Epigenetic dysregulation of hematopoietic stem cells and preleukemic state

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Abstract Recent genetic analyses have revealed that pre-malignant somatic mutations in hematopoietic cells are common in older people without an evidence of hematologic malignancies, leading to clonal hematopoietic expansion. This phenomenon has been termed clonal hematopoiesis of indeterminate potential (CHIP). Frequency of such clonal somatic mutations increases with age: in 5–10% of people older than 70 years and around 20% of people older than 90 years. The most commonly mutated genes found in individuals with CHIP were epigenetic regulators, including *DNA methyltransferase 3A (DNMT3A)*, *Ten–eleven-translocation 2 (TET2)*, and *Additional sex combs-like 1 (ASXL1)*, which are also recurrently mutated in myeloid malignancies. Recent functional studies have uncovered pleiotropic effect of mutations in *DNMT3A*, *TET2*, and *ASXL1* in hematopoietic stem cell regulation and leukemic transformation. Of note, CHIP is associated with an increased risk of hematologic malignancy and all-cause mortality, albeit the annual risk of leukemic transformation was relatively low (0.5–1%). These findings suggest that clonal hematopoiesis per se may not be sufficient to engender preleukemic state. Further studies are required to

decipher the exact mechanism by which preleukemic stem cells originate and transform into a full-blown leukemic state.

Keywords Hematopoietic stem cells · Clonal hematopoiesis · CHIP · Preleukemic state · Epigenetic regulators

Introduction

Recent studies using next-generation sequencing analysis have uncovered premalignant genetic changes in healthy individuals with no evidence of hematologic malignancies. This phenomenon has been termed clonal hematopoiesis of indeterminate potential (CHIP), which is associated with increased risk of developing hematologic malignancy and decreased overall survival [1, 2]. The most frequently mutated genes found in individuals with CHIP were epigenetic modifiers, including *DNA methyltransferase 3A (DNMT3A)*, *Ten–eleven-translocation 2 (TET2)*, and *Additional sex combs-like 1 (ASXL1)*. Notably, these epigenetic modifiers are also recurrently mutated in various myeloid malignancies, suggesting an essential role of these mutations in early phase of leukemia development. Indeed, functional correlation between epigenetic modifiers and normal/malignant hematopoiesis has been a focus of intense study for almost a decade. These studies have shown that mutations in epigenetic modifiers exert pleiotropic effect in leukemogenesis, including hematopoietic stem cell (HSC) self-renewal/differentiation, transcriptional regulation of oncogenes/tumor suppressors, and DNA damage response [3, 4]. In this review, we will focus on genetic basis and clinical features of CHIP, roles of mutations in epigenetic regulators in preleukemic stem cells (Pre-LSC)

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and myeloid transformation, and hypothetical model of leukemogenesis.

Clonal hematopoiesis of indeterminate potential and age-related clonal hematopoiesis

Early analysis of inactivation patterns of X-linked genes such as glucose-6-phosphate dehydrogenase (*G6PD*) or the androgen receptor (*AR/HUMARA*) revealed the clonal origin of myeloid malignancies [5, 6]. Although similar techniques have shown that age-related clonal skewing of hematopoietic cells is relatively common after the age of 55–65 years, until recently the general notion was that this clonal hematopoiesis is rarely associated with hematologic malignancy [7, 8]. Recent study has identified *TET2* mutations in 5.6% of older women with age-related X-inactivation skewing clonal hematopoiesis without evidence of hematologic malignancy, suggesting that initial driver mutations can drive age-related clonal hematopoiesis [9]. In addition, *DNMT3A* mutations, but not leukemic blast-specific *NPM1* mutations, were identified in non-neoplastic normal T cells derived from acute myeloid leukemia (AML) patients, further providing evidence that mutations in some epigenetic modifiers can serve as premalignant genetic changes [10].

