Cellular and Molecular Life Sciences (2019) 76:2511–2523
https://doi.org/10.1007/s00018-019-03084-7

REVIEW

The role of ASXL1 in hematopoiesis and myeloid malignancies

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Received: 25 December 2018 / Revised: 18 March 2019 / Accepted: 25 March 2019 / Published online: 30 March 2019

Abstract

Recent high-throughput genome-wide sequencing studies have identified recurrent somatic mutations in myeloid neoplasms. An epigenetic regulator, *Additional sex combs-like 1* (*ASXL1*), is one of the most frequently mutated genes in all subtypes of myeloid malignancies. *ASXL1* mutations are also frequently detected in clonal hematopoiesis, which is associated with an increased risk of mortality. Therefore, it is important to understand how *ASXL1* mutations contribute to clonal expansion and myeloid transformation in hematopoietic cells. Studies using *ASXL1*-depleted human hematopoietic cells and *Asxl1* knockout mice have shown that deletion of wild-type ASXL1 protein leads to impaired hematopoiesis and accelerates myeloid malignancies via loss of interaction with polycomb repressive complex 2 proteins. On the other hand, *ASXL1* mutations in myeloid neoplasms typically occur near the last exon and result in the expression of C-terminally truncated mutant ASXL1 protein. Biological studies and biochemical analyses of this variant have shed light on its dominant-negative and gain-of-function features in myeloid transformation via a variety of epigenetic changes. Based on these results, it would be possible to establish novel promising therapeutic strategies for myeloid malignancies harboring *ASXL1* mutations by blocking interactions between ASXL1 and associating epigenetic regulators. Here, we summarize the clinical implications of *ASXL1* mutations, the role of wild-type ASXL1 in normal hematopoiesis, and oncogenic functions of mutant ASXL1 in myeloid neoplasms.

Keywords ASXL1 · BAP1 · HOX · Acute myeloid leukemia · AML · Myelodysplastic syndrome · MDS · MPN · CMML

Introduction

Myeloid malignancies are characterized by aberrant clonal expansion and differentiation defects of hematopoietic stem cells (HSCs), hematopoietic stem progenitor cells (HSPCs) or myeloid progenitor cells. Most myeloid malignancies are associated with high mortality due to limitations of the available therapeutic agents and high relapse rate. To investigate the causative mutations of myeloid malignancies, genome-wide sequencing studies have been performed and have revealed the mutational landscape [1–4].

An epigenetic modulator, *Additional sex combs-like 1* (*ASXL1*), is one of the most frequently mutated genes in a variety of myeloid neoplasms such as myelodysplastic syndromes (MDS) [5–7], acute myeloid leukemia (AML) [7–9], myeloproliferative neoplasms (MPN) [10–16] and chronic myelomonogenous leukemia (CMML) [14, 17–20], and its mutations are always associated with poor prognosis. Additionally, *ASXL1* mutations are frequently found in clonal hematopoiesis (CH) [also called clonal hematopoiesis of indeterminate potential (CHIP)], precursor states for hematologic neoplasms with somatic mutations in the absence of diagnostic criteria for hematologic malignancies [21–23]. Therefore, understanding the mechanism by which *ASXL1* mutations contribute to myeloid transformation is clinically important. To understand the functions of ASXL1, ASXL1 knockdown or Asxl1 knockout mice studies have been performed [24–26]. These studies demonstrated that ASXL1 knockdown promoted the development of MDS/MPN disease and ASXL1 depletion resulted in impaired hematopoiesis due to loss of interaction with polycomb repressive complex 2 (PRC2). On the other hand, most *ASXL1* mutations exist in the last exon and would produce C-terminally truncated mutant proteins of ASXL1 (hereinafter referred as to mutant ASXL1) by escaping from nonsense-mediated-decay [8, 27]. Overexpression of mutant ASXL1 impaired myeloid differentiation and induced MDS in mouse transplantation

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models [28]. There is also growing evidence indicating that the physiological expression of mutant ASXL1 protein perturbs hematopoiesis and promotes myeloid transformation by altering histone modifications in both a dominant-negative and gain-of-function manner [29]. In addition, novel promising therapeutic strategies targeting *ASXL1* mutated malignancies have been investigated [30–33].

In this review, we will summarize the clinical significance of *ASXL1* mutations in myeloid malignancies. We will also describe recent findings of ASXL1 functions from biochemical and biological perspectives, and will then introduce potential targeted therapies for myeloid malignancies harboring *ASXL1* mutations.

Members of mammalian ASXL family

Mammalian ASXL family genes (*ASXL1*, *ASXL2* and *ASXL3*) are paralogs of *Drosophila Additional sex combs* (*Asx*) [34, 35]. *Asx* was originally identified as an enhancer of the trithorax and polycomb group (ETP) genes to regulate *Hox* gene expression [36, 37]. Polycomb group (PcG) genes repress [38, 39], while trithorax group (TrxG) genes activate *Hox* gene expression [40, 41]. Thus, *Drosophila Asx* is involved in both gene activation and repression. In addition, Schermann et al. revealed that Asx and Calypso, the human ortholog of BRCA1-associated protein 1 (BAP1), formed a Polycomb-repressive deubiquitinase (PR-DUB), which removes monoubiquitination of histone H2A at lysine 119 (H2AK119ub) [42]. Collectively, *Drosophila* Asx is now thought to integrally control gene expression through exerting a variety of epigenetic modifications.

