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The Role of ASXL1/2 and Their Associated Proteins in Malignant Hematopoiesis

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Abstract

Purpose of the Review Advances in genomic and epigenetic research have uncovered a central role for aberrant epigenetic regulation in the pathogenesis of myeloid malignancies. In the current review, we summarize the roles of ASXL1/2 and their associated proteins in normal and malignant hematopoiesis.

Recent Findings ASXL1/2 and their associated proteins, e.g., polycomb repressive complex 2 proteins, play key roles in regulating hematopoietic stem cell (HSC) functions. Genetic studies reveal that ASXL1/2 and their associated proteins play important roles for the establishment and maintenance of the cell fates of HSCs. Alterations of the genes coding ASXL1/2 and their associated proteins lead to the development of hematological malignancies.

Summary Epigenetic regulation is crucial for normal hematopoiesis. Alteration of multiple epigenetic modifiers contributes to myeloid malignancies. Understanding the molecular mechanisms is critical for further studying ASXL1/2 and their associated proteins in hematopoiesis and developing new therapeutic strategies to treat myeloid malignancies.

Keywords Hematopoiesis · Epigenetic regulation · ASXL1 · Hematopoietic stem cells · Myeloid malignancies

Introduction

Hematopoiesis is a precisely regulated process that continuously generates all lineages of blood cells from hematopoietic stem cells (HSCs) [1]. HSCs are multipotent precursors that have both self-renewal capacity and the ability to regenerate all the different cell lineages [2]. The cell fate of a given cell is fine-tuned by transcriptome status for genes that direct stem cell self-renewal, lineage commitment, and maturation. Abundant evidence indicates that epigenetic modifications play critical roles in the regulation of gene expression, stem cell self-renewal, and lineage commitment. Importantly, next-generation sequencing of hematological malignancies identified spectrum and frequent mutations of genes encoding the epigenetic regulators [3, 4]. These clinical findings result in a great interest in understanding the consequences of these gene mutations in the pathogenesis of myeloid malignancies. Deciphering the molecular mechanisms underlying the alterations of genes controlling epigenetic regulations provides new avenues for the development of novel targeted therapies to treat hematological malignancies.

Myeloid leukemia transformation is a stepwise process that is associated with molecular diversity and clonal selection of neoplastic stem cells [5]. Clonal hematopoiesis of indeterminate potential (CHIP) is defined by the presence of a hematologic neoplasm-associated somatic mutation in peripheral blood or bone marrow (BM) but the absence of definitive morphological evidence of a hematologic neoplasm. The most frequently mutated genes associated with CHIP are *DNMT3A*, *TET2*, and *ASXL1* [6–9]. Mutations in these genes are also common in myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Over time, the acquisition of additional somatic lesions (secondary mutations, such as *RUNX1*) contributes to malignant transformation.

Myeloid malignancies are clonal diseases of hematopoietic stem or progenitor cells (HSC/HPCs) that may result from

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genetic and epigenetic alteration-mediated HSC/HPC dysfunction. Myeloid malignancies include MDS, myeloproliferative neoplasms (MPN), MDS/MPN overlap syndrome (MDS/MPN), and AML. MDS comprises a heterogeneous group of myeloid neoplasms characterized by peripheral cytopenia, BM failure, morphologic dysplasia in one or more hematopoietic lineages, and genetic instability with increased risk to transform to secondary AML [10–12]. Morphologic dysplasia represents a major diagnostic criterion of MDS and can be detected in erythroid cells, neutrophilic cells, and megakaryocytes. AML refers to a group of clonal hematopoietic disorders characterized by proliferation of immature myeloid cells in the BM. The diagnosis of AML depends primarily upon detection of leukemic blasts of myeloid lineage (≥ 20%) in the BM.

It has been shown that the polycomb (PcG) and trithorax (Trx) complexes play crucial roles in the regulation of gene expression in mammals. The two major PcG protein complexes, polycomb repressive complex 1 and 2 (PRC1 and PRC2), are known to maintain transcriptional silencing through chromatin compaction and repressive histone post-translational modifications [13]. PRC1/2 activity is counteracted by the Trx complexes, which deposit activating histone marks, regulating gene transcription. Deletion of distinct PcG proteins results in embryonic lethality accompanied by differentiation biases [14]; thus, PcG proteins are fundamental for proper lineage commitment. ASXL1 (additional sex combs like 1) and ASXL2 are PcG proteins that putatively functions in chromatin-modifier complexes. Genomic studies revealed that the mutations of *ASXL1*/2 and other genes encoding PcG proteins are frequently identified in myeloid malignancies.

