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Clonal hematopoiesis in human aging and disease

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Abstract

As people age, their tissues accumulate an increasing number of somatic mutations. While most of these mutations are of little or no functional consequence, a mutation may arise that confers a fitness advantage on a cell. When this process happens in the hematopoietic system, a substantial proportion of circulating blood cells may derive from a single mutated stem cell. This outgrowth, called "clonal hematopoiesis," is highly prevalent in the elderly population. Here we discuss recent advances in our knowledge of clonal hematopoiesis, its relationship to malignancies, its link to non-malignant diseases of aging, and its potential impact on immune function. Clonal hematopoiesis provides a glimpse into the process of mutation and selection that likely occurs in all somatic tissues.

Introduction

Aging is associated with a steady increase in the number of somatic mutations in virtually all tissues [1–5]. These age-associated mutations fall into several classes. The most frequent class arises from the spontaneous deamination of 5-methylcytosine to thymine and primarily occurs at CpG dinucleotides, which are often in the methylated state [6]. If a cell has not repaired this error before replication, one daughter cell will have a thymine:adenine pairing of DNA bases instead of the parental cytosine:guanine. This process occurs at a linear rate with respect to time and is therefore considered a signature of aging [7]. A second class of mutation is small insertions and deletions (indels), which commonly arise from errors introduced during non-homologous end joining of DNA double-strand breaks and can result in frameshift mutations if the breaks occur in protein-coding portions of the genome [8]. Evidence from model organisms suggests that double-strand breaks may become more common as cells age [9]. A third mechanism of mutation is replication error by DNA polymerase, which also typically results in base substitutions or small indels. The error rate of eukaryotic DNA replication tends to be very low, except in the case where DNA

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mismatch repair function is compromised [10]Sanders, 2018 #305}. The number of replication cycles that a cell has undergone typically increases with age; therefore the number of polymerase errors also cumulatively increases [11]. The fourth type of age-associated mutation is large structural variation, such as insertions, deletions, loss-of-heterozygosity, or rearrangements spanning several kilobases or more, although these occur somewhat less commonly than base substitutions and small indels [12].

In combination, these mutational processes create a broad array of genetically distinct tissue stem cells. Through Darwinian selection, some of these stem cells gain a competitive advantage, a phenomenon that has been most extensively studied in the human hematopoietic system. It is estimated that humans have 50,000 to 200,000 hematopoietic stem cells (HSCs) [13]. As each HSC acquires ~1 exonic mutation per decade of life [3], by the age of 70 an average person would be expected to harbor up to 1.4 million protein-coding variants, corresponding to an average of 70 mutations per gene, in at least one HSC (Fig. 1). If just one of these mutations is capable of imparting a fitness advantage to the cell in which it arose, expansions of mutated HSCs, termed “clones”, should be common in aging humans. Indeed, numerous studies have now shown that such outgrowths of mutated blood cells, termed “clonal hematopoiesis,” are highly prevalent in the elderly. An equivalent state is also pervasive in the epithelium of skin [14] and esophagus [15,16], suggesting that somatic mutation-driven clonal expansions may be a characteristic of aging in several tissues. Here, we review recent developments in our understanding of clonal hematopoiesis and its implications for human health.

A brief history of clonal hematopoiesis

Clonal hematopoiesis is characterized by the over-representation of blood cells derived from a single clone. Blood cancers such as chronic myeloid leukemia were first demonstrated to be clonal from karyotypic analysis and are the prototypical example of clonal hematopoiesis [17]. But in the 1990s, studies of non-random X-chromosome inactivation (XCI) in women led to the discovery that clonal hematopoiesis also occurs in individuals without cancer [18]. One X-chromosome is randomly inactivated in each cell during female embryonic development and this epigenetic state is stably maintained across cell divisions. The demonstration of non-random XCI in a population of cells is therefore evidence of a clonal process. Importantly, non-random XCI in blood cells was observed to increase in frequency with age, although the underlying mechanism was unclear [19]. A more complete catalog of the genetic framework of blood cancers allowed investigators to look for cancer-associated somatic mutations in these cases. In 2012, mutations in *TET2* (a gene coding for an enzyme that oxidizes methylated DNA) were found in ~5% of elderly women with non-random XCI. This was the first demonstration that mutation-driven clonal hematopoiesis occurs in healthy persons [20].

A second line of evidence that supported the existence of pre-malignant clonal expansions in healthy individuals came from studies of patients who were in remission after being treated for acute myeloid leukemia (AML). Like most cancers, AML generally results from several driver mutations that occur sequentially in the same clone over time [3]. If the first mutation to be acquired leads to clonal expansion without malignant transformation, it should be

possible to identify a pre-malignant stage in which only the initiating lesion is present [21]. This was first demonstrated in a case study of a patient with AML in which the driver mutation of the leukemia, an *AML1*/*ETO* translocation, could be detected in a fraction of phenotypically normal HSCs and mature hematopoietic cells in remission samples [22]. In later studies, DNA sequencing of HSCs from AML patients in remission revealed that stem cells with only a single driver mutation were often present, suggesting that pre-malignant clonal hematopoiesis was a generalizable finding in AML [23–25]. However, the extent of mutation-driven clonal hematopoiesis in the healthy population, its full genetic spectrum, and its natural history remained unknown.