Mammalian *ASXL1* is ubiquitously expressed [43]. Human *ASXL1* gene is located on chromosome 20q11 and encodes a 1541 amino acids–protein [44]. ASXL1 has an N-terminus ASXN domain, an ASX homology (ASXH) domain at the N-terminus region, and a plant homeodomain (PHD) finger at the C-terminal region (Fig. 1). ASXN, ASXH, and PHD domains are shared among all three mammalian ASXL family proteins. The ASXN domain is structurally similar to a forkhead-box domain and predicted to be essential for the DNA-binding ability of ASXL family proteins [45]. The ASXH domain is highly conserved from Drosophila to mammalian and is also called as DEUBAD (deubiquitinase adaptor) because this domain binds a deubiquitinase BAP1 [42], suggesting the importance of the interaction between BAP1 and ASXL1. The PHD domain is a histone- or DNA-binding module, and recognizes different histone modification subtypes such as unmethylated H3K4 (H3K4me0) and trimethylated H3K4 (H3K4me3) [46, 47].

Germline mutations of *ASXL1* and *ASXL3* are identified in patients with Bohring–Opitz syndrome, which is characterized by severe developmental disorders [48, 49]. *ASXL2* germline mutations are associated with the Shashi-Pena syndrome, which is a neurodevelopmental syndrome [50]. *ASXL1* and *ASXL2* are ubiquitously expressed in a variety of tissue, whereas *ASXL3* expression is restricted to lymph node, eyes, lungs, skin, brain, and pituitary gland [43].

A recent study showed that ASXL2 was essential for cardiac development and skeletal or metabolic homeostasis [51]. In myeloid malignancies, *ASXL2* mutations are frequently found in AML harboring RUNX1-ETO fusion gene, whereas the frequency of *ASXL2* mutations in other myeloid malignancies is much lower than that of *ASXL1* mutations [52]. Interestingly, however, *ASXL2* mutations are more frequently associated with RUNX1-ETO than *ASXL1* mutations, making this particular fusion gene unique among many fusion genes. *Asxl2*-deficient mice showed more severe impaired hematopoiesis than *Asxl1*-deficient mice and development of MDS-like disease [53–55]. These results indicate that wild-type ASXL2 plays crucial roles as well as a tumor suppressor role in normal hematopoiesis. *ASXL3* mutations are mainly detected in prostate cancers and pancreatic cancers, whereas the mutations are rarely found in hematological malignancies [56]. Although, ASXL2 and ASXL3 share conserved critical domains with ASXL1, the frequency of *ASXL1* mutations are much higher than those of *ASXL2* and *ASXL3* mutations. The diversity of mutation frequencies within the *ASXL* family could be due to the differences in their unique binding partners, their binding sites on chromatin or histone modifications recognized by the PHD domain.

Clinical implications of *ASXL1* mutations in myeloid malignancies

Somatic *ASXL1* mutations are recurrently found in various myeloid malignancies including myelodysplastic syndromes (MDS) [5–7], acute myeloid leukemia (AML) [7–9] and myeloproliferative neoplasms (MPN) such as chronic myelogenous leukemia (CML), chronic neutrophic leukemia (CNL) and primary myelofibrosis (pMF) [10–16]. *ASXL1* mutations are most frequently identified in patients with MPN/MDS overlap syndrome including chronic myelomonocytic leukemia (CMML) (50%) [14, 17–20] and juvenile myelomonocytic leukemia (JMML) [57, 58]. *ASXL1* mutations are also detected in other myeloid malignancies such as blastic plasmacytoid dendritic cell neoplasm (BPDCN) [59] and systemic mastocytosis [60–62]. Additionally, *ASXL1* mutations are found in aplastic anemia, a common cause of acquired bone marrow failure [63, 64]. Conversely, *ASXL1* mutations are rarely found in lymphoid neoplasms [65].

The majority of *ASXL1* mutations are frameshift or nonsense mutations localized at the last exon, exon 12. *ASXL1*