In this review, we focus on recent findings on the pathological roles of PcG-related proteins, including EZH2, ASXL1, ASXL2, and BAP1 in myeloid malignancies.

EZH2 and PRC2

There are two well-characterized PcG complexes, PRC1 and PRC2, that incorporate multiple histone-modifying activities to mediate transcriptional repression. PRC1 and PRC2 mediate monoubiquitination on lysine 119 of histone H2A (H2AK119ub) and methylation on lysine 27 of histone H3 (H3K27me1/me2/me3), respectively [15]. The mammalian PRC2 complex consists of four core components: enhancer of zeste homolog 1 (EZH1) or its paralog, EZH2; embryonic ectoderm development (EED); suppressor of zeste 12 homolog (SUZ12); and retinoblastoma-binding protein 4 and 7 (RBBP4/7) [14, 16] (Fig. 1). EZH2 and EZH1 are the catalytic subunits of the PRC2 complex, and their C-terminal SET (Su(var)3–9, enhancer of zeste, and trithorax) domain exhibits methyltransferase activity that catalyzes the methylation of H3K27, a repressive chromatin mark [17, 18]. The catalytic activity of EZH2 requires the presence of SUZ12 and EED [19–21]. RBBP4 and the zinc finger protein AEBP2, two additional subunits of the PRC2 complex, together further stimulate EZH2 enzymatic activity [15]. These subunits are required to maintain the integrity of PRC2 complex. Mutations in any of these genes could impair the stabilization of the PRC2 complex.

EZH2 is the most frequently mutated PcG member in hematological malignancies [22]. Monoallelic gain-of-function mutations that result in substitution of Y641 within the SET domain of *EZH2* ($EZH2^{Y641}$) were found in 22% of germinal center-like diffuse large B-cell lymphoma and 7% of follicular B-cell lymphoma [23]. $EZH2^{Y641}$ mutations result in increased conversion of H3K27me1 to H3K27me2 and H3K27me3. In contrast, loss-of-function mutations were found both mono- and bi-allelically in myeloid malignancies [24]. Deletions, missense, and frameshift mutations in *EZH2* that abrogate its methyltransferase activity frequently occur in MDS (3–13%), MPN (3–13%), and MDS/MPN (8–16%) [24, 25]. *EZH2* mutations lead to the reduction of global levels of H3K27me3 and the increased levels of *EZH2* target genes, including potential oncogenes in tumor cells. Thus, *EZH2* has a cell type-dependent, multifaceted role and acts as either an oncogene or a tumor suppressor in hematological malignancies.

The pathological role of deregulated EZH2 has been deeply investigated in mouse model systems (Table 1). Deletion of *Ezh2* in mice leads to the development of various hematological malignancies, including myeloid malignancies (MDS and MDS/MPN, but not AML), lymphoma, and acute T-cell lymphoblastic leukemia (T-ALL) [26–28]. *EZH2* mutations co-occur with mutations of *TET2* (tet methylcytosine dioxygenase 2) or *RUNX1* (Runt-related transcription factor 1) in patients with myeloid malignancies. Concurrent loss of *Ezh2* and *Tet2* in mice markedly accelerated the development of MDS and MDS/MPN [38]. Sashida et al. showed that the loss of *Ezh2* promoted *RUNX1* mutant-mediated MDS but inhibits leukemic transformation [39]. This is consistent with the finding that MDS patients with *EZH2* mutations have a low risk of transformation to AML and *EZH2* mutations are rare in de novo AML [3]. The loss of *Ezh2* significantly promotes the development of $JAK2^{V617F}$ mutant-induced myelofibrosis, resulting, at least in part, from the enhancement of aberrant megakaryocytopoiesis [40–42]. Conditional loss of *Ezh2* in a hematopoietic system accelerates the onset of the early T-cell precursor ALL induced by oncogenic $NRAS^{Q61K}$ [43]. Collectively, loss of *Ezh2* leads to MDS and MDS/MPN diseases in mice, and combination of *Ezh2* loss with other gene mutations significantly accelerates the progression of myeloid malignancies. These findings indicate that EZH2 plays a tumor suppressive role in MDS and MPN. In contrast, similar to human patients, the mice with Y641F knocked in