More recently, three large studies have further uncovered the genome-wide premalignant genetic lesions responsible for age-related clonal hematopoiesis [11–13]. A group from Washington University analyzed The Cancer Genome Atlas blood sequencing data from 2728 patients without apparent hematologic malignancies and identified blood-specific clonal mutations in more than 2% of all individuals and 5–6% of people older than 70 years [11]. In the second study, whole-exome sequencing of peripheral blood DNA from 12380 Swedish patients without hematologic malignancies with median follow-up of 2–7 years after DNA sampling demonstrated clonal hematopoiesis with somatic mutations in 10% of persons older than 65 years but in only 1% of those younger than 50 years [12]. In the third analysis, investigation of whole-exome sequencing datasets derived from 17182 individuals in 22 population-based cohorts in type 2 diabetes association studies identified clonal somatic mutations in 9.5% of people aged 70–79, 11.7% of people aged 80–89, and 18.4% of people aged 90–108 years [13]. These data suggested that frequency of clonal somatic mutations increases with age. In two patients with CHIP who later developed AML, sequencing of bone marrow biopsy specimens revealed that their leukemia arose from the previously identified CHIP clones [12]. The most common recurrently mutated genes in these studies were *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *Janus kinase 2* (*JAK2*), and *SF3B1*, which are also recurrently mutated

in myeloid neoplasms (Fig. 1) [11–13]. Of note, mutations in *DNMT3A*, *TET2*, and *ASXL1* are commonly observed in these three studies, underscoring an essential role of epigenetic modifiers in the development of clonal hematopoiesis.

Clonal hematopoiesis and preleukemic state

The above studies have demonstrated that the presence of CHIP is a strong risk factor of developing hematologic malignancy [12, 13]. Along this line, individuals with clonal mutations in the Swedish cohort showed an increased risk of hematologic cancer diagnosis (hazard ratio (HR) 12.9) and death (HR 1.4) compared to age-matched individuals without mutations [12]. In addition, study in population-based cohorts revealed that the presence of CHIP is associated with an increase in the risk of hematologic malignancy (HR 11.1) and all-cause mortality (HR 1.4) compared to age-matched controls [13]. Importantly, however, annual risk of leukemic transformation in individuals with CHIP was only 0.5–1%. Moreover, death from hematologic malignancy alone could not explain the observed increase in overall mortality. Rather, the most significant cause of decreased overall survival associated with CHIP was an increased propensity for smoking, thrombosis, and cardiovascular diseases, including coronary artery disease and stroke [12, 13]. Indeed, atherosclerosis-prone low-density lipoprotein receptor-deficient (*Ldlr*^{−/−}) mice reconstituted with *Tet2*-deficient BM cells led to clonal hematopoietic expansion and a marked increase in atherosclerotic plaque in vivo due to increased NLRP3 inflammasome-mediated interleukin-1 β secretion in *Tet2*-deficient macrophages, providing functional relevance of somatic *TET2* mutations in hematopoietic cells in atherosclerosis development [14]. These data indicate that, although clonal genetic changes or cooperating mutations may lead to alteration of normal hematopoiesis, clonal hematopoiesis per se may not be sufficient to engender preleukemic state, which evolves with high frequency into full-blown leukemia.

Clonal somatic mutations in aplastic anemia

Clonal hematopoiesis is also identified in non-malignant hematologic diseases. Acquired aplastic anemia (AA) is characterized by peripheral blood cytopenia with hypocellular bone marrow. However, given the resemblance of clinical presentation and the lack of clear diagnostic criteria distinguishing AA and hypoplastic myelodysplastic syndromes (MDS), the exact clinical diagnosis is often challenging [15]. Previous studies have shown the evidence of clonal hematopoiesis in AA. Indeed, around 50% of patients with acquired AA have expanded populations of

Fig. 1 Recurrent mutations in CHIP, MDS, AML, and AA. Recurrently mutated genes are classified into functional categories. Mutational frequency data are derived from the following resources: CHIP [11–13], MDS [2], AML [35, 71], and AA [17, 18]

paroxysmal nocturnal hemoglobinuria (PNH) clones [16]. Moreover, transient cytogenetic abnormalities have been reported in acquired AA patients with no apparent evidence of MDS [16]. Despite these observations, until recently, the significance and molecular basis of clonal hematopoiesis in AA have been elusive.