Clonal hematopoiesis in the genome sequencing era

In 2014, three groups examined exome sequencing data from cases and controls within genetic association studies for diabetes, schizophrenia, and solid tumors that together comprised greater than 30,000 persons [26–28]. Importantly, these individuals were unselected for hematological phenotypes and the source of DNA was peripheral blood cells, thus permitting the study of mutation-driven clonal hematopoiesis on a larger scale than was previously possible. These studies can be thought of as a natural experiment of saturation mutagenesis in humans, and can be summarized in a simple postulate—given a sufficiently large population, every possible mutation that can occur will occur in some HSCs. Those cells carrying mutations that are neutral or deleterious will not expand, and therefore will not be detectable from blood DNA. Those mutations that are detectable are the ones that cause clonal expansion, and these will point to biological pathways that increase the fitness of HSCs.

The surprising result of this experiment of nature was that clonal hematopoiesis largely results from mutations in a very restricted set of genes. Mutations in classical oncogenes and tumor suppressors, such as those involved in cellular growth signaling (*JAK2*, *GNAS*, *GNB1*, *CBL*) and the DNA damage response (*TP53*, *PPM1D*), were seen but were not the most common. Instead, nearly two-thirds of clonal hematopoiesis could be accounted for by loss-of-function mutations in just two enzymes involved in DNA methylation, *DNMT3A* and *TET2*. The third most commonly mutated gene was *ASXL1*, a chromatin regulator, while mutations in splicing factors (*SF3B1*, *SRSF2*, *PRPF8*, *U2AF1*) were also frequent. Why these mutations cause clonal expansion remains an intense area of investigation (see ‘Mechanisms of Clonal Expansion’ below).

A second observation that emerged from these studies was that clonal hematopoiesis is an age-related phenomenon. Somatic clones were detectable in less than 1% of healthy individuals under age 40, but they increased in frequency with each decade of life. In contrast, 10–20% of individuals age 70 or older harbored a detectable clone. The size of these mutant clones was massive; in one study, a median of ~18% of blood cells carried the mutations [28]. For comparison, previously described mutations in the blood of healthy persons such as *BCR-ABL* or *BCL2* translocations were usually present in less than 0.01% of cells and were often transient [29,30]. One important consideration is that the prevalence of clonal hematopoiesis is highly dependent on the sensitivity of the method used to detect it (Fig. 2). These initial studies used whole-exome sequencing, which is relatively

insensitive to smaller clones. Subsequent studies in healthy persons using more sensitive approaches have found the prevalence of clonal hematopoiesis to be much higher, although the biological significance of the smaller clones is unknown [31–33].

Thus far our discussion of clonal hematopoiesis has focused on mutational changes that are limited to small stretches of DNA, such as base substitutions and small indels. But large structural variation, such as gains or losses of large segments of chromosomes, also increases with age and has clinically meaningful associations [34–36]. While the accumulation of both small and large somatic variants are linked to aging, the underlying biology of the two is generally non-overlapping. For example, copy number gains or losses of *DNMT3A*, *TET2*, and *ASXL1*, are rarely found in surveys of somatic large structural variation [35]. Instead, changes associated with chronic lymphocytic leukemia (CLL) and losses of sex chromosomes are the most common variants that accumulate in aging[36]. Mechanistic understanding of why these variants are positively selected during aging is lacking in the majority of cases. Further complicating the picture, clonal hematopoiesis has been observed in the absence of any known driver mutation [27,37]. What causes apparent clonal expansion in these cases is unknown, but could be due to mutations in genes not previously queried in surveys of clonal hematopoiesis, mutations in the non-coding genome, or even genetic drift due to age-related constriction of the stem cell pool [37].

Clonal hematopoiesis of indeterminate potential (CHIP)

Clonal hematopoiesis generally refers to any clonal outgrowth of hematopoietic cells, regardless of cause or disease state. Thus, someone with a frank malignancy like acute myeloid leukemia would be considered to have clonal hematopoiesis. Stochastic processes such as constriction of the stem cell pool with aging may also lead to clonal hematopoiesis in the absence of a known driver mutation. The term “clonal hematopoiesis of indeterminate potential” (CHIP) was introduced to distinguish non-malignant clonal hematopoiesis that is clearly linked to cancer-associated mutations from other forms of clonal hematopoiesis [38]. CHIP refers to the presence of a cancer-associated variant in the blood cells of a person without a frank malignancy or another recognized clonal entity, such as monoclonal B-lymphocytosis (MBL) or paroxysmal nocturnal hemoglobinuria [39]. The term “indeterminate potential” is intended to invoke the medical uncertainty associated with such a state. By this definition, cancer-free persons with somatic mutations in genes such as *TET2* or *TP53* would be considered to have CHIP. This term would not apply to persons with copy number abnormalities associated with MBL/CLL or clonal expansion in the absence of a known driver mutation. To meet the definition of CHIP, the clones must also meet a certain size threshold. If sequenced deeply enough, a cancer-associated mutation may be detectable in most people over the age of 50 [33], but only clones that reach a certain size are likely to be clinically meaningful. The threshold for CHIP was set at a variant allele fraction (VAF) of 2% (meaning 2% of the sequenced alleles contained the mutation, or roughly 4% of cells, assuming the mutation is heterozygous), but may be revised if it is demonstrated that there is prognostic significance for clones below this size.

Mechanisms of clonal expansion

The role of CHIP-associated genes in hematopoiesis has been extensively reviewed elsewhere [40,41]. For some of these genes, clear mechanisms for clonal expansion have been found. HSCs from mice with mutations in both *Tp53* [42] and *Ppm1d*, a gene encoding a regulator of p53 and other members of the DNA damage response pathway, [43,44] are able to enter cell cycle despite the presence of DNA damage, leading to a stem cell advantage in the setting of cytotoxic drugs. Activating mutations in *JAK2* allow constitutive signaling though growth factor receptors, thus leading to clonal expansion by enhanced proliferation of cells that carry this mutation [45].

