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Clonal Hematopoiesis in Aging

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Abstract

Purpose of Review Clonal hematopoiesis of indeterminate potential (CHIP) is a common, age-associated condition characterized by the acquisition of somatic mutations. This concise review explores our current understanding of the mechanisms that influence the development of clonality with aging and its potential malignant and non-malignant clinical implications.

Recent Findings Aging of the hematopoietic system results in phenotypic changes that favor clonal dominance. Cell-extrinsic factors provide additional selective pressures that further shape clonal architecture. Even so, small clones with candidate driver mutations appear to be ubiquitous with age and largely benign in the absence of strong selective pressures. Benign clonal expansion may compensate for the loss of regenerative HSC capacity as we age.

Summary CHIP is a marker of aging that reflects the biologic interplay between HSC aging and cell-extrinsic factors. The clinical significance of CHIP is highly variable and dependent on clinical context. Distinguishing the causal relationships and confounding factors that regulate clonal behavior will be essential to define the mechanistic role of CHIP in aging and potentially mitigate its clinical consequences.

Keywords Clonal hematopoiesis · CHIP · Aging · Somatic mutations · Clonal selection · Clonal evolution

Introduction

Hematopoietic stem cells (HSCs) must maintain adequate blood cell production throughout an individual's lifespan. With chronological aging, however, HSCs gradually lose their capacity for self-renewal and exhibit skewed differentiation that can result in increased risk of anemia and impaired adaptive and innate immunity [1]. These alterations are primarily driven by cell-intrinsic mechanisms that involve genetic and epigenetic changes that set the stage for clonal selection. While prior studies in mice have strongly suggested that HSC aging manifests largely as a consequence of cell-intrinsic molecular changes [2], the importance of microenvironmental influences may be underestimated in these short-lived animals with limited exposure to environmental stressors. The role of the bone marrow microenvironment in human hematopoiesis is likely much more pronounced. Emerging evidence has shown that external selective pressures such as chronic inflammation can affect normal hematopoiesis and remain underappreciated forces in the modeling of clonal architecture [3]. Despite rapid advances in the genetic understanding of clonal hematopoiesis in various malignant states, the clinical significance of clonality in the context of healthy aging remains largely undefined.

In this review, we discuss the cell-intrinsic molecular mechanisms that contribute to HSC aging and describe the clonal drivers seen in aging hematopoiesis. Specifically, we will review the effects of cumulative molecular damage on HSC senescence that occur as a function of increasing age. We describe various cell-extrinsic factors that could influence the bone marrow niche for CHIP development. We summarize how clonal hematopoiesis can arise in a wide variety of contexts as a derivative of dynamic selective forces, using patients receiving chemotherapy, autoimmunity, and a senescent immune system as salient examples. Although CHIP in aging can be depicted as a preleukemic state, we highlight the near universality of this finding in healthy individuals and how it

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might inevitably result from increases in our life expectancy. Importantly, the prevalence of clonal hematopoiesis is highly dependent on the methods used to detect it. Similarly, the clinical significance of clonal hematopoiesis depends greatly on its abundance and on the context in which it occurs. Arming healthcare providers with this knowledge will allow them to better personalize clinical management decisions and patient counseling.

Aging Hematopoietic Stem Cell Phenotypes

The functional superiority of young HSCs in serial transplantation assays performed in mice emphasizes the significance that age plays in HSC biology [4–7]. Appreciating the cell-intrinsic mechanisms that contribute to aging HSC phenotypes is key to understanding the clonal selection that follows the natural aging process. Evidence from more recent studies suggests aging hematopoiesis is the result of cumulative selection pressures at the level of the HSC [8]. Here, we focus on reduced self-renewal and lineage skewing with the caveat that most models of HSC aging are based on the murine experimental model.

