

Clonal hematopoiesis and associated diseases: A review of recent findings

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Abstract

Recent genome-wide studies have revealed that aging or chronic inflammation can cause clonal expansion of cells in normal tissues. Clonal hematopoiesis has been the most intensively studied form of clonal expansion in the last decade. Clonal hematopoiesis of indeterminate potential (CHIP) is an age-related phenomenon observed in elderly individuals with no history of hematological malignancy. The most frequently mutated genes in CHIP are *DNMT3A*, *TET2*, and *ASXL1*, which are associated with initiation of leukemia. Importantly, CHIP has been the focus of a number of studies because it is an independent risk factor for myeloid malignancy, cardiovascular disease (CVD), and all-cause mortality. Animal models recapitulating human CHIP revealed that CHIP-associated mutations alter the number and function of hematopoietic stem and progenitor cells (HSPCs) and promote leukemic transformation. Moreover, chronic inflammation caused by infection or aging confers a fitness advantage to the CHIP-associated mutant HSPCs. Myeloid cells, such as macrophages with a CHIP-associated mutation, accelerate chronic inflammation and are associated with increased levels of inflammatory cytokines. This positive feedback loop between CHIP and chronic inflammation promotes development of atherosclerosis and chronic heart failure and thereby increases the risk for CVD. Notably, HSPCs with a CHIP-associated mutation may alter not only innate but also acquired immune cells. This suggests that CHIP is involved in the development of solid cancers or immune disorders, such as aplastic anemia. In this review, we provide an overview of recent findings on CHIP. We also discuss potential interventions for treating CHIP and preventing myeloid transformation and CVD progression.

KEYWORDS

clonal hematopoiesis, hematopoietic stem cell, myeloid leukemia, solid tumor

1 | INTRODUCTION

Constant exposure of cells to stress or DNA damage-inducing chemical agents results in accumulation of somatic mutations in

an age-dependent manner. Some somatic mutations, namely driver mutations, provide a fitness advantage to cells under selective pressure. For example, chronic inflammation or exposure to specific environmental stimulus might allow such clones to become

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dominant. Consequently, when a single ancestor cell of the positively expanded clone gains additional driver mutations, cancer cells emerge. Notably, clonal expansion occurs even in tissues that appear normal.¹⁻³

Among the different forms of clonal expansion, clonal hematopoiesis (CH) has been most intensively studied.⁴⁻⁶ The term “CH of indeterminate potential (CHIP)” refers to the presence of at least one driver mutation in hematopoietic cells of peripheral blood, without hematological malignancy.⁷ These subpopulations are derived from hematopoietic stem cells (HSCs) that may have acquired driver mutations somatically. Similar to that in stem cells in other tissues, accumulation of somatic mutations in HSCs occurs in an age-dependent manner.⁸ CHIP is a significant risk factor mainly for myeloid malignancies such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasms (MPN), which indicates that it is a precursor state for hematological malignancy with lower frequency. In addition, some lymphoid malignancies seem to emerge from CHIP. Importantly, CHIP is associated with increased risk of all-cause mortality and cardiovascular disease (CVD).^{5,9} CHIP has also been detected in nonmalignant diseases, such as acquired aplastic anemia (AA), which is an autoimmune disorder. It is also prevalent in patients with solid tumors who have undergone chemotherapy and has been associated with poor prognosis.¹⁰ Therefore, it is clinically important to understand the interrelationship between CHIP and hematological malignancies as well as CVD, AA, and solid tumors.

Mouse models that recapitulate CHIP-associated mutations facilitate understanding of how each CHIP-associated mutation alters the function of HSCs and promotes myeloid transformation. Recent studies have revealed that inflammation plays a pivotal role in the pathogenesis of CHIP and its related diseases. Myeloid cells, especially macrophages, derived from HSCs with CHIP-associated mutations exhibit aberrant production of inflammatory cytokines,

which gives them a fitness advantage resulting in the propagation of CHIP clones and development of CVD. In addition, CHIP-associated mutations might alter the function of immune cells, such as T cells, thereby affecting the development of autoimmune diseases and anticancer immunity. Here, we reviewed recent findings on CHIP and described its association with hematological malignancy and nonhematological diseases such as CVD and solid tumors.