Less clear is why mutations in *TET2* or *DNMT3A* lead to clonal expansion. Especially perplexing is the fact that these two genes have ostensibly opposite biochemical functions. DNMT3A is one of the two enzymes responsible for *de novo* methylation of the fifth position in cytosine bases of DNA, a mark that is thought to influence gene expression [46,47]. TET2 is one of three enzymes responsible for catalyzing the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine and further intermediates, which can eventually lead to demethylation [48,49]. Mouse models carrying loss-of-function mutations in either of these genes clearly show an HSC competitive advantage *in vivo* as well as a propensity for leukemia when cooperating mutations are present [50–53]. Stem cells from these mice are able to serially replate in colony forming assays over many generations, whereas wild-type stem cells quickly lose this capacity, suggestive of enhanced self-renewal capacity in the mutant stem cells [54,55]. However, it is unknown why loss of cytosine methylation or hydroxymethylation, broadly or at specific genomic loci, leads to changes in self-renewal ability.

Mutations in genes encoding core members of the spliceosome are also common in CHIP. HSCs from mice mutant for these genes, *Sf3b1* [56], *Srsf2* [57], and *U2af1* [58], have a competitive disadvantage *in vivo*, unlike the clonal expansion observed in humans with these mutations. The reason for the disparity of phenotypes in humans and mice is unclear. Partly because of the inability to model clonal hematopoiesis in these mice, the exact mechanisms whereby these mutations lead to clonal expansion remains unknown.

Clonal hematopoiesis is also commonly found in aplastic anemia, a disorder caused by an autoimmune attack on bone marrow progenitor cells that results in severely depressed blood counts [59]. In contrast to the mutations associated with CHIP, the mutations most commonly seen in aplastic anemia affect the genes *PIGA*, *BCOR*, and *BCORL1*. The *PIGA* gene is required for the synthesis of glycophosphotidylinositol (GPI) and its loss results in down-regulation of several cell surface proteins that are GPI anchored [60]. Loss of some of these proteins may allow for immune escape, thus explaining selection for *PIGA*-mutated clones. The mechanism of selection for *BCOR*/*BCORL1* mutations in this setting is unknown.

The risk of developing clonal hematopoiesis is largely related to the stochastic acquisition of somatic mutations in HSCs during aging. However, some epidemiological studies have implicated environmental and heritable components as well. For example, CHIP has been reported to be more common in older men [28] and smokers [27] and less common in Hispanics

[28], although these associations are all relatively modest. Recent studies have implicated genetic predispositions [37,61] as well as the microbiome [62] in the etiology and progression of clonal hematopoiesis. More insights into the factors that influence clonal expansions are expected to emerge from genetic sequencing of large population cohorts with richly annotated clinical phenotypes.

CHIP and hematological malignancy

CHIP itself does not denote a malignancy, nor is it associated with clinically significant alterations in blood counts [28,63]. But many of the most commonly seen mutations in CHIP are also recurrent drivers of AML [64], myelodysplastic syndrome (MDS) [65–67], myeloproliferative neoplasms [68], and certain lymphomas [69,70]. Thus, one might predict that individuals with CHIP would develop hematological malignancies at a rate above background since they have the “first hit” needed for malignant transformation. Indeed, in population-based cohorts that underwent exome sequencing, the presence of CHIP was associated with ~10-fold increased relative risk of these malignancies over several years of follow-up [27,28]. In one study, 4% of CHIP carriers developed a blood cancer over the subsequent 8 years, corresponding to ~0.5% of CHIP cases converting to malignancy per year [28]. Notably, the risk of malignancy in the carriers of CHIP was associated with the size of the mutant clone, as those who went on to develop malignancy had substantially larger clone sizes than those who did not. Myeloid malignancies were most common, although some people with CHIP did develop lymphoid cancers [27,28].

To refine risk estimates for developing AML associated with clonal hematopoiesis, two groups performed nested case-control studies within large population-based cohorts that had several years of follow-up [71,72]. Both groups found that individuals with antecedent clonal hematopoiesis were at ~3 to 5-fold increased risk for developing AML in the subsequent years (Fig. 3A). The risk was lower than the ~10-fold increase seen in previous studies because clonal hematopoiesis was identified using methods that were more sensitive than exome sequencing, which resulted in the detection of more clones of smaller size, including clones below the size threshold for CHIP. Similar to previous studies, the risk of AML positively correlated with the size of the mutant clone. An intriguing finding from these studies was that mutations in *TP53*, *JAK2*, *SF3B1*, *SRSF2* and *U2AF1* were linked to a particularly high risk of developing AML; however, many of these individuals had multiple driver gene mutations, making the assessment of risk for singleton mutations challenging. Nonetheless, such studies could provide a rationale for population-wide screening for those at especially high risk for transformation, though it remains to be seen what interventions would be beneficial in these individuals.

A feared complication in patients who have been treated with cytotoxic drugs for solid cancers is therapy-related development of secondary AML and MDS. Several studies have found that patients with CHIP and solid tumors or lymphoma have an increased risk of these therapy-related myeloid neoplasms after treatment for the primary disease [73–75]. It is hypothesized that pre-existing mutant HSC clones selectively expand under the pressure of cytotoxic therapy, and can cause cancer several years later with the acquisition of subsequent mutations [76]. One study of a patient population treated for solid cancers found that CHIP

mutations were more prevalent in this group than in populations not selected for cancer [77]. Furthermore, the mutational spectrum in patients with non-hematologic cancers was altered: HSC mutations in DNA damage response genes such as *TP53* and *PPM1D* were far more prevalent in this setting, likely due to strong selective pressure from exposure to cytotoxic therapies. Patients with CHIP also had increased mortality, most often due to progression of their primary malignancy. These studies indicate that CHIP in the setting of other cancers is likely to be especially pervasive and portends a poor prognosis.