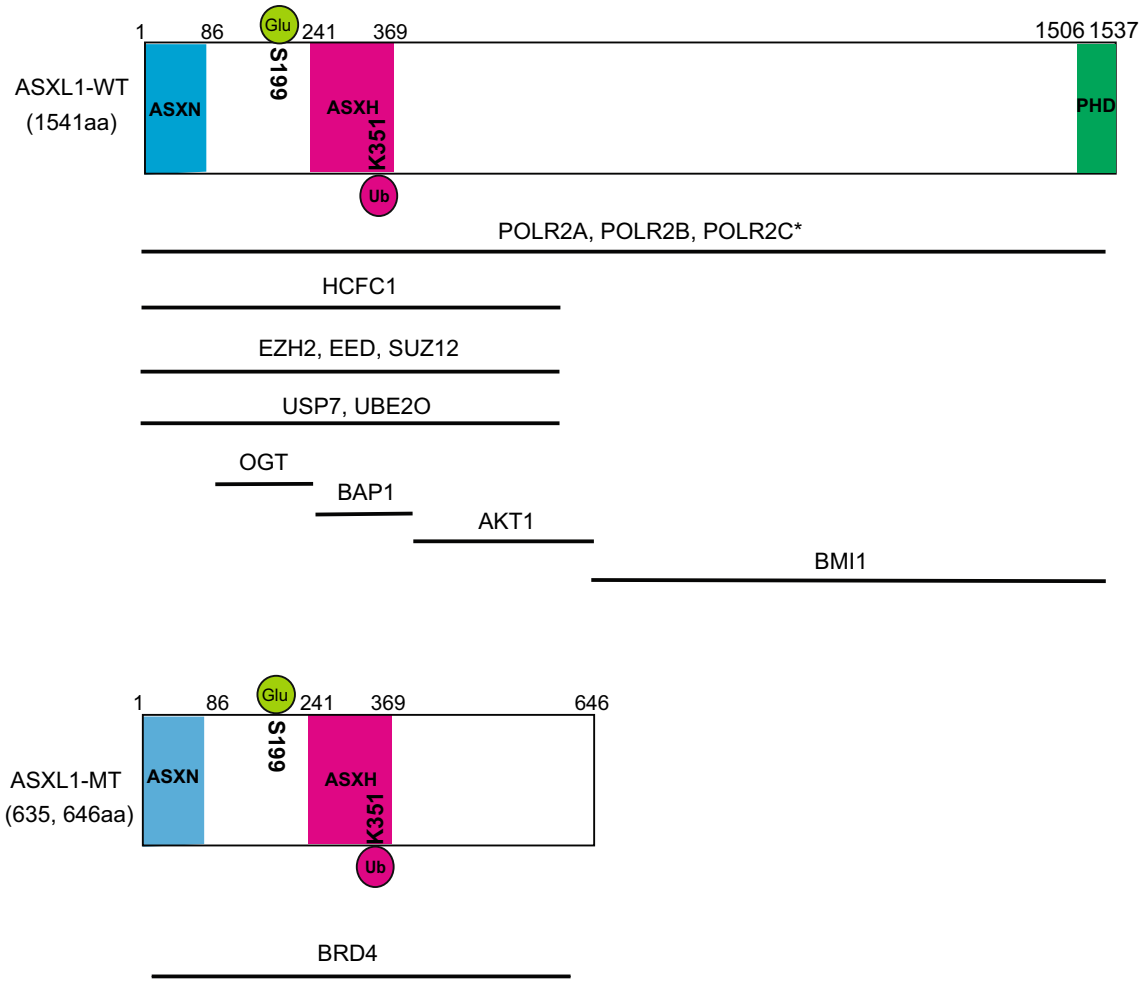

Fig. 1 Schematic representation of the structure of wild-type ASXL1 (ASXL1-WT) and C-terminally truncated mutant ASXL1 (ASXL1-MT). Their known interacting partners and post translational modifications are also shown. *Binding sites are not identified

mutations frequently coexist with the following mutations; DNA methylation-related genes (*TET2* [1], *IDH1* [66], *IDH2* [8, 66, 67]), spliceosomes (*U2AF1* [68], *SRSF2* [69]), transcriptional factors (*CEBPA* [9], *RUNX1* [8, 67, 70, 71], *GATA2* [72]), signal transducers (*NRAS* [14], *JAK2* [70]), *STAG2* [70] and *SETBP1* [73–76]. However, *ASXL1* mutations are mutually exclusive to *DNMT3A* [8, 67], *FLT3-ITD* [8, 67, 71, 77], *NPM1* [8, 71, 77, 78] and *SF3B1* [79] mutations. These positive and negative associations of mutations should be considered in functional analyses of these mutations.

ASXL1 mutations in acute myeloid leukemia

ASXL1 mutations are found in 5–11% of AML patients [71, 80] and independently confer poor prognosis [8, 9, 67, 71, 77]. *ASXL1* mutations in AML are more common in older patients [9, 67, 71], in secondary leukemia [67] and in male patients [9, 67, 71]. In AML, *ASXL1* mutations frequently coexist with *RUNX1* mutations [8, 67, 71] and *IDH2* mutations [67, 81], and are positively associated with FAB M0 karyotype [71, 77], *t*(8; 21) [52, 71, 82], trisomy 8 [67, 71] and del(7q)/−7 chromosomal aberrations [67].

Notably, *RUNX1* is the most frequently mutated gene in *ASXL1*-mutated AML. Coexistence of *ASXL1* and *RUNX1* mutations is related to poor prognosis in AML patients [67]. We previously reported that a RUNX1 frameshift mutation (RUNX1 S291fsX) indeed cooperates with an *ASXL1* mutation to develop MDS/AML in a mouse model [29]. Further studies are required to reveal the precise mechanism by which *ASXL1* mutation and *RUNX1* mutation cooperatively induce myeloid malignancies.

ASXL1 mutations in myelodysplastic syndromes

ASXL1 mutations are found in 11–21% of patients with MDS and are also associated with adverse outcomes in MDS patients [1, 5, 83]. *ASXL1* mutations are more frequently detected in patients with high-risk cases of MDS [6, 7]. DNA hypomethylating agents (HMA) such as azacitidine or decitabine are used for high-risk MDS patients. A recent study showed that *TET2* mutations confer improved response to HMA; however, there was no association between *ASXL1* mutations and response to HMA as there was with *TET2* mutations [84]. Another study demonstrated that *ASXL1* mutations are associated with shorter overall survival in MDS patients treated with HMA [85].

In MDS patients *ASXL1* mutations frequently coexist with *SETBP1* mutations [73–76]. *SETBP1* mutations are localized in the SKI homologous region, resulting in increased stability of the SETBP1 protein [73, 76]. The presence of *SETBP1* mutations is reported to be associated with quicker leukemic transformation of MDS and shorter survivals. In fact, Inoue et al. demonstrated that *SETBP1* mutations rapidly drive leukemic transformation of MDS with *ASXL1* mutations both in patients and in a mouse model [73].