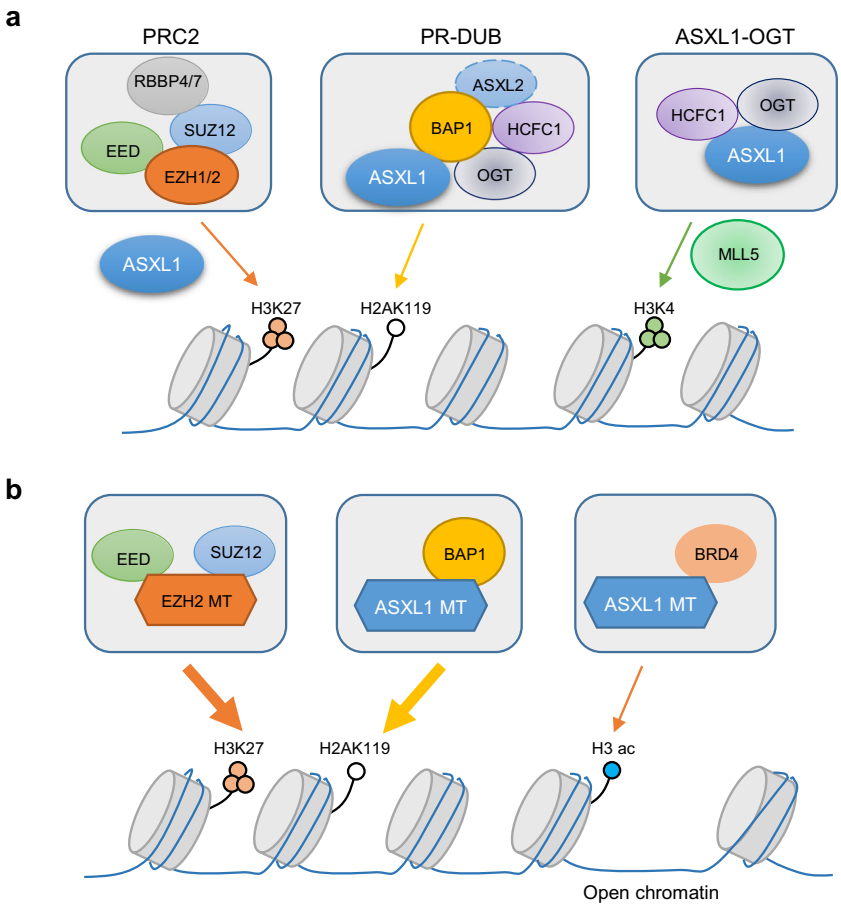

Fig. 1 Overview of effects on histone modifications by wild-type and truncated PcG proteins

the endogenous *Ezh2* locus exhibited lymphoma and melanoma accompanied with an excess of H3K27me3 activity [28].

These studies reinforce a dual role of EZH2 in the process of tumorigenesis, which is cell-lineage dependent.

ASXL1 and ASXL2

ASXL proteins (ASXL1, ASXL2, and ASXL3) are mammalian homologs of additional of sex combs (Asx), a protein that regulates the balance of PcG and Trx function in *Drosophila*. Three ASXL members share conserved domains, including N-terminal ASXN, ASXH domains, ASXM domain (containing the deubiquitinase adaptor, DEUBAD), and a C-terminal plant homeodomain (PHD) finger [44]. Somatic or de novo germline mutations in any of the three ASXL family members were identified in patients with myeloid malignancies or developmental syndromes, respectively [45]. These findings have resulted in a great interest in understanding the function of each ASXL protein, the protein complexes they exist in, and their roles and redundancies in normal hematopoiesis and their mutations in the pathogenesis of myeloid malignancies.

As a putative chromatin regulator, ASXL1 plays an important role in epigenetic regulation by activating or repressing the transcription of genes involved in proliferation and differentiation through its cooperative effect with other chromatin modifiers to regulate histone modifications [46–48]. ASXL1 and BAP1 formed a polycomb repressive deubiquitinase complex (PR-DUB), which removes monoubiquitin from H2AK119 [46]. ASXL1 represses its target genes through H3K27me3 by physically interacting with other PRC2 complex proteins [47]. Recent studies demonstrate a function of ASXL1 in the modulation of H3K4 methylation, an active histone mark on gene regulation [48•]. These results indicate that cooperating with other chromatin factors, ASXL1 plays pivotal roles in controlling the levels of H2AK119ub, H3K27me3, and H3K4me3 and in subsequent gene regulation (Fig. 1). In a separate study, we found that ASXL1 interacts with cohesin complex to maintain normal sister chromatid separation [49] and RNA polymerase II (RNAPII) complex to regulate RNAPII transcriptional activity [50]. These studies demonstrate multifaceted functions of ASXL1 in gene regulation by assembling epigenetic regulators and transcription factors at specific gene loci.