CHIP and non-malignant disease

Most clonal expansion states are expected to increase the risk of neoplasia in the tissues in which they arise. But might there be consequences of the mutant clones apart from cancer? While it will be fascinating to determine these consequences in all tissues, some characteristics of the hematopoietic system make it particularly noteworthy as a potential cause of non-malignant disease. First, tissue architecture constrains the extent of clonal expansion within tissues such as gut or skin epithelium to patches that are rarely larger than a few square millimeters [15], but there is no such spatial restriction on HSCs, which freely admix throughout the bone marrow and body [78]. Indeed, some individuals with clonal hematopoiesis have virtually all of their blood cells arising from a single mutated HSC [28]. Second, alterations in hematopoietic cells have the potential to impact a wide range of disease states. In contrast to tissue-specific cells or epithelia, immune cells such as lymphocytes, granulocytes, and monocytes can migrate to and influence nearly every organ. These immune effector cells are derived from HSCs, so any mutations that occur in HSCs can also potentially alter the immune response or baseline inflammatory state.

These observations have prompted an examination of the effects of mutation-driven clonal hematopoiesis on human health and disease beyond blood cancer. Several studies have found that CHIP is associated with a 30–40% increased mortality risk [27,28,37]. In an initial study, this risk could not be explained by cancer deaths but was instead related to increased cardiovascular mortality [28]. Further analysis revealed that the risk of future ischemic stroke and coronary heart disease was more than doubled in carriers of CHIP (Fig. 3A). In this study of primarily middle-aged individuals, the risk of coronary heart disease and ischemic stroke associated with CHIP was as great or greater than that conferred by well-known risk factors for cardiovascular disease, such as circulating LDL-cholesterol levels, smoking, and blood pressure (Fig. 3B). Replication studies in additional cohorts confirmed and extended the early work. In studies of middle-aged and older individuals, the risk for coronary heart disease was nearly twice as high for individuals with CHIP compared to individuals without CHIP. The risk for early-onset myocardial infarction (MI), defined as heart attack before age 40 in men or age 50 in women, was four times higher in those with CHIP compared to those without CHIP. The relative risk for coronary heart disease in individuals bearing mutations in *DNMT3A*, *TET2*, or *ASXL1* was roughly doubled compared to those without CHIP; in individuals bearing *JAK2* mutations, the relative risk was ~12-fold higher compared to those without CHIP. In addition, individuals with larger mutant clones had the greatest risk of cardiovascular disease, mirroring the situation for malignancy risk [79]. Just as CHIP is associated with an increased risk of cardiovascular disease, lower-risk subtypes of MDS are also associated with a doubling of the risk of dying from cardiovascular causes [80].

The link between CHIP and cardiovascular outcomes is not limited to atherosclerotic disease. Recent evidence suggests that individuals with post-MI related congestive heart failure who carry CHIP-associated *DNMT3A* or *TET2* mutations have worse survival outcomes than individuals without CHIP [81]. In a separate study (83), individuals with CHIP-associated *JAK2* mutations were reported to have a ~12 times greater risk of developing venous thrombosis than those without CHIP, whereas individuals with mutations in other CHIP-associated genes had a doubling of the risk [82]. Thus, CHIP is likely to be an indicator of poor prognosis for several distinct cardiovascular disorders.

While it is clear that CHIP-associated mutations play a causal role in the development of blood cancer, it is less clear, *a priori*, whether their role in non-malignant diseases is causal or merely correlative. Like several other well-described markers in blood cells, such as red cell distribution width [83], DNA methylation clocks [84], and loss of Y-chromosome [85], CHIP is associated with multiple adverse outcomes in epidemiological studies. One potential explanation is that all of these biomarkers are measures of some aspect of biological aging but are themselves not directly causal for health outcomes. What distinguishes CHIP from these other measures is the ability to manipulate model organisms experimentally to test causality.

In 2017, two research groups used mouse models to establish a causal role for CHIP in atherosclerosis. Both groups found that loss of *Tet2* in bone marrow cells led to an increase in the size of atherosclerotic lesions in hyperlipidemic mice, an effect that could not be explained by quantitative changes in blood cell parameters of the mutant mice [79,86]. Rather, the mutant bone marrow-derived macrophages upregulated many pro-inflammatory molecules, suggesting a potential mechanism for the increase in atherosclerosis. In support of this hypothesis, loss of *Tet2* in myeloid cells was sufficient to confer enhanced atherosclerosis. In addition, blockade of the pro-inflammatory cytokine IL-1B reversed the accelerated atherosclerosis seen in *Tet2* mutant mice [86].

Subsequent studies showed that loss of *Tet2* or *Dnmt3a* led to worsening heart function in a mouse model of congestive heart failure, corroborating the human genetic association [87,88]. Heart function in these studies was also improved with blockade of IL-1B, suggesting that this may be a common pathway for reversing the effects of CHIP in the heart. There is also evidence that *Jak2* mutations enhance atherosclerosis in mouse models by altering macrophage function [89]. Mutations in *JAK2* have a well-described role in activating STAT transcription factors, which are central to immune response in several cell types involved in atherosclerosis. Furthermore, *JAK2* mutations prime neutrophils to form neutrophil extracellular traps, leading to thrombosis, which may also contribute to poor cardiovascular outcomes [82]. Together, these studies provide strong evidence that somatic mutation-driven clonal hematopoiesis has a causal role in cardiovascular disease.