ASXL1 mutations in chronic myelomonocytic leukemia

The *ASXL1* mutation is the most frequently (40–50%) detected mutations in CMML patients. CMML patients harboring *ASXL1* mutations have poorer prognosis [17, 18, 86, 87] and are categorized as a high-risk leukemic transformation group [17, 18]. Prognostic scores, including *ASXL1* mutational status, divides CMML patients into three groups with distinct outcomes [17]. In CMML patients, *ASXL1* mutations frequently coexist with *TET2* mutations. Additional *TET2* mutations are associated with shorter survival in the presence of *ASXL1* mutations [88], while patients harboring *TET2* mutations in the absence of *ASXL1* mutations are categorized as favorable risk groups [89]. In CMML patients, hypomethylating agents are effective, but patients harboring *ASXL1* mutations present a lower overall response rate (ORR) [90].

ASXL1 mutations in clonal hematopoiesis

Along with *TET2* and *DNMT3A* mutations, *ASXL1* mutations are frequently detected in clonal hematopoiesis (CH) as well [22]. Especially, CH is characterized by the presence of a somatic mutation common with hematological neoplasia without cytopenia nor dysplasia. CH is an independent risk factor in progression of myeloid malignancies [21, 23]. CH is also prevalent in aplastic anemia, and clones carrying *ASXL1* mutations tend to increase in size over time [64].

A recent study revealed that CH carriers with *DNMT3A*, *TET2*, *ASXL1* and *JAK2* mutations are associated with atherosclerosis and coronary heart disease. Consistent with these clinical observations, *Tet2*-deficient mice showed enhanced progression of atherosclerosis than control mice [91, 92]. A recent study revealed that lack of *Dnmt3a* also accelerated atherosclerosis in mice [93]. Further studies are required to clarify whether CH with *ASXL1* mutations also accelerate the development of atherosclerosis.

CH is frequently detected in solid tumor patients, particularly after chemotherapy [94]. The presence of CH in solid tumors is associated with higher recurrence ratio and adversely affects survival. It seems that chemotherapy promotes CH; *PPM1D* and *TP53* mutations are particularly related to prior chemotherapy in CH with solid tumors [94]. Recently, there is a series of evidence that *PPM1D* mutations drive CH and confer resistance to chemotherapy [95, 96], but *ASXL1* mutations that are unassociated with prior chemotherapy are frequently found in CH with solid tumors. On the other hand, it is also possible that CH enhances the growth of solid tumors. It will be interesting to investigate whether CH with *ASXL1* mutations influence the growth of solid tumors.

The role of ASXL1 in normal hematopoiesis

To understand the roles of ASXL1 in normal hematopoiesis, several groups engineered and analyzed *Asxl1* knockout mice (Table 1). Fisher et al. engineered and analyzed a constitutive *Asxl1* knockout mouse. Constitutive disruption of *Asxl1* led to partial perinatal lethality. Constitutive loss of *Asxl1* also showed impaired B and T lymphopoiesis and impaired myeloid differentiation [97]. Wang et al. showed that heterozygous genetic *Asxl1* knockout mice (*Asxl1* $^{+/-}$) developed MDS/MPN [26]. *Asxl1* loss led to an increase in apoptotic and mitotic cells in the bone marrow. *Asxl1* loss also exhibited reduced hematopoietic stem cell (HSC)/hematopoietic stem progenitor cell (HSPC) populations and impaired hematopoietic repopulation ability. In addition, Zhang et al. demonstrated that deletion of *Asxl1* cooperated with *Nf1* haplo-insufficiency to activate multiple oncogenic pathways such as MYC, NRAS and BRD4, promoting myeloid transformation [98].

Abdel-Wahab et al. reported that hematopoietic cell-specific deletion of *Asxl1* induced an MDS-like disease. They generated conditional *Asxl1* knockout mice by crossing mice bearing floxed *Asxl1* alleles with *Vav-Cre* or

Table 1 Asxl1 knockout and mutant Asxl1 expressing mice studies

Mice	Peripheral blood phenotypes	HSC/HSPC phenotypes	Myeloid malignancies	Histone modifications	Gene expressions	References
Asxl1^{tm1Bc} mutant mice	Decreased matured B-cell	Decreased formation of myeloid/erythroid colonies	*Asxl1*^{tm1Bc} mutant mice did not develop AML up to 58 weeks	Not described	Not described	[97]
Mx1-Cre/*Vav*-Cre conditional Asxl1 knockout mice	Leukocytopenia and anemia in old (> 6 months) mouse	Increase in LT-HSCs and LSK fractions	Development of Progressive MDS-like disease	Reduced global level of H3K27me3	Increased expression of *Hoxa7/9*	[25]
Constitutive Asxl1 knockout mice	Leukocytopenia, anemia, thrombocytopenia	Decrease in LSK fractions, increase in GMP fractions	Asxl1$^{+/-}$ mice developed mild MDS-like disease	Reduced global level of H3K27me3/H3K4me3	Increased expression of *Hoxa5/7/9/10*	[26]
Retroviral mutant ASXL1 expression in mouse BMT	Leukocytopenia, anemia, thrombocytopenia	Not described	MDS-like disease after a long latency (> 1 year)	Reduced global level of H3K27me3	Increased expression of *miR125a* and *Hoxa9*	[28]
Asxl1 Y588X mutant expressing transgenic mice	Anemia	Increase in ST-HSCs and LSK fractions	A part of mice developed myeloid malignancies	Increased level of H3K122ac at *Prdm16* promoter locus	Increased expression of *Prdm16*	[33]
Vav-Cre Rosa26 mutant Asxl1 knockin mice	Decreased in RBC count	Decrease in LT-HSCs and LSK fractions	Knockin mice alone did not develop MDS/AML but promoted MDS/AML development with RUNX1 mutant	Reduced global level of H2AK119ub/H3K4me3, reduced level of H3K27me3 at *Hoxa* loci	Decreased expression of *Id3*, *Runx1*, *Sox6* and *Tjp1*	[29]
Constitutive locus Asxl1 G643Wfs mutant knockin mice	Leukocytosis and increase in RBC count in aged (18 months) male mice	Competitive serial transplantation assay showed disadvantage	Knockin mice alone did not develop myeloid malignancies within 18 months	Change in distribution of H3K27me3 peak	Not described	[115]
Constitutive locus Asxl1 G643Wfs mutant knockin mice	Leukocytopenia/thrombocytosis in aged (12 months) mouse	Decrease in LT-HSCs and LSK fractions	A part of mice developed MDS/MPN disease in long latency (18–24 months)	Reduced level of H2AK119ub at *p16Ink4a* locus	Increased expression of *Hoxa7/9/10* and *p16Ink4a*	[116]