Recent targeted deep sequencing study analyzed 150 acquired AA patients with no morphological evidence of MDS and detected clonal somatic mutations in 19% of these cases [17]. The most frequently mutated genes were *ASXL1*, *DNMT3A*, and *BCL6 corepressor (BCOR)* (Fig. 1), with the highest median mutant allele burden of 31% in *ASXL1* mutations [17]. In this study, patients with clonal somatic mutations had longer disease duration and shorter telomere lengths compared to patients without mutations. In addition, AA patients with clonal somatic mutations with disease duration of longer than 6 months were associated with 40% risk of transformation to MDS [17].

More recently, targeted deep sequencing of myeloid cancer candidate genes was performed in 439 patients with AA and identified mutations in these genes in approximately one-third of AA patients [18]. Consistent with previous study, the most commonly mutated genes were *DNMT3A*, *ASXL1*, *BCOR/BCORL1*, and *phosphatidylinositol glycan anchor biosynthesis class A (PIGA)* (Fig. 1), most of which showed less than 10% variant allele frequencies [17, 18]. Of note, *DNMT3A*-mutated and *ASXL1*-mutated clones tended to increase in size over time, whereas the size of *BCOR/BCORL1*-mutated and *PIGA*-mutated clones was more likely to decrease or remain stable [18]. Furthermore, mutations in *BCOR/BCORL1* and *PIGA* correlated with a better response to immunosuppressive therapy (IST) and favorable survival, whereas mutations in a subset of genes including *DNMT3A* and *ASXL1* were associated with a poorer response to IST, inferior overall survival, and progression to MDS or AML [18]. Together, these studies

suggest that a significant proportion of AA patients with no morphological evidence of MDS possess mutations in myeloid cancer-related genes including epigenetic regulators, indicating functional relevance of epigenetic modifiers in clonal hematopoiesis in AA. In the following chapter, we will review pleiotropic effect of major epigenetic modifiers in HSC regulation and leukemogenesis, mutations of which were reported in individuals with clonal hematopoiesis.

Roles of epigenetic modifiers in clonal hematopoiesis and leukemogenesis

DNMT3A

There are three active mammalian families of DNA methyltransferases that enzymatically add a methyl group

to cytosine in CpG dinucleotides in DNA. DNMT1 is a maintenance methyltransferase that methylates the newly synthesized CpG dinucleotides during DNA replication, whereas DNMT3A and DNMT3B are de novo DNA methyltransferases (Fig. 2) [19]. As mentioned in the previous chapter, *DNMT3A* is recurrently mutated in individuals with CHIP and AA patients with clonal hematopoiesis [11–13, 17, 18]. Originally, somatic mutations of *DNMT3A* were first reported in adult AML cases [20, 21]. In addition, *DNMT3A* mutations are associated with adverse prognosis and decreased overall survival in cytogenetically normal AML [22].

Around 50% of *DNMT3A* mutations in AML are heterozygous missense mutation in Arg882 (R882, most commonly R882H), which is located in the catalytic domain of the enzyme [20, 21]. In physiologic condition, DNMT3A functions as a tetramer, comprising either two homodimers