To date, few studies have demonstrated an unequivocal link between CHIP and other diseases of aging. CHIP has been found to be associated with a 30% increase in the likelihood of having type 2 diabetes (T2D) [28]. However, causality could not be established, and this could represent a case of reverse causation, as hyperglycemia might influence the development or expansion of clones by interfering with TET2 function [90]. Other studies

have found links between CHIP and chronic obstructive pulmonary disease (COPD) [37,63]. However, CHIP was also strongly linked to smoking in these studies, so the result could be confounded by this association.

Immune function and CHIP

As many of the genes associated with CHIP are involved in transcriptional regulation, one might expect them to have broad effects on immune function. Several recent studies have examined the role of *TET2* and *DNMT3A* in immunity (Fig. 4). One group found that mice deficient in *Tet2* developed more severe inflammation in several tissues upon challenge with bacterial endotoxin, and this was partially explained by increased expression of the pro-inflammatory cytokine *Il6* in dendritic cells and macrophages [91]. Surprisingly, this effect on *Il6* expression was independent of the catalytic function of Tet2, and was instead reported to be related to a direct interaction between Tet2 and histone deacetylases, resulting in transcriptional repression. Subsequent studies have confirmed the over-expression of *Il6* and also found that *Il1b*, IL8 family chemokines, and other inflammatory mediators show increased expression in *Tet2*-deficient macrophages challenged with low-density lipoprotein or endotoxin [79,86,92]. Humans with mutations in *TET2* are also reported to have increased levels of circulating IL8 protein [79]. The changes in expression are relatively modest for each gene (~2 to 3-fold increase) but appear to be biologically significant given the number of genes that are dysregulated and the breadth of phenotypes seen in *Tet2* knockout mice. These molecules are thought to enhance inflammation locally within atherosclerotic plaques by increasing chemotaxis of leukocytes to arterial intima, which potentially explains the accelerated atherosclerosis seen with loss of *Tet2* [93].

Less is known about the role of *DNMT3A* in innate immune function, but most studies to date have found evidence of enhanced inflammation when its function is perturbed. For example, one study found that mast cells from mice that lacked *Dnmt3a* produced higher levels of IL-6, TNF-alpha, and IL-13 in response to stimulation with IgE *in vitro*, and enhanced mast cell activity was also seen in a mouse model of allergy [94]. Another study used CRISPR to mutate *Dnmt3a* in mouse RAW 264.7 macrophages, and observed increased expression of *Cxcl1*, *Cxcl2*, and *Il6* in response to endotoxin [87]. Mechanistically, very little is known about why these specific gene expression changes are seen with loss of *Dnmt3a*.

CHIP may be relevant in the adaptive immune response as well. Bone marrow transplant recipients with hematological malignancies who received donor marrow from carriers of CHIP had higher rates of graft-versus-host disease and reduced relapse rates [95]. One possible explanation for this finding is that the donor cells harboring the mutations were capable of mounting stronger immune responses against both normal host tissues and the tumor. There is also a report of an exceptional response in a patient with CLL treated by infusion of chimeric antigen receptor (CAR) T-cells in which the CAR construct disrupted one copy of *TET2*, while the other copy had been previously mutated somatically [96]. The authors speculate that the resulting *TET2*-deficient CAR-T clone was more effective at eliminating the tumor cells because of an expanded central memory CD8+ T-cell population, reduced T-cell exhaustion in response to stimulus, as well as enhanced cytokine production

in T-cells. Several studies have found additional roles for *TET2* and *DNMT3A* in normal T- and B-cell function (Figure 4).

In summary, growing evidence supports a role for the commonly mutated CHIP genes in immune function, and CHIP may underlie some part of the phenomenon termed "inflammaging", the age-associated increase in systemic inflammation [97].

Future outlook

Clonal hematopoiesis provides a fascinating glimpse into the end result of decades of mutation and natural selection within a tissue. The potential health implications of CHIP are broad. It is associated with blood cancers, cardiovascular disease, and overall mortality. While our knowledge of this condition has increased exponentially over the last several years, this research has also highlighted fundamental biological questions and opened new pathways for translational discovery.

A major area of uncertainty is the range of disease states associated with CHIP. As CHIP is linked to enhanced inflammation, it is possible that links between CHIP and several diseases of aging will be found. Does CHIP influence the risk of Alzheimer's disease, autoimmunity, liver disease, or others? As ever larger genetic cohorts with rich phenotypic information are assembled, these links may be systematically discovered.

While CHIP as a whole is clearly linked to cancer, cardiovascular disease, or death, the level of risk for any given individual is likely to be variable. The effect of specific gene variants on various outcomes may be quite different, as has been found in AML [71]. Furthermore, the interaction of CHIP with other risk factors, such as having type 2 diabetes or elevated serum levels of C-reactive protein, may be synergistic for adverse outcomes. To have power to detect such associations, very large population-based cohorts are needed. Additional biomarkers such as plasma proteins, metabolites, and DNA methylation may prove useful for risk stratification as well. Studies of serial samples have shown that the trajectory of a clone can vary between people. Some people have clones that remain stagnant in size for many years, whereas other people have clones that show steady growth [28,33]. Since clone size associates with the risk of leukemia and other adverse outcomes, it is imperative to understand this situation. It is likely that cell extrinsic factors, like the microenvironment, the microbiome, or diet, will play a role.