2 | EMERGENCE OF CHIP IN HEALTH AND DIFFERENT DISEASES

In 2012, presence of somatic mutations in the *TET2* gene was evaluated in 182 elderly women with skewed X-linked inactivation and identified in the genomes of 10 women.¹¹ This was the first study that used next-generation sequencing to reveal that blood cells with gene mutations underwent clonal expansion.¹¹ In 2014, three groups reported that healthy individuals aged >60 years carried somatic mutations associated with hematological malignancy.⁴⁻⁶ Another report suggested that the prevalence of CH increases with age, and it was present in 62% of individuals aged ≥80 years.¹² Importantly, CH is associated with increased risk of developing myeloid malignancy and all-cause mortality.⁵

Although CH appears during the preleukemic state, it is unclear whether it leads to benign or malignant neoplasms. To distinguish the state of nonmalignancy from hematological cancers, the term “CHIP” was coined. CHIP is defined by the presence of somatic mutations in peripheral blood at a variant allele frequency (VAF) of ≥0.02, in the absence of apparent hematological disorders.⁷ The most common mutation observed in CHIP is in the C to T single-nucleotide substitution in the coding region, which occurs due to an age-dependent increase in the rate of spontaneous deamination of 5-methyl-cytosines at the CpG loci. This is consistent with the

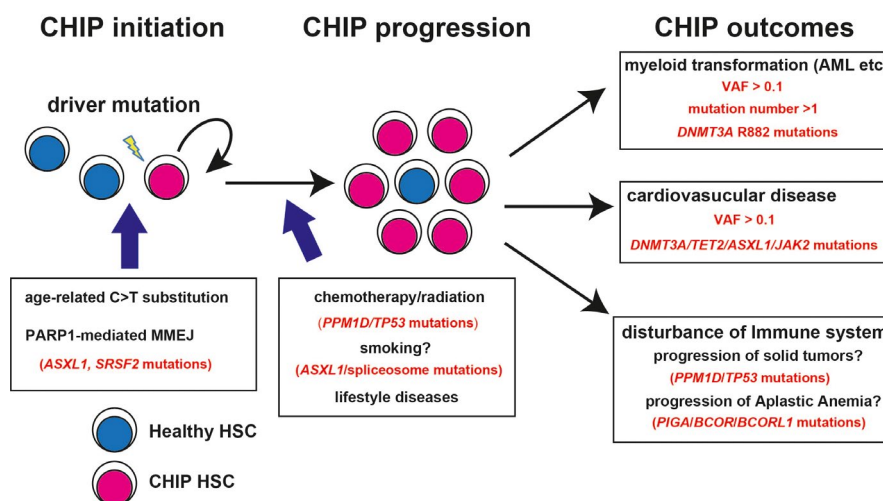


FIGURE 1 Overview of clonal hematopoiesis and its implication. Clonal hematopoiesis of indeterminate potential (CHIP) is initiated by acquiring some driver mutations in hematopoietic stem cells (HSCs) due to aging and MMEJ-mediated double-strand break repair. HSCs carrying CHIP-associated mutations gain a fitness advantage under the selective pressures such as chemotherapy/radiation and lifestyle diseases. Consequently, CHIP progression promotes myeloid transformation, cardiovascular diseases, and potentially disturbance of immune systems. Risk factors and associated gene mutations for each category are highlighted in red font

clinical data, which suggest that aging is the strongest risk factor for CHIP.¹³ In addition to age, the second most important risk factor for CHIP is a history of chemotherapy and radiation. Therapy-related CH is frequently detected in patients with solid tumors (~25%) and is associated with adverse outcomes.¹⁰ CHIP has been observed in patients who undergo autologous stem cell transplantation (ASCT) due to nonmyeloid hematological malignancies, such as multiple myeloma and lymphoma,^{14,15} whereas the impact of CHIP after ASCT is still under debate. CHIP is also prevalent in other nonmalignant hematological diseases, such as AA, Erdheim-Chester disease, and inherited bone marrow failure syndromes.¹⁶⁻¹⁸ These findings indicate that there is a need for future studies to focus on the implications of CHIP (Figure 1).

3 | RECURRENT MUTATIONS IN CHIP

Genes coding for the epigenetic regulators DNMT3A, TET2, and ASXL1 (DTA) are most frequently mutated in healthy elderly individuals with CHIP. Mutations in *DNMT3A* and *TET2* influence DNA methylation whereas those in *ASXL1* alter histone modifications, and thereby influence hematopoiesis.¹⁹⁻²¹ In CHIP, although mutations in the genes coding for other epigenetic regulators, such as *IDH1* and *IDH2*, are also reported, they occur at lower frequencies than those in *DTA*. Moreover, mutations in genes related to signaling pathways (*JAK2*, *CBL*, *GNB1*, and *GNAS*), spliceosome (*SRSF2*, *SF3B1*, and *U2AF1*), and cohesion (*STAG2*, *RAD21*, and *SMC3*) are also associated with CHIP. In addition to the exonic mutations described above, an intronic mutation in the telomerase reverse transcriptase (*TERT*) gene is strongly associated with CHIP.²² Mutations in *PIGA*, *BCOR*, or *BCORL1* are frequently and exclusively detected in patients with AA and CHIP.¹⁶ Furthermore, mutations in *DNMT3A* and *ASXL1* are frequently detected in patients with AA.