Fig. 2 DNA methylation and demethylation pathway. The biochemical process of DNA methylation/demethylation and the enzymes involved in each steps are shown. Cytosine can be methylated into 5-methylcytosine (5mC) by DNMTs, and 5mC can be iteratively oxidized into 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) by TETs. 5caC can be converted to unmethylated cytosine by TDG-mediated BER pathway. Alternatively, 5hmC can be deaminated into 5-hydroxymethyluracil (5hmU) by AID/APOBEC, followed by TDG- or SMUG1-mediated BER to generate unmethylated cytosine. Oncometabolite 2-HG, which can be produced from α -KG by mutant IDH1/2, inhibits TET enzymes and other α -KG-dependent oxygenases. Note that enzymes affected by somatic mutations in myeloid malignancies are enclosed in squares

or heterodimers with DNMT3L, a DNMT family member that lacks a methyltransferase catalytic domain [23]. Heterodimerization of DNMT3A with DNMT3L enhances its methyltransferase activity [23, 24]. Although mouse Dnmt3a R878H (corresponding to human R882H) mutant protein can still interact with wild-type Dnmt3a and Dnmt3b, co-expression of wild-type and mutant form in murine embryonic stem (ES) cells led to inhibition of the wild-type DNA methylation ability, consistent with dominant-negative effect of *DNMT3A* R882H mutations [25]. Moreover, DNMT3A R882H mutant was shown to inhibit wild-type de novo methylation activity by disrupting the formation of its functional tetramers [26], further confirming the dominant-negative role of this mutant.

Several studies have reported the functional relevance of *DNMT3A* in hematopoiesis. Initial study demonstrated that *Dnmt3a/Dnmt3b* double-knockout HSCs, but not single deficient HSCs, show disrupted HSC self-renewal [27]. More extensive analysis in vivo using hematopoietic tissue-specific conditional *Dnmt3a*-deleted animals revealed progressive expansion of long-term HSC compartment with impaired differentiation in *Dnmt3a*-null cells by incomplete epigenetic repression of HSC-specific genes [28]. More recently, hematopoietic tissue-specific conditional loss of both *Dnmt3a* and *Dnmt3b* resulted in enhanced HSC self-renewal and a more severe differentiation block than *Dnmt3a* single-null cells, partly due to activated β -catenin signaling [29]. In agreement with the murine data, recent targeted deep sequencing study has identified recurrent *DNMT3A* mutations at high allele frequency in highly purified HSC population as well as in normal T-cell compartment in AML patients [10]. In addition, *DNMT3A* mutant HSCs showed a multilineage repopulation advantage over non-mutated HSCs in xenografts, suggesting a fundamental role of *DNMT3A* mutation in establishing Pre-LSCs [10]. Given *DNMT3A* is also one of the most recurrently mutated genes in individuals with CHIP and AA patients with clonal hematopoiesis [11–13, 17, 18], these evidences indicate that *DNMT3A* mutation is clearly responsible for clonal hematopoietic expansion.

Further studies focused on the function of *DNMT3A* mutation in leukemogenesis. *In vivo* analysis using a retroviral transduction of *DNMT3A* R882H mutant construct and bone marrow transplantation (BMT) assay showed development of chronic myelomonocytic leukemia (CMML)-like disease in mutant recipients, possibly through increased CDK1 protein level and enhanced cell-cycle activity [30]. Similarly, mice transplanted with *Dnmt3a*-null whole bone marrow (BM) or HSCs developed a spectrum of hematologic malignancies, including MDS, AML, myeloproliferative neoplasms (MPN), and T- and B-cell acute lymphocytic leukemia (ALL) [31, 32]. Furthermore, primary mice with conditional hematopoietic *Dnmt3a* loss

caused fully penetrant MPN with myelodysplasia (MDS/MPN) in vivo, with cell-autonomous aberrant tissue tropism and marked extramedullary hematopoiesis with liver involvement [33]. These studies underscore a significant relevance of *DNMT3A* as a tumor suppressor in vivo. Of note, recent study demonstrated that *DNMT3A*^{R882}-mutated AML cells show chemoresistance to anthracyclines by impaired nucleosome eviction and chromatic remodeling in response to anthracyclines, which resulted from attenuated recruitment of histone chaperone SPT-16, leading to defective DNA damage response [34]. This result, at least partially, explains why *DNMT3A*^{R882}-mutated AML patients show an inferior outcome when treated with standard-dose daunorubicin-based induction chemotherapy [35, 36] and how *DNMT3A*^{R882} mutant cells persist and drive relapse after induction therapy [10].