IFN-α-inducible *Mx1-Cre* transgenic mice [25]. Deletion of *Asxl1* in hematopoietic cells resulted in age-dependent leukopenia and anemia with dysplasia. In the bone marrow of *Asxl1*$^{-/-}$ mice, the number of HSC/HSPC was increased, but the repopulating ability of these cells were impaired. They also showed that *Asxl1* and *Tet2* double knockout mice developed MDS more rapidly than *Asxl1*$^{-/-}$ or *Tet2*$^{-/-}$ mice. Zhang et al. found that systemic deletion of *Asxl1* produced more severe hematological phenotypes than conditional deletion of *Asxl1*, implicating an important role for *Asxl1* in the microenvironment to support hematopoiesis. They further showed that bone marrow stromal cells derived from CMML patients had decreased expression of *ASXL1*, and that loss of *Asxl1* in the bone marrow niche led to a decrease in long-term (LT)-HSCs and myeloid lineage skewing in mice [99]. In human CD34-positive cord blood cells, it was shown that ASXL1 knockdown resulted in reduced erythropoiesis and impaired erythrocyte enucleation [100].

Taken together, these studies demonstrated an essential role of wild-type ASXL1 in maintaining normal hematopoiesis. *Asxl1* deletion leads to impaired progenitor differentiation and often promotes the development of myeloid malignancies.

ASXL1 interaction partners

Schermann et al. revealed that, the mammalian ASXL1, like *drosophila* Asx and a deubiquitinase Calypso, bound the mammalian BAP. They also showed that ASXL1 and BAP1 formed a Polycomb-repressive deubiquitinase (PR-DUB), which removes monoubiquitination of histone H2A at lysine 119, catalyzed by PRC1 complexes [42]. Wild-type ASXL1 interacts with EZH2, EED and SUZ12 as well, main components of the polycomb repressive complex (PRC) 2 to help PRC2 functions [24]. Wild-type ASXL1 protein contributes to repress their target genes such as posterior *HOXA* genes via collaboration with PRC2 to induce a representative histone repressive mark H3K27me3. Therefore, ASXL1 depletion results in global reduction of the trimethylation of histone H3 at lysine 27 (H3K27me3), a representative repressive mark, leading to derepression of posterior *HOXA* genes. It was also reported that knockdown of wild-type *Asxl1* caused myeloid transformation in concert with a NRAS mutant [24]. In addition, Wang et al. revealed that lineage^{-} c-Kit^{+} cells of *Asxl1*-knockout bone marrow cells exhibited global reduction of both H3K27me3 and H3K4me3 [26]. Inoue et al. showed that ASXL1 interacted with OGT and HCFC1 by mass spectrometry, and found that the knockdown of ASXL1, OGT or HCFC1 decreased global levels of H3K4me3 and attenuated myeloid differentiation of HL-60 cells [31]. Previous reports showed that the OGT/HCFC1 complex bound and recruited trithorax homologues, such as MLL1, SET1/COMPASS and MLL5 [101–103]. These results indicate that wild-type ASXL1 could play pivotal roles as a scaffold to control the levels of H2AK119ub, H3K27me3 and H3K4me3, leading to epigenetic control of gene expression.

In addition, wild-type ASXL1 was shown to interact with non-histone proteins; ASXL1 directly bound AKT1 and ASXL1 deficiency led to p27-dependent cell cycle arrest, resulting in cellular senescence [104]. ASXL1 also interacts with the cohesion complex, including SMC1A, SMC3, and RAD21, and ASXL1 depletion leads to impaired telophase cohesion separation [105]. Moreover, ASXL1 interacts with RNA polymerase II (RNAPII) complex to regulate RNAPII transcriptional activity [99].

These findings demonstrated that ASXL1 interacts with a variety of molecules, important for transcription and translation, and that its loss or mutations cause aberrant histone modifications and dysregulated transcription as well as other cellular functions such as cell division and cell signaling, leading to various diseases (Fig. 2).