Specific DNA-damaging agents or environmental stimuli may differentially affect the frequency of mutations in CHIP. For example, a history of chemotherapy can increase the prevalence of mutations in DNA damage response genes (*CHEK2*, *PPM1D*, and *TP53*) due to the selective pressure induced by cytotoxic conditions.^{23,24} Another study showed that mutations in *ASXL1* and genes coding for spliceosomes are strongly associated with exposure to DNA-damaging agents due to substance abuse such as smoking.¹² Interestingly, some CHIP-associated mutations in genes such as *ASXL1* and *SRSF2* are caused by PARP1 dependent microhomology-mediated end joining (MMEJ) double-stranded break repair, indicating that DNA damage repair can also contribute to mutations associated with CHIP.²⁵

Although clonal expansion can be detected in noncancerous tissues,^{1,26} the mutation profiles of such clones are different from the ones observed in CHIP. For example, the frequency of mutations in signaling pathway-associated genes or oncogenes in CH is lower than that in cells undergoing clonal expansion in other tissues such as esophageal epithelium, colorectal epithelium, and skin.¹⁻³ This difference could be because hematopoietic cells can expand freely without being limited in space, unlike cells in other tissues.

4 | MOUSE MODELS RECAPITULATE HUMAN MUTATIONS-ASSOCIATED CHIP

To understand the implications of CHIP, mouse models recapitulating human CHIP have been generated (Table 1). Knocking out *Dnmt3a*, which is the most frequently mutated gene in CHIP, endowed a strong competitive advantage to long-term HSCs (LT-HSCs).^{27,28} In contrast, *Dnmt3a* R878H knockin (KI) mice, which mimic the human *DNMT3A* hotspot mutation R882H, showed modest LT-HSC expansion compared with *Dnmt3a* knockout mice.²⁸ The *Dnmt3a* knockout mice showed increased self-renewal capacity in the LT-HSC fraction, whereas the *Dnmt3a* R878H KI mice showed increased self-renewal capacity in the LT-HSC as well as the multipotent progenitor (MPP) fraction.²⁹ Given that leukemia-initiating cells arise from progenitor population rather than stem cell population in most human primary AML cases,³⁰ these findings are consistent with the clinical data that suggest that although the *DNMT3A* R882 mutation is less frequent in CHIP, it markedly increases the risk of myeloid transformation.³¹⁻³³ Loss of *TET2*, the second most commonly mutated gene, also increased the self-renewal capacity of HSCs.³⁴ Contrastingly, *Tet2* catalytic-dead mutant mice showed significant but weaker HSC expansion than the *Tet2* knockout mice and had a myeloid-specific phenotype.³⁵ This indicated that the catalytic activity of *TET2* is not important for HSC expansion. Although it remains elusive how mutations in *DNMT3A* or *TET2* affect the progression of CHIP, recent findings suggest that alterations in DNA methylation at the enhancer loci might contribute to a lineage bias.²¹

Although *ASXL1* is the third most frequently mutated gene in CHIP, *ASXL1*-mutated CHIP showed a high odds ratio for AML and an increased hazard ratio for CVD compared with *DNMT3A*- or *TET2*-mutated CHIP.³⁶ To understand the characteristics of *ASXL1* mutations in CHIP, we generated *Asxl1* mutant KI (*Asxl1*-MT KI) mice that mimicked the human *ASXL1* mutation frequently found in CHIP.³⁷ The *Asxl1*-MT KI mice developed an MDS-like phenotype after a long period of latency but did not undergo complete myeloid transformation. In addition, unlike HSCs from *Dnmt3a* and *Tet2* mutant mice, those from *Asxl1*-MT KI mice did not have a repopulating advantage in competitive transplantation assays. Notably, *Asxl1*-MT KI mice showed age-related expansion of phenotypic HSCs via activation of the AKT/mTOR pathway.³⁸ Overactivation of AKT or mTOR has also been observed in *Dnmt3a* R878H KI mice,³⁹ which indicates that this is a common mechanism promoting human CHIP.