TET2

The TET family of proteins was first reported with the cloning of *TET1* as a fusion partner of *MLL1* in patients with t(10;11)(q22;q23) AML [37]. TET proteins (TET1–3) are Fe(II)- and α -ketoglutarate- (α -KG)-dependent mammalian DNA oxidases that catalyze the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) (Fig. 2) [38]. The discovery of this new modification on DNA methylcytosine has provided a novel insight into DNA demethylation pathways. The TET enzymes can also oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [39]. Furthermore, 5caC can be directly recognized and repaired by thymine DNA glycosylase (TDG)-mediated base-excision repair (BER) to generate unmethylated cytosine, leading to active DNA demethylation (Fig. 2) [40]. Alternative active DNA demethylation through the activation-induced cytidine deaminase (AID)-APOBEC DNA repair pathway has also been reported. The first step of this pathway is the conversion of 5hmC to 5-hydroxymethyluracil (5hmU) by AID/APOBEC, followed by TDG- or single-strand-selective monofunctional uracil DNA glycosylase (SMUG1)-mediated BER to generate unmethylated cytosine (Fig. 2) [41, 42]. Of note, *Aid*-deficient BM cells demonstrated a significant accumulation of 5hmC compared to wild-type cells, consistent with deaminase activity of *Aid* on 5hmC [43]. On the other hand, 5hmC may also lead to passive DNA demethylation, as DNMT1, a maintenance methyltransferase that methylates unmethylated cytosine in the daughter strand upon DNA replication, cannot recognize 5hmC [44]. Although these studies suggest a critical role of TET enzymes in active/passive DNA demethylation, the exact biochemical mechanism of demethylation process, the exact genetic loci that are affected by TET-mediated demethylation, biological significance of each chemically

modified cytosine, and their functional relevance in cancer are still under active investigation. Recent studies have also uncovered a novel role of TET proteins in chromatin modifications. Several groups have reported that TET proteins interact with O-linked β -*N*-acetylglucosamine (O-GlcNAc) transferase (OGT), tethering OGT to the target gene promoters and regulating gene transcription through histone H2B O-GlcNAcylation or H3K4 trimethylation [45–47]. These data indicate that TET also modifies chromatin landscape as well as DNA methylation, thereby regulating gene transcription.

TET2 is one of the major epigenetic modifiers recurrently mutated in individuals with CHIP [11–13]. Previous studies using high-throughput genome-wide sequencing have identified somatic deletions and loss-of-function mutations in the *TET2* gene in 10–20% of patients with MDS/MPN [48, 49], in 10–20% of patients with AML and in 40–50% of patients with CMML [50, 51]. Additionally, *TET2* mutations are also reported in lymphoid malignancies, especially at high frequency in angioimmunoblastic T-cell lymphoma [52–55], suggesting a common key role of *TET2* as a tumor suppressor in hematologic malignancies. Large series of clinical correlative study has demonstrated that *TET2* mutations are associated with poorer prognosis in patients with intermediate-risk, cytogenetically normal AML [35]. Although several studies have shown a decrease in global 5hmC levels in patients with *TET2*-mutated myeloid malignancies, changes in global levels of 5mC in these patients are not conclusive [56, 57]. While transcriptional silencing through DNA methylation at promoter CpG islands has been well characterized, the effect of *TET2*-mediated demethylation reactions on methylation change at CpG islands is controversial. Recent bisulfite pyrosequencing study detected hypermethylation at non-CpG island promoters in some gene loci but not at promoter CpG islands in *TET2*-mutated CMML patient samples [58]. In addition, depletion of *Tet2* in *AML1-ETO*-induced murine pre-leukemic hematopoietic cells had little impact on the methylation change at CpG islands and promoters but rather showed hypermethylation at enhancers [59].