Hematopoietic stem cell exhaustion is a hallmark of biological aging and refers to the loss of capacity for self-renewal, resulting in deregulation of steady-state hematopoiesis. Decades of research have enabled scientists to phenotypically identify HSCs and surprisingly discover their numbers increase with age in both humans and mice [9–15]. Methods for defining HSCs have evolved from limiting dilution assays to identify transplantable cells capable of in vivo hematopoietic reconstitution to HSC purification strategies based on surface antigen profiles [16]. Despite the greater number of HSCs, serial transplantation assays have shown these aged cells exhibit several functional defects, including a reduced regenerative potential [4]. This observation remains poorly understood but may be related to quiescence of the most primitive and pluripotent HSCs [17–19]. Under this concept, subsequent HSC generations lose developmental potential with each cell division [20]. Consequently, the youngest HSC pool is relatively small, but with higher long-term repopulating potential whereas the HSC pool in older individuals has expanded but is more functionally restricted. This deterioration in HSC fitness during aging may be due to senescent cell-intrinsic mechanisms as we discuss below. The decline in functional HSCs may underlie the increasing marrow hypocellularity and impaired bone marrow recovery seen with age.

Older HSCs are not only less regenerative; they are skewed toward the myeloid lineage. This myeloid skewing seems to involve disturbances in early lineage specification. Microarray analyses of HSCs from young and old mice found aged mice to have increased numbers of common myeloid progenitor cells compared with young mice [9]. Interestingly, aging HSCs with loss-of-function mutations in epigenetic regulator genes such as *TET2* have been associated with enhanced self-renewal and preferential myeloid commitment in murine models [21]. The preponderance of these mutations in aging hematopoiesis and CHIP [22] suggests clonal dominance and myeloid bias are linked. The aged marrow may be more favorable toward myeloid skewed colonies regardless of how they achieve that state. A cross-sectional study of 198 unrelated women found age-associated declines in global 5-hydroxymethylcytosine levels were not solely accounted by acquired mutations in *TET2*; rather, cumulative stochastic changes in methylation, termed epigenetic drift, were primarily responsible for this reduction [23]. The clonal expansion of intrinsically myeloid skewed *TET2* mutant clones may be in part driven by this interaction with the permissive aged microenvironment.

Cell-Intrinsic Mechanisms of Hematopoietic Stem Cell Aging

Retention of an aging phenotype after transplantation of aged HSCs into young mice arguably supports that HSC aging is primarily driven by intrinsic changes [9, 24]. Cell-intrinsic mechanisms experimentally implicated in HSC aging, described below, may drive clonal selection with aging.

DNA Damage Age-dependent accumulation of DNA damage has been proposed as an irreversible cause of HSC aging. Mutations in genes encoding proteins involved in the DNA damage response (DDR) may induce HSCs to senesce, depleting them from the functional pool [25]. Higher levels of histone 2A family, member X, foci formation, a sensitive marker for DNA double-strand breaks, in aged HSCs support this theory [26–28]. Mice deficient in several DNA repair genes were shown to retain HSC reserves with age, but their HSCs displayed severe functional limitations, ultimately culminating in functional exhaustion. HSCs from *BRCA1*-deficient mice expanded in the bone marrow but formed fewer hematopoietic colonies in vitro and failed to reconstitute hematopoiesis in lethally irradiated mice [29]. HSC depletion due to stochastic dropout where remaining cells have gained no selective advantage is one route to clonally restricted hematopoiesis. Alternatively, somatic mutations in malignancy-associated genes could provide a selective growth advantage leading to clonal expansion. These paths to clonal hematopoiesis are not mutually exclusive and both are likely to be influenced by external conditions. However, the clinical implications for these two distinct paths could be very different.

Telomere Attrition Telomere erosion is a specific form of DNA damage linked to HSC aging [30]. These repetitive DNA sequences located at the chromosome ends prevent

activation of the DDR. With each cell division, telomere length shortens and eventually produces unprotected chromosome ends that are recognized as DNA damage, triggering apoptosis or senescence [31]. Successive breeding of telomerase mRNA knockout mice eventually results in critical shortening of telomeres leading to reduced long-term cell viability including compromised HSC renewal [32, 33]. Stochastic loss of HSCs via telomere attrition would allow for the expansion of clones with growth advantages. Recent evidence that telomerase activity may play a role in the development of clonal hematopoiesis comes from two studies reporting germline variants in the *TERT* gene locus [34, 35••].