The spliceosome mutant models, ie, *Srsf2* P95H and *Sf3B1* K700E KI mice showed progressive macrocytic anemia but did not develop leukemia till 1 year.^{40,41} In addition, HSPCs from *Srsf2* P95H or *Sf3B1* K700E KI mice showed a decrease in their ability to repopulate and an increase in their sensitivity to inflammatory stimuli via activation of NF- κ B signaling.⁴² As the activation of NF- κ B signaling promotes survival of leukemia-initiating cells,⁴³ these findings are in line with the clinical data, which indicate that spliceosome mutations, although not common in CHIP, confer a higher likelihood of myeloid transformation than mutations in *DNMT3A* or *TET2*.³⁶

TABLE 1 The phenotypes of CHIP model mice

Mice	PB phenotypes	HSC/HSPC phenotypes	Competitive repopulation	Mechanisms for HSC phenotypes	References
<i>Dnmt3a</i> knockout mice	A slight bias toward B cell differentiation relative to controls (in transplantation model)	Increased only LT-HSCs	Enhanced	Losing focal DNA methylation at key regulatory regions wherein self-renewal genes (<i>RUNX1</i> , <i>GATA3</i> etc) located	27,28
<i>Dnmt3a</i> R878H knockin mice	No differences in CD11b+, B220+, and CD3+ fraction	Increased LT-HSCs, ST-HSCs, and MPP3s	Enhanced (to lesser extent than <i>Dnmt3a</i> knockout mice)	N/A	29
<i>Tet2</i> knockout mice	No significant changes (4–6 weeks old). Increase in WBC and monocytes (20 weeks old)	Increased LT-HSCs, MEP, and CLP fraction	Enhanced	Enhanced quiescence due to increased susceptibility to hypermethylation of <i>Myc</i> and <i>Myb</i>	21,34,35
<i>Tet2</i> catalytic mutant mice	No significant blood count abnormalities but multilineage dysplastic features (10 months old)	Increased LT-HSCs (to lesser extent than <i>Tet2</i> knockout mice) and GMP fraction	Enhanced	N/A	35
<i>Asx1</i> -MT knockin mice	No significant changes in young (6–12-week-old) mice. Leukocytopenia, anemia, and thrombocytosis in aged (20–24-month-old) mice	Decreased LT-HSCs and MPPs in young mice. Increased phenotypic LT-HSCs in aged mice	Impaired	Overactivation of Akt/mTOR signaling via deubiquitinating Akt1 by <i>Asx1</i> -MT/Bap1 complex	37,38
<i>Srsf2</i> P95H knockin mice	Leukocytopenia and macrocytic anemia	Increased LT-HSCs	Impaired	Overactivation of NF- κ B signaling via aberrant <i>Casp8</i> splicing	40,42
<i>Sf3b1</i> K700E knockin mice	Macrocytic anemia	Increased LT-HSCs	Impaired	Overactivation of NF- κ B signaling via aberrant <i>Map3k7</i> splicing	41,42

Abbreviations: CHIP, clonal hematopoiesis of indeterminate potential; CLP, common lymphoid progenitor; HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cell; LT, long-term; MEP, megakaryocyte-erythroid progenitor; MPP, multipotent progenitor; PB, peripheral blood; ST, short-term; WBC, white blood cell.

Overall, these studies involving mouse models recapitulating human CHIP revealed that single CHIP-associated mutations generally show weak phenotypes in hematopoiesis and are insufficient for myeloid transformation. Although the mechanism by which HSCs with CHIP-associated mutations induce clonal expansion remains largely elusive, these mouse-based studies delineate the effects of different mutations on the characteristics of CHIP.

5 | MYELOID TRANSFORMATION OF HSPCs CARRYING CHIP-ASSOCIATED MUTATIONS

Two recent reports evaluated the clonal architecture and mutational histories of AML patients using single-cell DNA sequencing technique.^{31,44} These studies suggest that cells carrying single mutations in the *DNMT3A*, *TET2*, or *ASXL1* genes are frequently detected in AML, in addition to the leukemic cells that harbor multiple mutations. This suggests that CHIP clones with these additional mutations are likely to be the disease-initiating cells in AML. Moreover, a previous report showed that additional driver mutations, together with an initiating mutation, are sufficient to establish malignant founder clones in AML.⁸ Consistent with these clinical data, recent experimental studies have highlighted the mechanisms by which additional mutations or the overexpression of oncogenes might promote myeloid transformation in CHIP.