A number of studies have explored the functional role of *TET2* in hematopoiesis and leukemogenesis. *Tet2*-silenced murine BM hematopoietic stem/progenitor cells (HSPCs) showed preferential differentiation toward myeloid lineage in vitro [56]. Consistent with murine data, *TET2*-silencing in human cord blood (CB) CD34⁺ cells led to skewed differentiation toward CD14⁺ monocytic lineage in ex vivo culture experiment [60]. Indeed, *Aid*-deficient mice as well as *AID*-silenced human BM CD34⁺ cells also demonstrated myeloid skewing, suggesting that *TET2/AID* active DNA demethylation pathway might play an essential role in myeloid fate commitment [43]. In addition, oncometabolite

2-hydroxyglutarate (2-HG) produced by *Isocitrate dehydrogenase 1 and 2* (*IDH1/2*) mutations, recurrently seen in AML patients, inhibits *TET2* function as well as other α -KG-dependent oxygenases (Fig. 2) [57, 61]. It has been shown that Wilms tumor 1 (*WT1*) recruits *TET2* to regulate its target gene expression [62] and *WT1* mutations lead to reduced *TET2* function and decreased 5-hmC levels [63]. These studies revealed that *IDH1/2* mutations and *WT1* mutations share, at least partially, common epigenetic pathogenesis in AML as *TET2* mutations through altered DNA hydroxymethylation. Several groups have generated mouse models of germline or conditional *Tet2* loss and reported common features in these mice, including disrupted hematopoietic differentiation, expansion of HSPC (LSK, Lin[−] Sca-1⁺ c-Kit⁺) compartment, and enhanced HSC self-renewal [52, 64–68]. Furthermore, some *Tet2*-null mice eventually developed myeloid malignancies in vivo [52, 64, 65]. Of note, *Tet2*-disrupted fetal liver common myeloid progenitors (FL-CMPs) exhibited enhanced replating capacity in vitro, implicating that *Tet2* loss may potentially transform more differentiated myeloid progenitors [69]. Additionally, microRNA-22 (miR-22), which targets *TET2*, was reported to be upregulated in MDS patient samples [70]. Mice conditionally expressing miR-22 displayed increased HSC self-renewal with defective hematopoietic differentiation and developed MDS similar to *Tet2*-deficient mice, confirming the functional relevance of miR-22/*TET2* regulatory network in myeloid transformation [70]. Together, these data clearly show that *Tet2* regulates both myeloid differentiation and clonal hematopoietic expansion, thereby functioning as a tumor suppressor.

Given that most of the individuals with CHIP alone do not progress to hematologic malignancy [12, 13] and long latency is required for developing myeloid disease in *Tet2*-deficient mice [52, 64, 65], *TET2* loss itself is insufficient to cause hematopoietic transformation. In fact, previous studies have uncovered co-occurrence of other disease alleles with *TET2* mutations in myeloid malignancies [35, 71, 72], underscoring a critical role of convergent cooperative effects of these alleles. Recent study has demonstrated cooperative function of *Tet2* loss and *Flt3*^{ITD} mutations in AML development in vivo through combinatorial epigenetic remodeling of specific genetic loci [73]. In agreement with co-occurrence of *TET2* and *EZH2* mutations in MDS and MDS/MPN patients, concurrent depletion of *Tet2* and *Ezh2* in mice developed MDS or MDS/MPN by derepression of oncogenic polycomb targets [74]. In addition, combined *Tet2* loss with *AML1-ETO* leads to fully penetrant AML in vivo, partially due to hypermethylation of enhancer regions thereby silencing tumor suppressors [59, 75]. Furthermore, studies from two independent groups have shown that combination of *Tet2* loss and *Jak2V617F* resulted in aggressive MPN phenotype through both clonal

HSC dominance and expansion of downstream precursor populations [76, 77]. Notably, *TET2* mutations also co-occur with changes in other *TET* members or *DNMT3A* mutations in human acute B-lymphocytic leukemia and T-cell lymphoma [78, 79]. Concordant with this, *Tet1/2* double-knockout mice developed lethal B-cell malignancies, and *Tet2* loss combined with *Dnmt3a* mutation caused T-cell lymphoma/leukemia in vivo, possibly owing to dysregulated Bcl6/Myc and Notch pathway, respectively [78, 80, 81]. Collectively, these data suggest that functional convergent cooperativity of *TET2* mutations and co-occurring disease alleles drives hematopoietic transformation.