Posttranslational modifications of ASXL1

Notably, posttranslational modifications of ASXL1 influence its stability and function. Inoue et al. demonstrated that ASXL1 was ubiquitinated at lysine 351. The deubiquitinase USP7 stabilizes ASXL1 by removing polyubiquitin chain [106]. ASXL1 lysine 351 is subject to not only polyubiquitination but also monoubiquitination, in the presence of BAP1 [30]. Interestingly, monoubiquitination of mutant ASXL1 at lysine 351, in turn, activates the catalytic function of associating BAP1. Recent mechanistic analysis of mutant ASXL1 protein revealed the 'gain of function' features of *ASXL1* mutations. BAP1, a strong interacting partner of ASXL1, is frequently mutated in renal cell carcinoma, mesothelioma and uveal melanoma, implicating BAP1 as a tumor suppressor [107–109]. However, *BAP1* is rarely mutated in acute myeloid leukemia [110]. There are a series of experimental evidence that BAP1 plays tumor-promoting roles in myeloid neoplasms. Balasubramani et al. showed that the cancer-associated ASXL1 mutant protein aberrantly enhanced the catalytic function of BAP1, leading to a profound decrease in H2AK119ub [111]. Sahtoe et al. also biochemically demonstrated that the ASXH domain of ASXL1 was essential in increasing BAP1′s affinity to ubiquitin on H2A [112]. We showed the mutually reinforcing effects between the monoubiquitinated form of mutant ASXL1 and BAP1 in myeloid leukemogenesis by dysregulating *HOXA* and *IRF8* genes [30], which are responsible for leukemogenesis and monopoiesis, respectively. We also demonstrated that depletion of endogenous *BAP1* abrogated the leukemogenesis induced by mutant ASXL1, demonstrating pivotal roles of BAP1 in

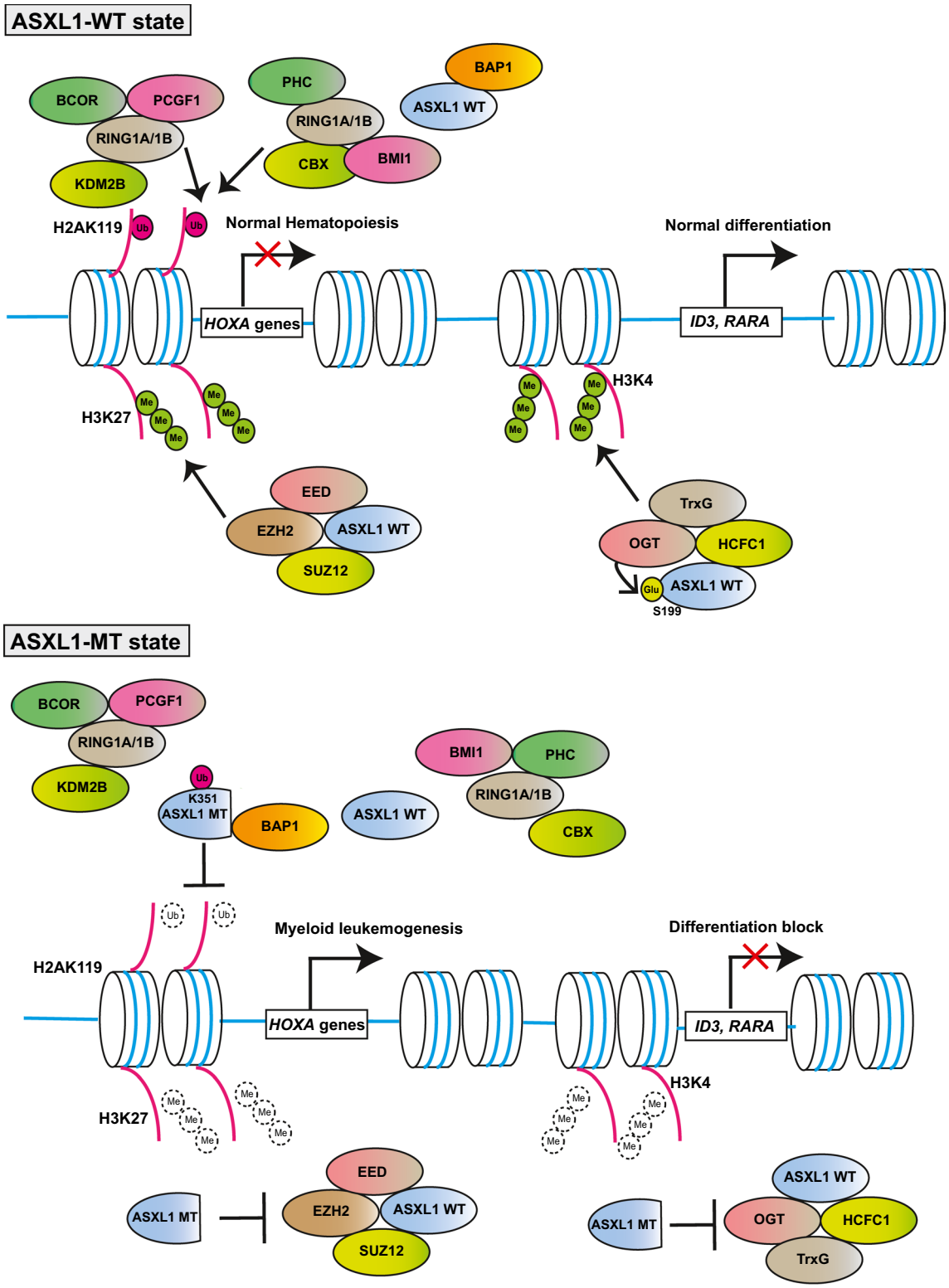

Fig. 2 Overview of effects on histone modifications by wild-type ASXL1 (ASXL1-WT) and C-terminally truncated mutant ASXL1 (ASXL1-MT)