Table 1 Selected genetically engineered murine models to study the functions of PcG genes (*Ezh2*, *Asxl1/2*, and *Bap1*) in hematological malignancies

Mouse model	HSC phenotypes	Disease phenotypes	Disease type	Histone modifications	Refs
Mx1Cre Ezh2$^{f/f}$	Not described	Enlarged lymph nodes and spleens; leukemic cells infiltrated the BM, spleen, liver, and kidney	T-ALL	Reduced H3K27me2/3	[26]
CreERT Ezh2$^{fl/fl}$	Increased LSK fractions in MDS/MPN mice	Morphologic dysplasia of hematopoietic cells; splenomegaly with extramedullary hematopoiesis	MDS and MDS/MPN	Changed the distribution of H3K27me3	[27]
CD19Cre Ezh2^{Y641F}	Not described	Disruption of the splenic architecture and expansion of abnormal, large lymphoid cells in the white pulp	B-cell lymphoma	Increased H3K27me3 and change the distribution of H3K27me3	[28]
Asxl1$^{fl/fl}$ *Mx1Cre or VavCre*	Increased LT-HSC and LSK fractions	Leukopenia and anemia; myeloid and erythroid dysplasia in hematopoietic tissues	MDS-like disease	Reduced H3K27me3	[29]
Asxl1$^{-/-}$ and *Asxl1*$^{+/-}$	Decreased LSK fractions in *Asxl1*$^{-/-}$	Multiple cytopenias; dysplastic features; myeloid cell infiltration in spleen and liver	MDS-like and MDS/MPN-like disease	Reduced H3K4me3 and H3K27me3 in *Asxl1*$^{-/-}$	[30]
Asxl1^{Y588X}Tg	Increased ST-HSC and LSK fractions; and enhanced HSC self-renewal	Anemia; myeloid cell infiltration in spleen and liver; >20% blast cells in BM of leukemia mice	AML, MPN, MDS, and MDS/MPN	Increased H3K27ac and H3K122ac	[31]
VavCre Asxl1-MT$^{fl/fl}$	Decreased LT-HSC and LSK fractions	Decreased RBC and increased platelets	No disease alone	Reduced H3K4me3 and H2AK119ub	[32]
Asxl1$^{G643fs/+}$	Decreased LT-HSC and LSK fractions	Leukopenia, dysplastic features in peripheral blood (PB)	MDS and MDS/MPN-like disease	Reduced H2AK119ub	[33]
Mx1Cre Asxl2$^{fl/fl}$	Decreased HSC and LSK fractions	Leukopenia and thrombocytopenia in recipient mice	Not described	Increased H3K27ac and H3K4me1 s	[34•]
Asxl2$^{-/-}$	Increased HSC and LSK fractions; and enhanced HSC self-renewal	Pancytopenia; dysplastic features in PB and BM; splenomegaly and disrupted splenic architecture with myeloid cell infiltration	MDS-like disease	Changed the distribution of H3K27ac, H3K4me1 and H3K3me2	[35•]
CreERT2 Bap1$^{fl/fl}$	Increased LSK fraction	Splenomegaly; leukocytosis, anemia, thrombocytopenia; erythroid dysplasia	MDS/CMML-like disease	Not described	[36]
Mx1Cre Bap1$^{f/f}$	Increased LSK fraction	Splenomegaly; leukocytosis and anemia	Myeloid malignancies	Increased H3K27me3 and reduced H4K20me1	[37]