Previous reports suggested that the *Dnmt3a* knockout and *Idh2* mutants synergistically promoted leukemogenesis by overproducing prostaglandin E2. These double-mutant cells were sensitive to the inhibition of prostaglandin synthesis.⁴⁵ In addition, a mutation in the *Npm1* gene was shown to drive myeloid transformation in *Dnmt3a* R878H expressing murine HSPCs.²⁹ *TET2* knockout also collaborated with the *NRAS* mutant in inducing myeloid transformation via synergistic activation of MAPK signaling. The *TET2/NRAS* double-mutant cells showed preferential sensitivity to MAPK kinase (MEK) inhibition.⁴⁶ Concerning *ASXL1* mutations, we also showed that mutating the *RUNX1* gene could promote the myeloid transformation of cells from the *Asxl1*-MT KI mice.³⁷ Recently, we reported that the overexpression of a homeobox transcriptional factor, *HHEX*, promotes myeloid transformation in HSPCs of *Asxl1*-MT KI mice via the upregulation of *MYB/ETV5*.⁴⁷ Inhibiting the *HHEX-MYB/ETV5* axis is a potential therapeutic strategy for treating *ASXL1*-mutated AML. Taken together, by demonstrating that HSPCs carrying CHIP-associated mutations undergo myeloid transformation, these studies have enabled the development of therapeutic strategies for AML.

6 | POSITIVE FEEDBACK LOOP BETWEEN CHRONIC INFLAMMATION AND CHIP

The relationship between chronic inflammation and hematopoiesis has been studied extensively in the last decade. The exposure of HSCs to chronic inflammation leads to loss of quiescence and

induction of a myeloid-biased lineage output,^{48,49} which results in impaired HSC function. In contrast, CHIP-associated mutations in genes such as *DNMT3A* and *TET2* provide HSCs with a competitive advantage under inflammatory conditions.^{50,51} In addition, activation of Toll-like receptor (TLR) signaling, which is frequently observed in MDS patients, confers a fitness advantage to HSCs under inflammatory conditions.⁵²

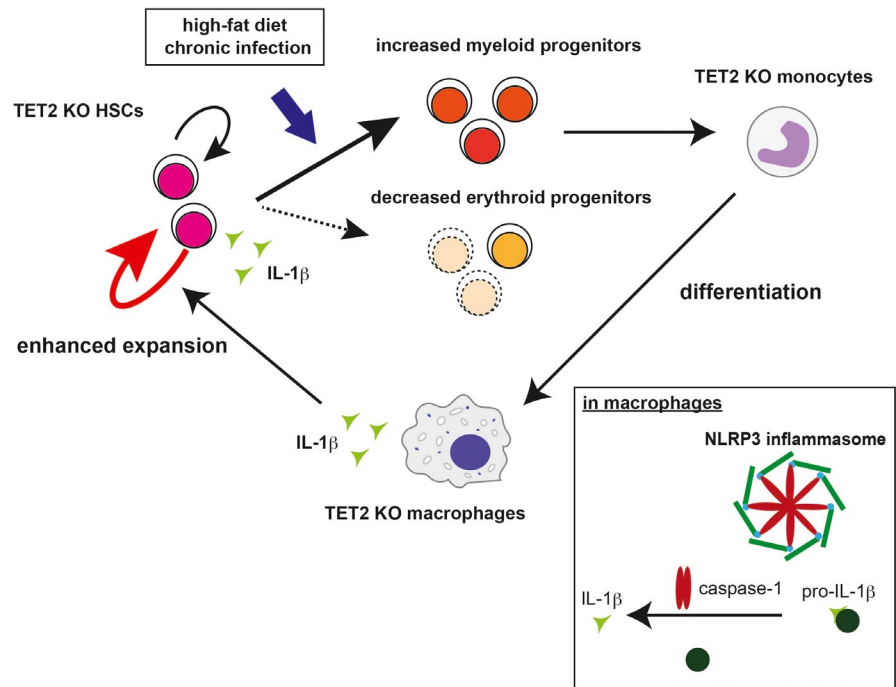
Moreover, aging or CHIP-associated mutations might also accelerate chronic inflammation. A recent report showed that aging increases the frequency of CD61-high myeloid-biased LT-HSCs that further promote a myeloid output in response to inflammatory stimuli.⁵³ In addition, a CRISPR-Cas9-mediated sequential KI developed in human cells showed that mutations in *ASXL1*, *SRSF2*, and *NRAS* activated the innate immunity signaling⁵⁴ pathways. Therefore, a combination of aging and CHIP-associated mutations might activate inflammation by increasing the production of inflammatory cytokines. In agreement with this hypothesis, *Tet2*-deficient HSPCs produced increased levels of monocytes and inflammatory cytokines such as IL-1 β and IL-6.⁵⁵ In fact, a recent study in a large cohort showed that serum levels of IL-6 are generally high in people with CHIP.⁵⁶ In addition, it is well known that obesity or aging induces inflammation in the bone marrow by promoting accumulation of adipocytes.^{57,58} *TET2* deficiency aggravated age- and obesity-related insulin resistance by increasing IL-1 β secretion in the adipose tissue⁵⁹ and thereby established a causal relationship between CH and type 2 diabetes mellitus.⁵ These findings indicate that CHIP progresses due to a positive feedback loop between HSCs carrying CHIP-associated mutations and chronic inflammation (Figure 2).