ASXL1

ASXL1 is the human homolog of *Drosophila Additional sex combs* (*Asx*). Recently, it has been demonstrated that *Drosophila* *Asx* forms a complex with the chromatin deubiquitinase, Calypso, to form the Polycomb-repressive deubiquitinase (PR-DUB) complex, which removes monoubiquitin from histone H2AK119 thereby repressing Hox gene expression [82]. The mammalian homolog of Calypso, BRCA-1-associated protein 1 (BAP1), also associates with ASXL1, and the mammalian BAP1–ASXL1 complex was shown to harbor deubiquitinase activity in vitro [82].

Analogous to *DNMT3A*, *ASXL1* is recurrently mutated in individuals with CHIP and AA patients with clonal hematopoiesis [11–13, 17, 18]. Multiple studies have also identified mutations in *ASXL1* in 15–20% of MDS, 25% of AML, and 40–60% of MDS/MPN patients [83–85]. Of note, *ASXL1* mutations seem to be enriched in AML secondary to a preexisting MPN rather than in de novo AML [86], suggesting a crucial role of *ASXL1* in myeloid transformation. Indeed, several clinical studies found that *ASXL1* mutations are associated with adverse outcome in AML and MDS [35, 87, 88].

Recent works have investigated the molecular mechanisms of clonal expansion and myeloid transformation by *ASXL1* mutations. Initial study showed that human myeloid leukemia cells harboring endogenous *ASXL1* mutations are associated with loss of ASXL1 expression, indicating that these mutations are bona fide loss-of-function mutations [89]. This study also revealed that ASXL1 physically interacts with EZH2, a member of the polycomb-repressive complex 2 (PRC2), in human myeloid leukemia cells and that ASXL1 loss results in loss of transcriptionally repressive H3K27me₃, leading to derepression of posterior *Homeobox A* (*HOXA*) cluster (Fig. 3) [89]. Subsequent study also reported that C-terminal-truncating *Asxl1* mutant causes derepression of *Hoxa9* and miR-125a expression through inhibition of PRC2-mediated methylation of H3K27 (Fig. 3), inducing MDS-like disease in vivo [90]. Notably, recipients of *Asxl1* mutant cells showed clonal

Fig. 3 Schematic presentation of *ASXL1* mutant-mediated derepression of PRC2 target oncogenes. In physiologic condition, ASXL1 and EZH2, a member of the PRC2, directly interact and cooperatively methylate histone H3K27, leading to repression of PRC2 target oncogenes. However, in *ASXL1* mutant context, ASXL1 loss results in loss of transcriptionally repressive H3K27me₃, thereby causing the derepression of PRC2-target oncogenes, including posterior *HOXA* cluster and *miR-125a*

advantage compared to wild-type recipients in vivo [90], consistent with functional relevance of *ASXL1* mutations in clonal hematopoietic expansion. Moreover, mice with constitutive loss of *Asxl1* (*Asxl1*^{−/−}) resulted in developmental abnormalities and onset of MDS-like disease, including dysplastic neutrophils and multiple lineage cytopenia [91]. Mice with hematopoietic-specific conditional deletion of *Asxl1* also developed MDS-like disease with multilineage cytopenias and dysplasia in the context of increased numbers of HSPCs [92], underscoring the importance of *ASXL1* in MDS pathogenesis. More recently, knockdown of *ASXL1* in human CB CD34⁺ cells or hematopoietic-specific deletion of *Asxl1* was shown to cause reduced erythropoiesis and impaired erythrocyte enucleation, further clarifying

