Strullu M, Guidez F, et al. Juvenile myelomonocytic leukemia displays mutations in components of the RAS pathway and the PRC2 network. *Nat Genet*. 2015;47(11):1334-1340.

75. De Raedt T, Beert E, Pasmant E, et al. PRC2 loss amplifies Ras-driven transcription and confers sensitivity to BRD4-based therapies. *Nature*. 2014;514(7521):247-251.

76. Yoshida K, Toki T, Okuno Y, et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders. *Nat Genet*. 2013;45(11):1293-1299.

77. Zhang J, Ding L, Holmfeldt L, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature*. 2012;481(7380):157-163.

78. Ntziachristos P, Tsirigos A, Van Vlierberghe P, et al. Genetic inactivation of the polycomb

repressive complex 2 in T cell acute lymphoblastic leukemia. *Nat Med*. 2012;18(2):298-301.

79. Huether R, Dong L, Chen X, et al. The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. *Nat Commun*. 2014;5:3630.

80. Simon C, Chagraoui J, Krosl J, et al. A key role for EZH2 and associated genes in mouse and human adult T-cell acute leukemia. *Genes Dev*. 2012; 26(7):651-656.

81. Danis E, Yamauchi T, Echanique K, et al. Ezh2 controls an early hematopoietic program and growth and survival signaling in early T cell precursor acute lymphoblastic leukemia. *Cell Reports*. 2016;14(8):1953-1965.

82. Carbuccia N, Murati A, Trouplin V, et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Leukemia*. 2009;23(11):2183-2186.

83. Abdel-Wahab O, Gao J, Adli M, et al. Deletion of Asxl1 results in myelodysplasia and severe developmental defects in vivo. *J Exp Med*. 2013; 210(12):2641-2659.

84. Damm F, Chesnais V, Nagata Y, et al. BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. *Blood*. 2013;122(18):3169-3177.

85. Gao Z, Zhang J, Bonasio R, et al. PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. *Mol Cell*. 2012;45(3):344-356.

86. Xu B, Konze KD, Jin J, Wang GG. Targeting EZH2 and PRC2 dependence as novel anticancer therapy. *Exp Hematol*. 2015;43(8):698-712.

87. Kim KH, Roberts CW. Targeting EZH2 in cancer. *Nat Med*. 2016;22(2):128-134.

88. McCabe MT, Ott HM, Ganji G, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature*. 2012; 492(7427):108-112.

89. Kim KH, Kim W, Howard TP, et al. SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. *Nat Med*. 2015;21(12): 1491-1496.

90. Bitler BG, Aird KM, Garipov A, et al. Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. *Nat Med*. 2015;21(3):231-238.

91. Fillmore CM, Xu C, Desai PT, et al. EZH2 inhibition sensitizes BRG1 and EGFR mutant lung tumours to TopoII inhibitors. *Nature*. 2015; 520(7546):239-242.

92. Xu B, On DM, Ma A, et al. Selective inhibition of EZH2 and EZH1 enzymatic activity by a small molecule suppresses MLL-rearranged leukemia. *Blood*. 2015;125(2):346-357.

93. Scott MT, Korfi K, Saffrey P, et al. Epigenetic reprogramming sensitizes CML stem cells to combined EZH2 and tyrosine kinase inhibition. *Cancer Discov*. 2016;6(11):1248-1257.

94. Mohammad F, Weissmann S, Leblanc B, et al. EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas. *Nat Med*. 2017;23(4):483-492.

95. Kim MJ, Cao L, Sheedy J, et al. PTC596-induced Bmi1 hyper-phosphorylation via CDK1/2 activation resulting in tumor stem cell depletion [abstract]. *Cancer Res*. 2014;74. Abstract 5517.

96. Göllner S, Oellerich T, Agrawal-Singh S, et al. Loss of the histone methyltransferase EZH2 induces resistance to multiple drugs in acute myeloid leukemia. *Nat Med*. 2017;23(1):69-78.

97. Ariës I, Chonghaile TN, Karim S, et al. PRC2 mutations induce resistance to conventional chemotherapy by inhibiting mitochondrial apoptosis in T-cell acute lymphoblastic leukemia [abstract]. *Blood*. 2016;128(22). Abstract 604.