ASXL1 is one of the most frequently mutated genes in a variety of myeloid malignancies, including ~15–21% of MDS [51, 52], ~8–10% of MPN [53, 54], ~43–49% of chronic myelomonocytic leukemia (CMML) [55, 56], ~7–8% of juvenile myelomonocytic leukemia (JMML) [57, 58], and ~3–10% of AML [59, 60]. Most importantly, *ASXL1* mutations are associated with poor prognosis [61]. Several clinical studies demonstrate that *ASXL1* mutations have been repeatedly identified in individuals with CHIP, a condition whose frequency increases with advanced age [6–8], indicating that *ASXL1* is one of the earliest genetic events during the process of myeloid transformation. Therefore, understanding the mechanism by which *ASXL1* mutations contribute to myeloid transformation is clinically important. *ASXL1* mutations have also been described in some rare myeloid malignancies, such as aplastic anemia [62]. Interestingly, *ASXL1* mutations have only been sporadically observed in lymphoid leukemia [63]. Compared with *ASXL1* mutations which found in all spectrum of myeloid malignancies, *ASXL2* mutations are restricted in a very specific subset of AML, ~23% of AML with *t*(8;21) translocation [64, 65]. Interestingly, *ASXL1* and *ASXL2* mutations are mutually exclusive in *t*(8;21) AML, suggesting that the mutations may have convergent downstream and/or are synthetic lethal effects with each other.

To understand the functions of ASXL family members in myeloid malignancies, several murine models targeting *Asxl1* and *Asxl2* have been described (Table 1) [29, 30, 34•, 35•]. Constitutive homozygous loss or hematopoietic-specific deletion of *Asxl1* impairs the self-renewal capacity of HSCs and leads to MDS-like disease in mice [29, 30]. Loss of *Asxl1* in the hematopoietic system alone also leads to the development of MDS with a longer latency compared to global deletion of ASXL1 in mice. *Asxl1*-deficient HSC/HPCs have reduced global levels of H3K27me3 and altered the expression of posterior *Hoxa* genes due to impaired recruitment of the PRC2 complex [47].

ASXL1 is known to co-occur with other gene mutations. Inactivating mutations of *NF1* (neurofibromatosis type I) are common genetic events in JMML and AML [66]. We showed that RAS pathway gene mutations occurred in 25.4% of *ASXL1*-mutated myeloid malignancies with poorer prognosis [67]. Using mouse models of *ASXL1* and *NF1*, we showed that loss of *Asxl1* cooperates *Nf1* haploinsufficiency to accelerate myeloid transformation, verifying the cooperative effect of mutations in *ASXL1* and RAS pathway genes in aggressive forms of myeloid malignancies [67]. Somatic mutation of *JAK2*V617F is considered to be the most notable landmark in the diagnosis of the classic Philadelphia chromosome-negative MPNs and is present in > 95% of polycythemia vera patients and in ~50% of essential thrombocythemia and primary myelofibrosis (MF) [68]. Polycythemia vera patients with co-mutations of *Asxl1* and $JAK2^{V617F}$ had a poor MF-free survival. Our recent studies show that heterozygous deletion of *Asxl1* promoted MF in $JAK2^{V617F}$-driven MPN in mice [68]. These studies indicate that alterations of *ASXL1* cooperate with the mutations of other genes critical for hematopoiesis to accelerate leukemogenesis.

The majority of patient-derived *ASXL1* mutations is nonsense or frameshift causing truncation of downstream of the ASXH domain with consequent loss of the PHD domain [61, 69]. These mutations are always heterozygous, leaving one wild-type allele intact, implying either a dominant-negative or gain-of-function character. Mutant *ASXL1* transcripts are predicted to produce C-terminally truncated ASXL1 protein by escaping from nonsense-mediated decay [70]. Inoue et al. reported that the truncating *ASXL1* mutant can be detected in MDS cells, which may play a role in MDS pathogenesis [70]. Despite this being widely acknowledged, it continues to remain controversial as to whether truncating mutations in *ASXL1* result in gain- or loss-of-function or if they confer dominant-negative activity in vivo. Hence, several groups including ours have investigated whether the presence of the truncated forms of ASXL1 protein induces myeloid malignancies.

We recently established a *Vav1* promoter-driven $Asxl1^{Y588X}$ transgenic mouse model ($Asxl1^{Y588X}$Tg) expressing the analogous protein product of the mutant $ASXL1^{Y591X}$, frequently seen in human patients. $Asxl1^{Y588X}$Tg mice had shortened survival rates and predisposition to a spectrum of myeloid malignancies [31••], closely recapitulating the characteristics of myeloid malignancy patients with *ASXL1* truncation mutations. We found that ASXL1 truncation exerts an oncogenic role in HSCs, at least in part through the gained interaction with BET bromodomain-containing protein 4 along with altered gene expression (Fig. 1). More importantly, $Asxl1^{Y588X}$Tg BM cells are sensitive to BET bromodomain inhibitors, suggesting the potential novel therapies targeting the cells bearing the truncation mutation of *ASXL1*.