mutant ASXL1-induced cell transformation. Recently, Daou et al. showed that monoubiquitination of wild-type ASXL2 at lysine 370, which corresponds to lysine 351 of ASXL1, was indispensable for activation of the catalytic function of BAP1, and was catalyzed by UBE2E family proteins [113]. Whether monoubiquitination of mutant ASXL1 at lysine 351 is also catalyzed by UBE2E family proteins remains to be elucidated. In addition to ubiquitination, Inoue et al. demonstrated that glycosylation of ASXL1 at serine 199 by OGT (O-linked *N*-acetylglucosamine transferase) was important for its stability [31]. Functional significance of other modifications of ASXL1 such as phosphorylation, sumoylation, and methylation remains to be elucidated.

Mutant ASXL1 protein gains functions leading to myeloid transformation

As described above, *Asxl1* deficiency leads to the development of myeloid diseases in mouse models, suggesting that *ASXL1* mutations are loss-of-function mutations. However, accumulating evidence suggests that mutant ASXL1 proteins gain functions that promote myeloid leukemogenesis. Most ASXL1 mutations in myeloid malignancies are heterozygous frameshift or nonsense mutations localized near the 5′ end of the last exon [20]. Mutant ASXL1 transcripts are, therefore, predicted to escape from nonsense-mediated decay, resulting in production of the C-terminally truncated ASXL1 protein [114]. In cell lines derived from patients with hematological malignancies, mutant ASXL1 proteins were indeed detected by western blot and mTRAQ-based mass spectrometric analyses [27].

Hence, several groups have investigated whether the presence of the C-terminally truncated forms of ASXL1 protein induce myeloid transformation. Inoue et al. showed that mutant ASXL1 proteins (ASXL1-MT) interacted with PRC2 components and interfere with its catalytic activity. Forced expression of ASXL1-MT inhibited wild-type ASXL1 functions and caused MDS/AML development in mouse bone marrow transplantation models via derepression of *miR125a* and *Hoxa* genes caused by decreased H3K27me3 [28]. Yang et al. established C-terminally truncated mutant of Asxl1(Asxl1^{Y588X})-expressing transgenic mice mimicking human *ASXL1* Y591X mutation and demonstrated that transgenic *Asxl1*^{Y588X} expression led to myeloid malignancies [33]. Nagase et al. engineered a conditional Rosa26 locus *ASXL1-MT* knock-in mice (*Asxl1-MT* KI mice) mimicking human *ASXL1* E635RfsX15 mutation, derived from patients with MDS/AML, and characterized the phenotype [29]. *Asxl1-MT* KI mice showed mild anemia and a modest block in erythroid differentiation associated with increased number of platelets, and repopulation ability of HSCs was attenuated. However, *Asxl1-MT* KI mice did not develop any hematological malignancies. Co-expression of a RUNX1 frameshift mutation cooperatively induced MDS/AML in *Asxl1-MT* KI mice. In addition, a retrovirus-mediated insertional mutagenesis study exhibited the susceptibility of *Asxl1-MT* KI bone marrow cells to myeloid leukemia. Thus, mutant Asxl1 promotes leukemia susceptibility.

Several groups generated and analyzed *Asxl1* mutant knock-in mice at the endogenous *Asxl1* locus. Hsu et al. established endogenous locus *Asxl1*^{G643fs} mutant knock-in mice mimicking human *ASXL1* G646WfsX12 mutation (*Asxl1*$^{tm/+}$) [115]. *Asxl1*$^{tm/+}$ mice showed enhanced colony-forming activity of HSPCs and modestly impaired repopulation ability of HSCs. They showed that *MN1* overexpression was observed in patients harboring ASXL1 mutations, and that *MN1* overexpression increased the frequency of long-term culture initiation cells. However, *Asxl1*^{G643fs} mutant knock-in mice alone did not develop hematological malignancies within 18 months of follow-up. On the other hand, Uni et al. generated endogenous locus knock-in mice of *Asxl1*^{G643fs} mutant and identified different phenotypes [116], although it is not clear why theoretically the exact same KI mice gave different phenotypes. The locus KI mice developed by Uni et al. presented decreased number of HSC and increased apoptotic cells, and leukopenia and thrombocytosis were observed at 12 months old, with some mice developing MDS/MPN-like disease after a long latency period (about 18–24 months). Consistent with the previous mouse studies of mutant ASXL1, expression of *Hoxa* genes in *Asxl1*$^{G643fs/+}$ mice was dysregulated. In addition, they focused on upregulation of senescence-related genes including *p16Ink4a* in *Asxl1*$^{G643fs/+}$ mice because young *Asxl1*$^{G643fs/+}$ mice (3 months old) showed myeloid-skewing differentiation like aged mice. In relation to this observation, it was previously reported that the ASXL1/BAP1 axis was implicated in upregulation of *p15Ink4b,* supported by the fact that the promoter activity of INK4B-ARF-INK4A locus was suppressed by H2AK119ub modification [117]. Uni et al. demonstrated that wild-type, but not mutant ASXL1 proteins, interacted with BMI1, a key component of PRC1. The level of H2AK119ub was decreased at the *p16Ink4a* promoter locus, and *Ink4a* expression was derepressed in *Asxl1*^{G643fs} mutant knock-in mice. They also found that *p16Ink4a* knockout rescued decreased HSC numbers and aberrant apoptosis in *Asxl1*^{G643fs} mutant knock-in mice.