The mechanisms by which the CHIP-associated mutations cause clonal expansion and enhanced inflammation is also a central unanswered question. It is striking that the two most commonly mutated genes in CHIP, *DNMT3A* and *TET2*, are opposing enzymes in DNA methylation, yet both lead to convergent phenotypes in stem cell biology and immunity. It is also uncertain why some people have apparent clonal hematopoiesis in the absence of a known driver mutation. This may be due to undiscovered mutations, but could some of these clonal expansions result purely from selection on a heritable epigenetic state?

Most importantly, we need to find ways to reverse the pathogenic effects of CHIP. Blockade of downstream inflammatory molecules may be one way to treat CHIP-associated atherosclerosis. But ideally drugs will be found that can suppress the mutant clones directly,

which could potentially mitigate the risk of both cancer and cardiovascular disease. The positive effects of CHIP on anti-tumor immunity may also one day be harnessed for therapy.

The process of mutation and clonal selection is likely to be universal across all organs and tissues. Understanding the causes and consequences of clonal hematopoiesis may provide a framework to understand this process, and aging, more broadly.

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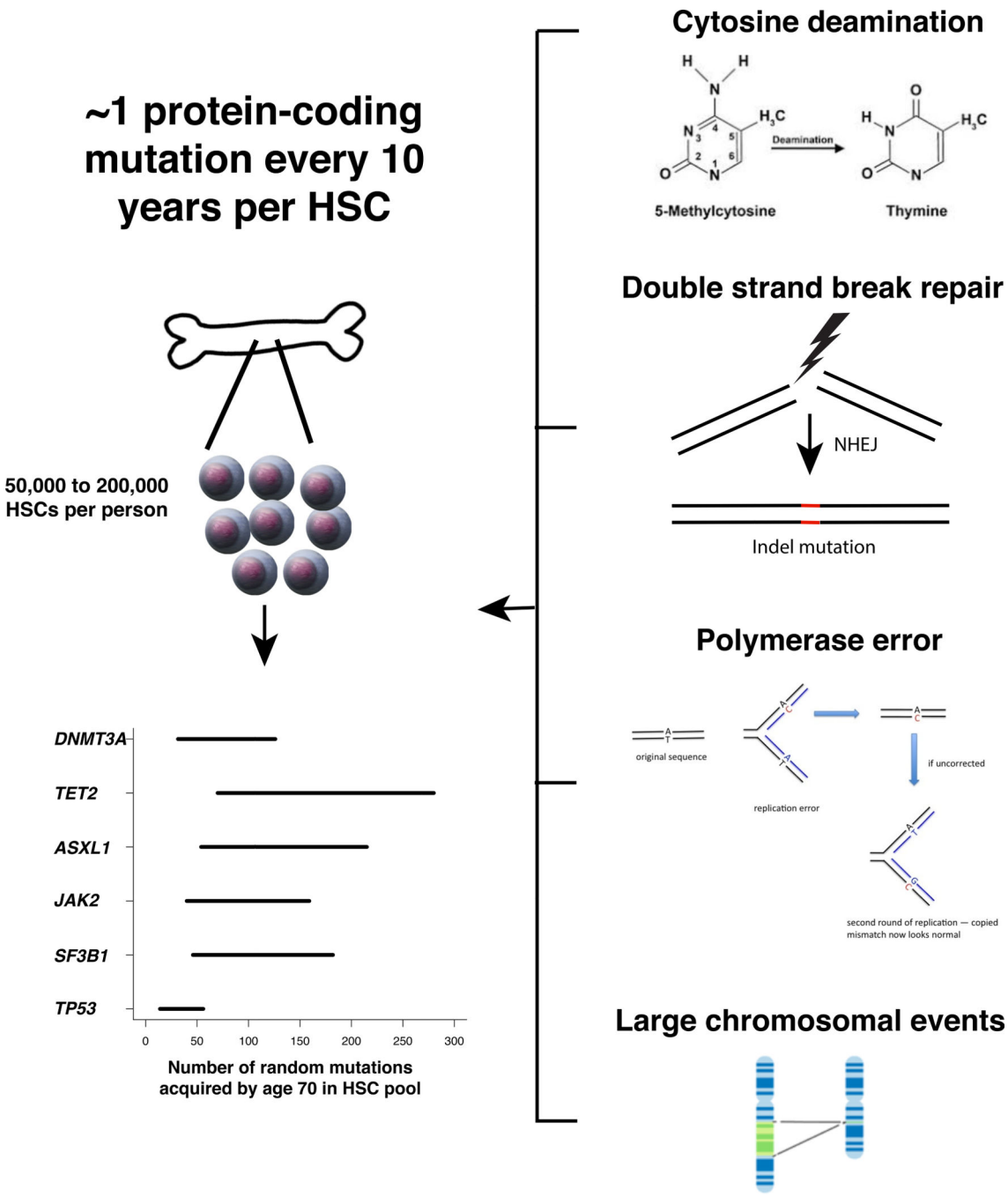


Fig. 1.

A single HSC in a healthy person acquires approximately one protein-coding mutation per decade of life (00). Four mutational processes contribute to the bulk of these age-associated mutations (right). Assuming there are 50,000 to 200,000 HSCs in an average person (00), we estimate that by age 70, an average person will harbor 350,000 to 1.4 million protein coding mutations in his/her HSC pool. Shown in the bottom left is the expected range of random mutations in HSCs in the exons of *DNMT3A*, *TET2*, *ASXL1*, *JAK2*, *SF3B1*, and *TP53* by age 70 per person. A subset of these mutations may lead to clonal expansions.