7 | INVOLVEMENT OF CHIP IN ATHEROSCLEROSIS PROGRESSION

Lifestyle diseases such as diabetes and hypercholesterolemia were reported to cause alterations in HSPC function and monocytosis.^{60,61} Inflammatory monocytes and their differentiation into macrophages underlies the pathogenesis of atherosclerosis.⁶² Consistent with this, in 2014 it was reported that CHIP-associated mutations were associated with the risk of CVD.⁵ In 2017, another study showed that CHIP, together with the mutations in *DNMT3A*, *TET2*, *ASXL1*, or *JAK2* genes, was associated with an increased risk of CVD.⁹ In recent years, mouse models have been used to study how these mutations promote atherosclerosis and CVD.

Low-density lipoprotein receptor (*Ldlr*) knockout mice are commonly used as an atherosclerosis model. It was observed that the atherosclerotic plaque size was larger in *Ldlr* knockout mice transplanted with *Tet2*-deficient bone marrow cells than in those transplanted with control bone marrow cells on high-fat diet.^{9,63} Importantly, *Ldlr* knockout mice transplanted with myeloid-specific *Tet2*-depleted cells also showed aggravation of atherosclerosis on atherogenic diet, which suggests that myeloid cells, such as monocytes and macrophages, were responsible for the pathogenesis.^{9,63} Mechanistically, inflammasomes are activated in *Tet2*-depleted

FIGURE 2 A positive feedback loop between self-renewal of hematopoietic stem cells (HSCs) and progression of inflammation in *TET2*-mutated clonal hematopoiesis of indeterminate potential (CHIP). *TET2*-mutated HSCs preferentially produce myeloid progenitors. As a result, monocytes and macrophages are increased in *TET2*-mutated CHIP. In macrophages with *TET2* mutations, the NLRP3 inflammasome pathway is activated and cleavage of pro IL-1 β is promoted, leading to overproduction of IL-1 β . In turn, IL-1 β enhances the self-renewal of HSCs with *TET2* mutations. This positive feedback loop would be responsible for the pathogenesis of atherosclerosis in *TET2*-mutated CHIP



macrophages, which causes an increase in the secretion of inflammatory cytokines such as IL-1 β and IL-6.^{9,63} In macrophages, CRISPR-Cas9-mediated DNMT3A depletion also resulted in increased production of cytokines such as IL-6.⁶⁴

In addition, mutations in *DNMT3A* and *TET2* in peripheral blood cells have been clinically associated with the progression and poor prognosis of chronic heart failure.⁶⁵ Experimentally, a chronic heart failure model showed that myeloid-specific *Tet2* depletion had a detrimental effect on cardiac remodeling and function.⁵⁵ MCC950, an NLRP3 inhibitor, reduced IL-1 β secretion in *Tet2*-depleted macrophages and ameliorated atherosclerosis or heart failure driven by *Tet2*-deficient myeloid cells.⁵⁵ Moreover, a recent report showed that a macrophage-restricted JAK2 mutant induces DNA replication stress and activates the AIM2 inflammasome, thereby aggravating atherosclerosis.⁶⁶ Collectively, these findings suggest a causal relationship between inflammasome activation in macrophages due to CHIP-associated mutations and the pathogenesis of CVD.