Collectively, these findings indicate that mutant ASXL1 at physiological expression levels alone is insufficient to induce myeloid transformation but impairs hematopoiesis and promotes susceptibility to myeloid malignancies by altering histone modifications. The distinct phenotypes of *Asxl1* mutant knock-in mice among several groups could be caused by the differences in the cites of *Asxl1* mutations or the levels and the hematopoietic lineages of *Asxl1* expression.

Potential therapies for myeloid malignancies harboring *ASXL1* mutations

Recent studies pave the way to novel therapeutic strategies for *ASXL1*-mutated myeloid malignancies. First, ASXL proteins/BAP1 complex promotes gene activation via opposing PRC1-mediated monoubiquitination of H2AK119 [118]. As described above, ASXL1-MT, but not wild-type ASXL1, strongly enhanced the catalytic activity of BAP1, resulting in profound reduction of H2AK119ub [30, 111]. In hematopoietic cells, hyperactive ASXL1-MT/BAP1 complex upregulates *HOXA* genes resulting in myeloid transformation [30]. Therefore, enzymatic activity of BAP1 or BAP1–ASXL1 binding is a potential therapeutic target for *ASXL1*-mutated myeloid malignancies. Guo et al. also revealed that the endogenous Bap1 activity is essential for pathogenesis of myeloid malignancies of $Asxl1^{Y588X}$ transgenic mice [119].

In addition, it has been shown that ASXL1-MT, but not wildtype ASXL1, bound Bromodomain-containing 4 (BRD4) [33], a well-known oncoprotein in myeloid malignancies [120]. BRD4 activates pTEFb complex and induces acetylation of H3 at lysine 122 (H3K122Ac), resulting in phosphorylation of RNA polymerase II and gene activation. In the $Asxl1^{Y588X}$ transgenic mice, the level of H3K122Ac at the promoter locus of *Prdm16* was increased, resulting in dysregulated expression of *Prdm16* [33]. Bone marrow cells from $Asxl1^{Y588X}$ transgenic mice showed higher sensitivity to the BRD4 inhibitor than those from normal mice.

A previous study showed that combined expression of ASXL1-MT and SETBP1-MT rapidly developed MDS/AML in mice and the leukemia cells showed repression of TGFβ pathway genes [73]. Nano-liquid chromatography–mass spectrometry analysis revealed physical interaction between mutant ASXL1 and HDAC1 [30]. Saika et al. demonstrated that decrease in acetylation levels of histone H3K14 and H4K5 at TGFβ pathway genes in leukemia cells transformed by ASXL1-MT and SETBP1-MT [32]. They also showed that mutant ASXL1-induced leukemia conferred high sensitivity to an HDAC inhibitor, vorinostat. Vorinostat restored acetylation of histone H3K14 and H4K5 and the expression of TGFβ pathway genes.

On the other hand, it is effective to reactivate the functions of wild-type ASXL1 which are weakened by hemizygous *ASXL1* mutations. Wild-type ASXL1/OGT complex is required for maintaining the level of H3K4me3 [31]. Depletion of ASXL1 or OGT led to impaired myeloid differentiation and global loss of the level of H3K4me3. In addition, OGT directly bound and stabilized wild-type ASXL1. Therefore, enhancing OGT activity is a reasonable strategy for restoring tumor suppressive functions of wild-type ASXL1. Intriguingly, an OGA inhibitor, which elicits the OGT activity, was effective in suppressing growth of leukemia cells expressing the mutant ASXL1 by restoring the tumor suppressor roles of wild-type ASXL1–OGT axis [31].

Taken together, inhibition of either BAP1, BRD4, HDACs or OGA has been shown to suppress leukemia with *ASXL1* mutations in mouse models. These findings need to be validated using patient derived xenograft (PDX) models in future studies.

Conclusions and future perspectives

ASXL1 mutations are often associated with poor prognosis. Therefore, it is important to understand the precise mechanisms by which *ASXL1* mutations contribute to myeloid transformation. Recent biological analyses demonstrated that mutant ASXL1 plays pivotal roles in leukemogenesis and leads to increased susceptibility to myeloid transformation by altering histone modifications. Meanwhile, unlike other epigenetic factors such as EZH2 and TET2, ASXL1 itself has no catalytic function. Hence, ASXL1 binding partners have been intensively investigated and biochemical analyses of these binding partners have shed light on the potential therapeutic strategies for myeloid malignancies harboring *ASXL1* mutations.

While mutant ASXL1 causes dysregulations of histone modifications, resulting in myeloid malignancies, wild-type ASXL1 should also play crucial roles in epigenetic regulations under the physiological conditions via interacting a variety of epigenetic factors. In addition, ASXL1 have various post-transcriptional modifications probably induced by outside stimuli. Therefore, investigation of epigenetic control by wild-type ASXL1 may clarify how the outside stimuli are converted to the transcriptional profiles via altering epigenetics.

Acknowledgements This work was supported by a Grant-in-Aid Scientific Research B from the Ministry of Education, Culture, Sports, Science and Technology of Japan (15H04855, TK), a Grant from the Tokyo Biochemical Research Foundation (TK), and a Grant from the Uehara Memorial Foundation (TK).

Compliance with ethical standards

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

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