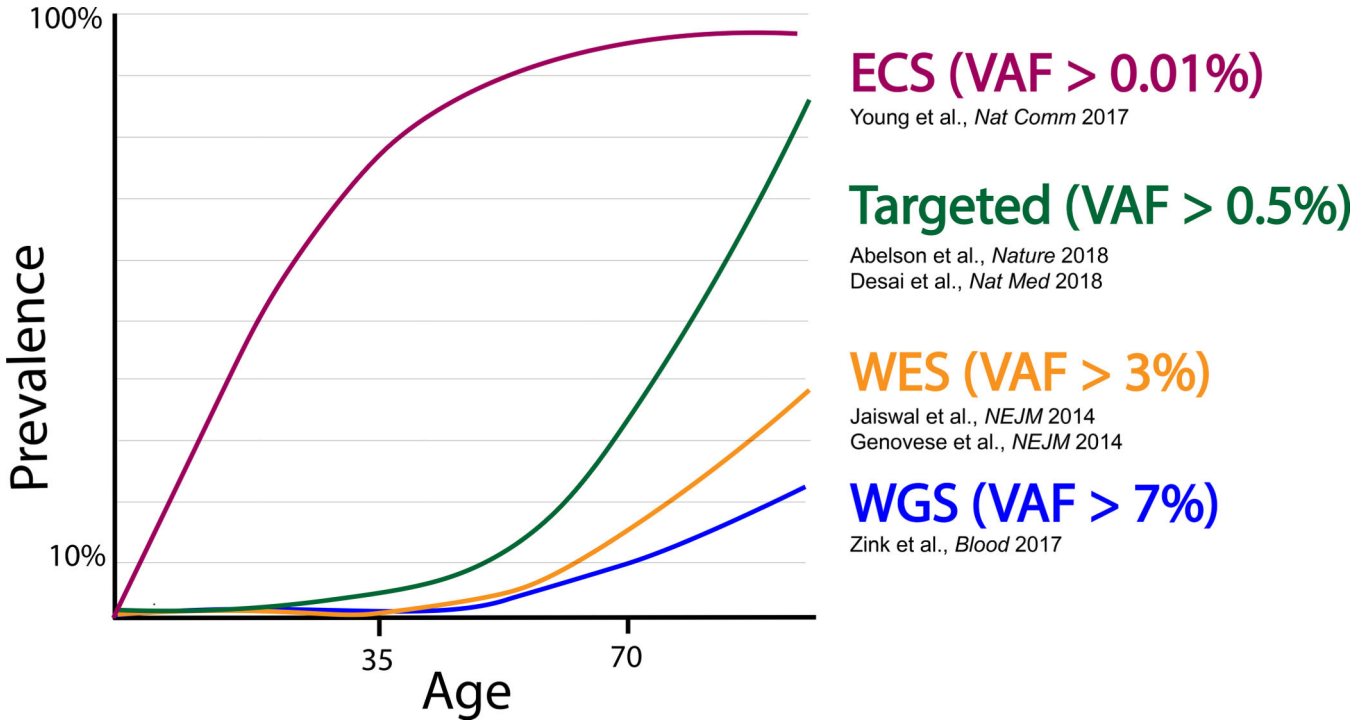
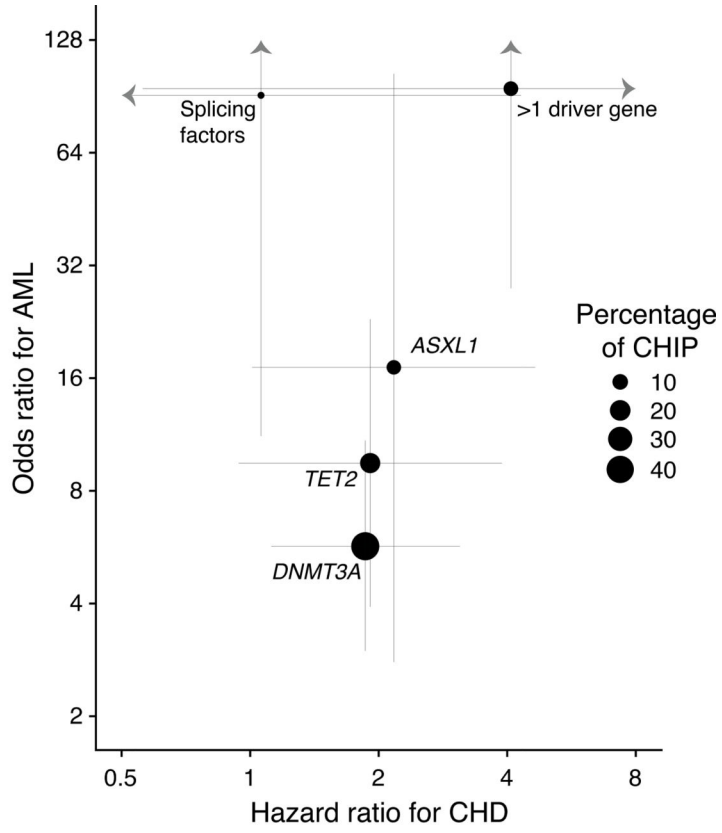


Fig. 2.

The estimated prevalence of CHIP as a function of age varies according to the sequencing method used.