8 | IMPLICATIONS OF CHIP FOR SOLID TUMORS AND AA

As mentioned above, CHIP is also prevalent in solid tumors and may contribute toward adverse outcomes.⁶⁷ Moreover, chemotherapy may induce the expansion of clones with mutations in specific genes, such as *TP53* or *PPM1D*. Therefore, follow-up evaluation for hematological malignancy is recommended in cancer patients with high-risk CHIP.⁶⁸

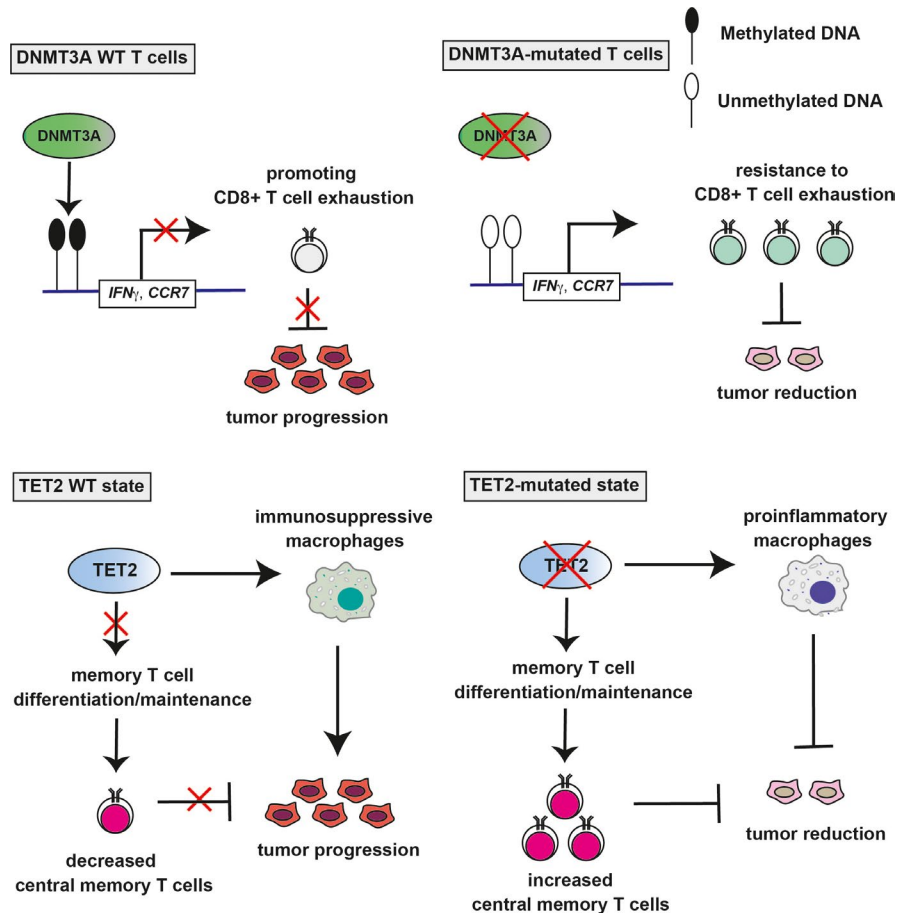
Given that HSCs harbor driver mutations in CHIP, it is plausible that the immune cells produced by these HSCs, such as B and T lymphocytes, and NK cells are also affected by the mutations. Recent experimental studies have shown that loss of DNMT3A induces

the accumulation of double-negative 2 (DN2) thymocytes (DN2; Lineage⁻ c-Kit⁺ CD25⁺),⁶⁹ whereas *TET2* depletion enhances CD8⁺ memory T cell differentiation.⁷⁰ Clinically, individuals who received allogeneic bone marrow transplants from donors carrying CHIP-associated mutations were more likely to experience graft-versus-host disease and had a low relapse rate.⁷¹ This reinforces the notion that CHIP-associated mutations alter the immune system.

A recent report showed that DNMT3A depletion in CD8⁺ T cells confers resistance to the exhaustion induced by chronic stimulation. This can be explained by the failure to methylate CpG sites de novo at the *IFN γ* and *CCR7* loci.⁷² *TET2* inactivation also enhances the antitumor activity of tumor-infiltrating lymphocytes.⁷³ In addition, *TET2*-depleted CAR-T cells showed increased therapeutic efficiency.⁷⁴ In fact, CHIP-associated mutations also affect other immune cells, in addition to T cells. Myeloid-specific *TET2* depletion suppressed the growth of melanoma cells by increasing the number of proinflammatory macrophages.⁷⁵ These findings indicate that CHIP in combination with *TET2* or *DNMT3A* mutations may suppress rather than promote the development of solid tumors (Figure 3). Thus, it is mysterious that worse prognosis is associated with CHIP in cancer patients, while the T cell immunity seemed to be enhanced by CHIP mutations. Further studies will be warranted to clarify the mechanisms for this discrepancy.