Nagase and colleagues also reported that expression of a C-terminal truncated *Asxl1* mutant in vivo using conditional knock-in mice results in impaired HSC function and mutant *RUNX1* promotes MDS/AML development in the *Asxl1* mutant background [32••]. They also found that BM $cKit^+$ cells from these mice had substantial reductions in the levels of H3K4me3 and H2AK119Ub, while the level of H3K27me3 was similar to that of control cells. This is different from the effects of *Asxl1* loss on the reduction of H3K27me3 level in HSC/HPCs [30]. Further analysis demonstrated the opposing effects of wild-type versus mutant *Asxl1* on H3K4me3 [32••]. These findings reveal that *ASXL1* mutations confer HSCs with an altered epigenome and increase susceptibility for leukemic transformation. Taken together, these studies demonstrated that the truncation mutation of *ASXL1* promotes the development of myeloid malignancies.

In addition to somatic mutations in hematological malignancies, de novo germline mutations of *ASXL1* have been

discovered in patients with Bohring-Opitz syndrome [71], a disease with severe developmental defects and early childhood mortality. The mouse models developed by our group and others showed that loss of *Asxl1* leads to Bohring-Opitz-like syndrome in mice by altering self-renewal and cell fate of BM stromal cells [29, 30, 72]. BM stromal cells are the major component of the BM microenvironment that maintain and regulate the HSC pool throughout life. We found that systemic deletion of *Asxl1* leads to more severe hematological phenotypes than conditional deletion of *Asxl1*, implicating an important role for *Asxl1* in the microenvironment to support hematopoiesis. Interestingly, germline mutations of *ASXL1* were associated with the patients with MDS and AML [73], further confirming the important role of *Asxl1* in BM microenvironment for normal hematopoiesis.

ASXL2 is another family member of ASXL proteins. The important role of ASXL2 in hematopoietic functions has recently been investigated by two independent groups [34•, 35•]. Using systemic deletion of *Asxl2* mouse model, Li et al. found that loss of *Asxl2* in mice leads to the development of MDS-like disease [35•]. ASXL2 is a critical regulator for self-renewal of HSCs, since *Asxl2* loss enhances the relating capacity of HSCs. Deletion of *Asxl2* alters the expression of genes critical for HSC self-renewal, differentiation ,and apoptosis which is associated with dysregulated H3K27ac and H3K4me1/2 [35•]. *ASXL2* is frequently mutated in AML patients bearing the RUNX1-RUNX1T1 (AML1-ETO) fusion. Micol et al. reported that *Asxl2* loss promoted AML1-ETO leukemogenesis [34•]. Moreover, ASXL2 target genes strongly overlapped with those of RUNX1 and AML1-ETO, and ASXL2 loss was associated with increased chromatin accessibility at putative enhancers of key leukemogenic loci. Interestingly, ASXL2 is required for normal hematopoiesis with distinct, non-overlapping effects from ASXL1 [34•]. These data demonstrate that ASXL2 is required for normal hematopoiesis and promote leukemogenesis driven by AML1-ETO.

BAP1

BAP1 is a deubiquitinase that is a part of the ubiquitin C-terminal hydrolase (UCH) family [74]. The BAP1 protein has three critical domains: a UCH N-terminal catalytic domain which contains the enzymatic active site, a central linker region which modulates binding to host cell factor-1, and a C-terminal ULD domain which potentially coordinates protein-protein interactions. As mentioned above, ASXL1 uses its DEUBAD domain to assemble PR-DUB complex to regulate H2AK119ub with the C-terminal domain of BAP1. This interaction stimulates ubiquitin binding and DUB activity [75]. However, unlike *ASXL1* mutations, *BAP1* somatic mutations are more common in solid tumors than hematological malignancies, indicating the independent roles of BAP1 in regulating epigenetic state and malignant transformation. Indeed, murine models with conditional knockout of *Bap1* versus *Asxl1* show distinct phenotypes (Table 1) [36, 37]. Mechanistic study revealed that loss of *Bap1* increased the expression of *Ezh2* with a higher level of H3K27me3 and enhanced repression of PRC2 targets in HSC/HPCs. Genetic deletion of *Ezh2* further showed an abrogation of *Bap1* loss-mediated myeloid malignancy in vivo [37]. These findings are in contrast with the reduction in H3K27me3 seen with *Asxl1* loss and raise the possibility that myeloid transformation resulting from ASXL1 and BAP1 loss could be independent of the function of the BAP1-ASXL1 complex.