an essential role of *ASXL1* in erythropoiesis [93]. Mutational cooperativity of *ASXL1* mutations with other disease alleles has also been investigated. Recipients of cells with *Nras*^{G12D} expression combined with *Asxl1* loss led to a progressive MPN-like disease in vivo [89]. Additionally, mutation in *SET-binding protein 1* (*SETBP1*), recurrently co-mutated with *ASXL1* in MDS patients, was reported to cooperate with *ASXL1* mutation to enhance expression of posterior *Hoxa* genes, activate stem cell signature, and repress tumor growth factor- β signaling, thereby inducing AML in vivo [94]. Together, these data suggest that functional cooperativity of *ASXL1* mutations with other genetic lesions plays a critical role in leukemogenesis.

The nuclear deubiquitinase enzyme BAP1 utilizes ASXL1 as an essential cofactor. Recent genetic studies have identified recurrent *BAP1* mutations in various cancers, including mesothelioma, renal cell carcinoma, and metastatic uveal melanoma [95–97]. Hematopoietic-specific conditional loss of *Bap1* resulted in retention of monoubiquitinated H2AK119 and decreased expression levels of transcriptional regulator host cell factor-1 (HCF1) and OGT, leading to MDS/MPN-like disease in vivo [98]. However, in contrast to *Asxl1* loss, *Bap1* deletion was shown to increase *Ezh2* and H3K27me3 levels and enhance

repression of PRC2 targets, sensitizing *BAP1*-deficient mesothelioma to pharmacologic EZH2 inhibition [99]. These data indicate that *ASXL1* and *BAP1* loss may function in independent manner in myeloid transformation.

Conclusion

Recent findings in genetic, functional, and clinical studies on human clonal hematopoiesis, Pre-LSC and epigenetic modifiers have further expanded our insights into the exact mechanism of leukemogenesis. Accumulating evidence suggests that primary mutations in epigenetic modifiers that occurred in normal HSPCs lead to clonal hematopoietic expansion, causing CHIP. CHIP clones are often considered equivalent to Pre-LSCs, since individuals with CHIP are associated with increased risk of hematologic malignancies. However, as mentioned earlier, the overall frequency of disease onset is relatively low (0.5–1% annually), and majority of individuals with CHIP who possess primary mutations will complete their lives without hematologic malignancies [12, 13]. In addition, functional studies have shown that mutations in epigenetic modifiers alone are insufficient to drive hematopoietic transformation.

Fig. 4 Hypothetical model of leukemogenesis from preexisting CHIP. Accumulating evidence suggests the following process as a hypothetical model of leukemogenesis from preexisting CHIP. Primary mutations in epigenetic modifiers that occurred in normal HSPCs lead to clonal expansion, causing CHIP. Given these primary mutations alone are insufficient to cause hematopoietic transforma-

tion, the majority of individuals with CHIP do not develop hematologic malignancy. In minority of individuals with CHIP, a subset of mutated HSPCs may turn into Pre-LSCs through an as yet unknown mechanism. Once these Pre-LSCs gain additional secondary mutations, these clones may fully drive hematopoietic transformation

Taken together, it may be reasonable to consider that a subset of HSPC clones harboring primary mutations turn into Pre-LSCs, cells that can potentially generate malignant leukemic clone, in minority of individuals with CHIP (Fig. 4) [100]. Once these Pre-LSCs gain additional secondary mutations, such as mutations in signaling factors, they may turn into a full-blown leukemic state. It is important to note that not all the patients with MDS or AML have preexisting CHIP. Furthermore, the exact mechanism of how Pre-LSCs originate from normal HSPCs harboring primary mutations remains to be elucidated. Further studies are definitely required to decipher the entire perspective of leukemogenesis, which will eventually allow us to improve our patient care in the future.

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