As CH can be detected in acquired AA, it is plausible that some CHIP-associated mutations disrupt the homeostasis of the immune system.¹⁶ Somatic mutations in the JAK-STAT and MAPK pathways, which confer clonality to CD8⁺ T cells,⁷⁶ are frequently found in the CD8⁺ cells of patients with AA. In the same study, mutations in *BCOR/BCORL1* were exclusively found in the CD3⁺ fraction but not in the CD3⁻ fraction of some AA patients,⁷⁶ suggesting that T cells with such mutations can eradicate normal HSCs. These findings are consistent with the clinical data of patients with AA; mutations in

FIGURE 3 DNMT3A- or TET2-mutated clonal hematopoiesis of indeterminate potential (CHIP) may augment antitumor activity. DNMT3A mutations in CD8⁺ T cells lead to hypomethylation at the CpG island of the *IFN γ* and *CCR7* gene, resulting in the exhaustion induced by chronic stimulation (upper). On the other hand, *TET2* mutations lead to polarization of tumor-infiltrating macrophages toward proinflammatory (M1-like) phenotype and also promote the differentiation and maintenance of memory T cell (lower). Taken together, DNMT3A or *TET2* mutations may enhance antitumor immunity by altering the number and function of immune cells



PIGA, *BCOR*, or *BCORL1* lead to a better response to immunosuppressive therapy. Moreover, these patients are less likely to undergo myeloid transformation compared with AA patients with mutations in *DNMT3A* or *ASXL1*.⁷⁷ In addition, HSCs with mutations in specific genes, such as *DNMT3A* or *ASXL1*, might escape the attack from cytotoxic T cells in AA patients. Future studies will have to investigate the role of each mutation in the pathogenesis of AA.

9 | POTENTIAL THERAPEUTIC APPROACHES FOR CHIP

To date, there are no definite criteria for determining which patients are eligible for therapy against CHIP. Recent studies have shown that a *DNMT3A* R882 mutation, more than one driver mutations, or a higher VAF could be a significant risk factor for AML transformation.^{33,78} In addition, a high VAF in CHIP patients was found to be directly proportional to the risk of CVD.⁹ Therefore, it could be suggested that CHIP partially predisposes patients to AML or CVD. Currently, there are no established therapies for CHIP; however, recent studies support the development of effective therapeutics.

Some studies have investigated treatment strategies for specific mutations. For example, treating Tet2-deficient mice with high-dose vitamin C reversed the aberrant self-renewal of HSPCs by mimicking Tet2 restoration.⁷⁹ Using aged *Asx1*-MT KI mice,³⁸ we have shown

that rapamycin, an mTOR inhibitor, efficiently blocked the expansion of phenotypic HSCs, which are driven by the overactivation of the AKT/mTOR pathway. Given that the expansion of phenotypic HSCs led to a reduction in the regenerative capacity in aged bone marrow,⁸⁰ preventing expansion of these "impaired" HSCs would enable the "true" HSCs to perform healthy hematopoiesis and limit CHIP progression.

As mentioned above, activation of the signaling pathways in innate immunity would confer a competitive advantage to HSCs with CHIP-associated mutations. Recently, a report showed that blocking signaling pathways in innate immunity with the help of agents, such as IRAK1/4 inhibitors, had prophylaxis effects on leukemogenesis.⁵⁴ In addition, age-associated changes in the bone marrow microenvironment could serve as promising therapeutic targets for treating CHIP. Upregulation of TGF β and IL-6 signaling has been observed⁸¹ in aged bone marrow stroma. Therefore, inhibitors that target the TGF β 1 and IL-6 pathways could be good candidate drugs in ameliorating an age-related lineage bias.⁸¹ Importantly, these findings could contribute to the prophylactic strategies for AML and CVD, in CHIP.

10 | CONCLUSION

Here, we reviewed the recent findings in CH and discussed its impact on the pathogenesis of myeloid malignancy and CVD. We also

outlined the probability of an association between solid tumors and AA. Because cancers and CVD are the leading causes of death in our aging society, CHIP has been actively studied in recent years. To the best of our knowledge, both CHIP and the associating diseases have causative relationship with the increase of inflammation. Moreover, alterations in the immune system caused by CHIP-associated mutations might play pivotal roles in the pathogenesis of autoimmune diseases and solid tumors. Therefore, targeting inflammation with the help of immunomodulatory agents may be a promising therapeutic strategy to improve the clinical outcomes of CHIP-related diseases. In addition, a more accurate risk classification of CHIP and advancements in sequencing technologies would help enable early intervention to people with CHIP.

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CONFLICT OF INTEREST

The authors have no competing interests to declare.

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