Recent biochemical characterization of BAP1 complexes suggests that BAP1 forms two mutually exclusive complexes with either ASXL1 or ASXL2 [76, 77] and both complexes can stimulate H2AK119 deubiquitination in vitro [78]. However, it is unclear how much redundancy in function occurs between ASXL1 and ASXL2 in their cooperative effect with BAP1. The discrepant phenotypes between the losses of *Bap1* versus *Asxl1* in vivo suggest that loss of *Asxl1* function may be compensated for by its paralog *Asxl2* and/or that *Bap1* and *Asxl1* affect hematopoiesis through divergent downstream effects.

Interestingly, overexpression of truncated forms of ASXL1, but not full-length ASXL1, in combination with overexpression of BAP1 resulted in clear depletion of global H2AK119ub as well as a striking reduction of H3K27me3 [79]. Further studies showed that BAP1 is required for the mutant *ASXL1* in the leukemogenesis [80], and deleting one allele of *Bap1* delays the truncated ASXL1-driven myeloid malignancies in vivo [81]. These data suggest that BAP1 has multifaceted functions in malignant hematopoiesis.

Open Questions

While the advances in genomic and epigenetic research have uncovered a central role for aberrant epigenetic regulation in the pathogenesis of myeloid malignancies, the molecular mechanisms underlying the alterations of epigenetic modifiers in myeloid malignancies remain largely unknown.

Chromatin looping is known to bring promoters and enhancers in proximity to activate gene transcription [82]. ASXL1 interacts with cohesin complex, suggesting that ASXL1 may regulate gene expression by modulating the chromatin architecture [49]. Recent studies showed that the loss of *Asxl2* resulted in the dysregulated gene expression by affecting histone enhancer marks including H3K4me1 and H3K27ac [34, 35]. Thus, the role of ASXL1/2 in the connection of promoters and enhancers is an interesting question. Although phylogenetic analyses suggest that the PHD domain of ASXL1 and ASXL2 protein may bind to H3K4me3 [83], the exact function of the PHD domain of ASXL1 and ASXL2 remains unclear. Moreover, it remains unknown whether the

ASXL1 or ASXL2 PHD domain binds to non-histone proteins. The majority of *ASXL2* mutations was out-of-frame frameshift mutations [64]; it is interesting to investigate if the truncated *ASXL2* exists in hematopoietic cells of human patients and whether the truncated ASXL2 protein contributes to the pathogenesis of myeloid malignancies. Furthermore, several reports show that ASXL1 is one of the key aging factors that is associated with clonal hematopoiesis. However, the investigation of *ASXL1* alterations in clonal hematopoiesis and aging is still missing. A more in-depth understanding of the roles of the PcG-related proteins in hematopoiesis and their alterations in the pathogenesis of myeloid malignancies is necessary.

Conclusion

ASXL1/2 and their associated proteins are essential regulators of hematopoiesis, and alterations of *ASXL1/2* and other genes encoding PcG proteins contribute to the pathogenesis of hematological malignancies. Given that PcG genes establish crosstalk with numerous epigenetic regulators in malignant diseases, the inhibitors can potentially be applied to a broader spectrum of hematopoietic neoplasms. Despite the immense progress that has been achieved, we are still at an early stage in understanding the complex epigenetic regulation in hematopoiesis. Epigenetic aberrations are potentially reversible and can be restored by epigenetic therapies. The understanding of regulatory networks involving in hematopoiesis and their impact on gene expression is crucial to decipher the molecular mechanisms that control hematopoietic development in physiological and pathological conditions and to develop novel therapeutic strategies. Since many of the mutations of genes encoding chromatin modifiers found in hematological malignancies can also be identified in solid tumors, the identification of the molecular basis of hematological cancers could also be informative for developing novel therapeutic strategies for other cancers with mutations in these epigenetic modifiers.

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Compliance with Ethical Standards

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

Human and Animal Rights and Informed Consent All reported studies and experiments involving human or animal subjects performed by the authors have been previously published and complied with applicable ethical standards as defined in the Helsinki declaration and its amendments, institutional and national research committee standards, and international/national/institutional guidelines.

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- • Of importance
- •• Of major importance

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