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
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.](image-url)
clonal expansion can also contribute to mutations associated with CHIP.

are caused by PARP1 dependent microhomology-mediated end join-

is still under debate. CHIP is also prevalent in other nonmalignant

eloma and lymphoma,14,15 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 (SRFS2, 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, PPMD1, 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.25 Interestingly, some CHIP-associated mutations in genes such as ASXL1 and SRFS2 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.25 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, Srfs2 P95H and SF3B1 K700E KI mice showed progressive macrocytic anemia but did not develop leukemia till 1 year.40,41 In addition, HSPCs from Srfs2 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-κB signaling.42 As the activation of NF-κB 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
| Mice                              | PB phenotypes                                                                 | HSC/HSPC phenotypes                      | Competitive repopulation | Mechanisms for HSC phenotypes                                                                 | References |
|----------------------------------|-------------------------------------------------------------------------------|------------------------------------------|--------------------------|------------------------------------------------------------------------------------------------|------------|
| **Dnmt3a 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 (RUNX1, GATA3 etc) located | 27,28      |
| **Dnmt3a R878H knockin mice**    | No differences in CD11b+ , B220+, and CD3+ fraction                           | Increased LT-HSCs, ST-HSCs, and MPP3s    | Enhanced (to lesser extent than Dnmt3a knockout mice) | N/A                                                                                           | 29         |
| **Tet2 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 Myc and Myb            | 21,34,35   |
| **Tet2 catalytic mutant mice**   | No significant blood count abnormalities but multilineage dysplastic features (10 months old) | Increased LT-HSCs (to lesser extent than Tet2 knockout mice) and GMP fraction | Enhanced                 | N/A                                                                                           | 35         |
| **Asxl1-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 Asxl1-MT/Bap1 complex          | 37,38      |
| **Srsf2 P95H knockin mice**      | Leukocytopenia and macrocytic anemia                                         | Increased LT-HSCs                        | Impaired                 | Overactivation of NF-κB signaling via aberrant Casp8 splicing                                   | 40,42      |
| **Sf3b1 K700E knockin mice**     | Macrocytic anemia                                                            | Increased LT-HSCs                        | Impaired                 | Overactivation of NF-κB signaling via aberrant Map3k7 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 hematopoeis 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. 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 CHIP, 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-1β and IL-6. In fact, a recent study in a large cohort showed that serum levels of IL-6 are generally high in people with CHIP. In addition, it is well known that obesity or aging induces inflammation in the bone marrow by promoting accumulation of adipocytes. TET2 deficiency aggravated age- and obesity-related insulin resistance by increasing IL-1β secretion in the adipose tissue and thereby established a causal relationship between CH and type 2 diabetes mellitus. These findings indicate that CHIP progresses due to a positive feedback loop between HSCs carrying CHIP-associated mutations and chronic inflammation (Figure 2).

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, 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. 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.

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. In addition, a CRISPR-Cas9-mediated sequential KI developed in human cells showed that mutations in ASXL1, SRSF2, and NRAS activated the innate immunity signaling 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-1β and IL-6. In fact, a recent study in a large cohort showed that serum levels of IL-6 are generally high in people with CHIP. In addition, it is well known that obesity or aging induces inflammation in the bone marrow by promoting accumulation of adipocytes. TET2 deficiency aggravated age- and obesity-related insulin resistance by increasing IL-1β secretion in the adipose tissue and thereby established a causal relationship between CH and type 2 diabetes mellitus. 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. Inflammatory monocytes and their differentiation into macrophages underlies the pathogenesis of atherosclerosis. Consistent with this, in 2014 it was reported that CHIP-associated mutations were associated with the risk of CVD. 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. 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-depleted bone marrow cells than in those transplanted with control bone marrow cells on high-fat diet. 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, are responsible for the pathogenesis. Mechanistically, inflammasomes are activated in Tet2-depleted
macrophages, which causes an increase in the secretion of inflammatory cytokines such as IL-1β and IL-6. In macrophages, CRISPR-Cas9-mediated DNMT3A depletion also resulted in increased production of cytokines such as IL-6.

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. Experimentally, a chronic heart failure model showed that myeloid-specific Tet2 depletion had a detrimental effect on cardiac remodeling and function. MCC950, an NLRP3 inhibitor, reduced IL-1β secretion in Tet2-depleted macrophages and ameliorated atherosclerosis or heart failure driven by Tet2-deficient myeloid cells. Moreover, a recent report showed that a macrophage-restricted JAK2 mutant induces DNA replication stress and activates the AIM2 inflammasome, thereby aggravating atherosclerosis. 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. 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.

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+), whereas TET2 depletion enhances CD8+ memory T cell differentiation. 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. 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 of interferon-γ (IFNγ) and CCR7 loci. TET2 inactivation also enhances the antitumor activity of tumor-infiltrating lymphocytes. In addition, TET2-depleted CAR-T cells showed increased therapeutic efficiency. 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. 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. Somatic mutations in the JAK-STAT and MAPK pathways, which confer clonality to CD8+ T cells, 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, 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
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. 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. In addition, a high VAF in CHIP patients was found to be directly proportional to the risk of CVD. 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 HSCs by mimicking Tet2 restoration. Using aged Asxl1-MT KI mice, 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, 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. In addition, age-associated changes in the bone marrow microenvironment could serve as promising therapeutic targets for treating CHIP. Uregulation of TGFβ and IL-6 signaling has been observed in aged bone marrow stroma. Therefore, inhibitors that target the TGFβ1 and IL-6 pathways could be good candidate drugs in ameliorating an age-related lineage bias. 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 associated 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.

ORCID
Shuhei Asada https://orcid.org/0000-0001-6116-7996

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