VAF, variant allele fraction. ECS, error corrected sequencing. WES, whole- exome sequencing. WGS, whole-genome sequencing.

A



B

	HR (95% CI)
Age 50-59	**2.2** (1.3-3.7)
Age 60-69	**2.4** (1.4-4.0)
Age ≥70	**6.3** (3.8-10.4)
Female	**0.7** (0.5-0.9)
Has T2D	**2.2** (1.6-3.0)
Former or current smoker	**1.4** (1.0-1.9)
Hypertension stage II-IV	**1.4** (1.0-1.9)
TC >200 mg/dL	**1.4** (1.0-1.9)
HDL<35 mg/dL	**1.4** (1.0-2.2)
HDL>60 mg/dL	**0.8** (0.5-1.1)
CHIP present	**1.8** (1.1-2.9)

Fig.3.

(A) Forest plots for odds ratio for developing acute myeloid leukemia (AML) and hazard ratio for developing coronary heart disease (CHD) in those with mutations in the genes listed, adapted from Abelson et al. Nature 2018 and Jaiswal et al., NEJM 2017. Only those mutations with a variant allele fraction greater than 2% (meeting the definition of CHIP) were included. Individuals with mutations in more than one driver gene were analyzed as a separate category. Lines represent the 95% confidence interval for odds or hazard ratios and

the sizes of the dots are proportional to the number of mutations in each gene in the general population.

B) Hazard ratio (HR) and 95% confidence interval (CI) for developing CHD based on Framingham risk factors plus presence of CHIP mutations. Data taken from population-based cohorts unselected for CHD status (just give ref numbers).

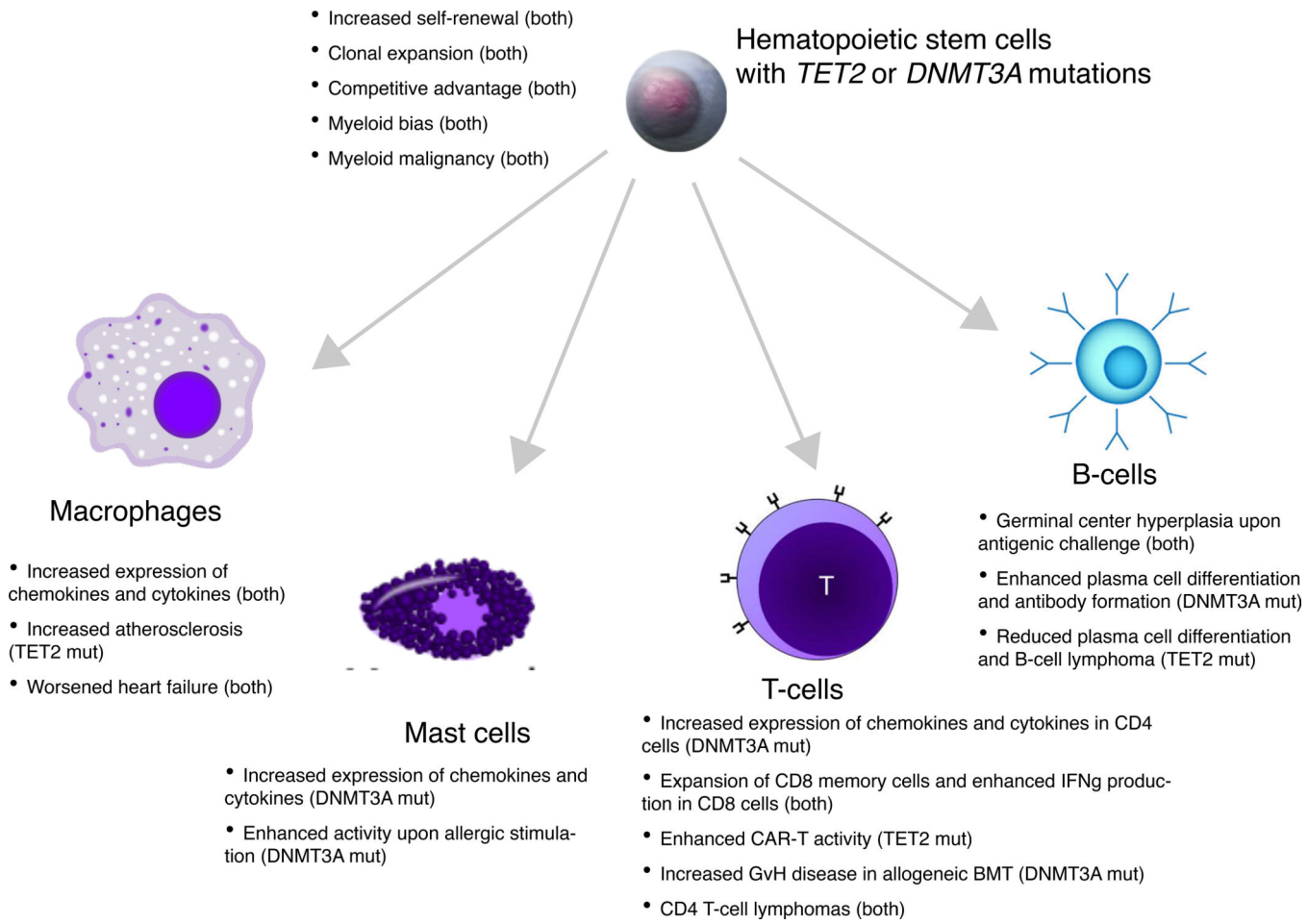


Fig. 4.

Phenotypic changes in HSCs and immune cells with TET2 or DNMT3A mutations.