Telomeropathies such as dyskeratosis congenita are caused by mutations in genes encoding components of the telomere complex such as *TERT* and *TERC* [36]. Children with short telomere syndromes often exhibit early-onset bone marrow failure and an increased propensity to develop myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) in adult life [37]. Accelerated telomere attrition due to underlying genetic mutations in conjunction with overproduction of cytokines in response to marrow failure may deplete the HSC pool and promote premalignant clonal expansion.

Epigenetics Age-associated clonal mutations primarily involve two genes that modulate DNA methylation, *DNMT3A*, and *TET2* [22]. This suggests that maintenance of epigenetic regulation is necessary for proper HSC regulation. Array-based DNA methylation profiling on young and old human HSCs identified hypomethylation of genes that are normally hypomethylated and activated during myeloid differentiation [38, 39]. Such alterations of the epigenomic landscape during HSC aging might explain the reduced differentiation potential characteristic of older HSCs. Diminished capacity for HSC self-renewal appears to be partially linked to the higher replicative history induced by age. Inaccurate transmission of epigenetic elements to daughter cells can result in the gradual and random erosion of epigenetic marks as HSCs divide and age [40, 41]. Failure to preserve transcriptional fidelity may provide a theoretical backdrop that underlies the aberrant epigenetic status of aged HSCs. The inability of daughter cells to maintain the HSC pool may pave the way for clonal selection, which may be accelerated in the presence of somatically mutated epigenetic regulators.

Aging Hematopoiesis

Clonality has long held negative connotations in the field of hematology despite our knowledge that the hematopoietic system normally becomes increasingly oligoclonal with age (Fig. 1a). Studies in healthy women more than two decades ago provided the first evidence for age-related clonal hematopoiesis by demonstrating skewed X-inactivation patterns in unfractionated leukocytes [42]. A seminal study shortly thereafter confirmed the frequent presence of excessive skewing indicating a remarkably high rate of acquired clonal hematopoiesis [43]. Several mechanisms were proposed to account for this finding, including the acquisition of somatic mutations and the notion of aging-associated HSC depletion leading to stochastic differentiation and clonal dominance by chance [43, 44]. It was not until many years later that somatic mutations like those in *TET2* were found in some normal elderly women with acquired clonal hematopoiesis characterized by skewed X-inactivation [45]. This discovery supported a model in which somatic mutations confer a growth advantage to mutant clones resulting in clonal bias.

More recently, advances in genetic techniques have detected a high rate of somatic mutations in myeloid-malignancy-associated genes in healthy individuals. A series of large, population-based genetic analyses have revealed that somatic mutations in HSCs are commonly acquired during aging [46–48]. Mutations in this context were often in genes known to be recurrently mutated in MDS. Most individuals harbored a single mutation in epigenetic regulators with *DNMT3A*, *TET2*, and *ASXL1* being the most frequently affected [46–48]. Variant allele frequencies (VAFs) in the peripheral blood were about 9–12%, representing a sizeable proportion of total hematopoietic output. Strikingly, the prevalence of such mutations in unselected individuals was remarkably high. With a VAF cutoff of 2%, mutations indicative of clonal hematopoiesis could be identified in 10–15% of 70-year-olds [46, 47], the typical age of diagnosis for patients with MDS. This is more than an order of magnitude greater than the incidence of blood cancers in this population making it clear that mutations were not predestined to evolve into a frank malignancy. For this reason, somatic variants with a VAF of ≥ 2% in an otherwise healthy population were described as having indeterminate potential or CHIP [49••]. While CHIP is largely benign in an oncogenic sense, conferring only a small increase in absolute malignancy risk [46, 47], its presence is associated with an increased risk of all-cause mortality that is ascribed to an increase in cardiovascular mortality comparable to that associated with elevated cholesterol and hypertension.

Clonal hematopoiesis associated with a selective growth advantage is often identified by somatic mutations in candidate driver gene exons. However, this may underestimate the rate of CHIP since stochastic dropout of HSCs leading to residual oligoclonal hematopoiesis may not require driver gene mutations. Since DNA replication and repair are imperfect processes, HSCs invariably acquire mutations over their life history. The accumulation of mutations is an age-dependent process and the majority of random mutations will lie outside of coding regions [50]. Whole-genome sequencing (WGS) of a highly polyclonal population would not be expected to find passenger mutations associated with any individual clone since they would be unique to that clone and

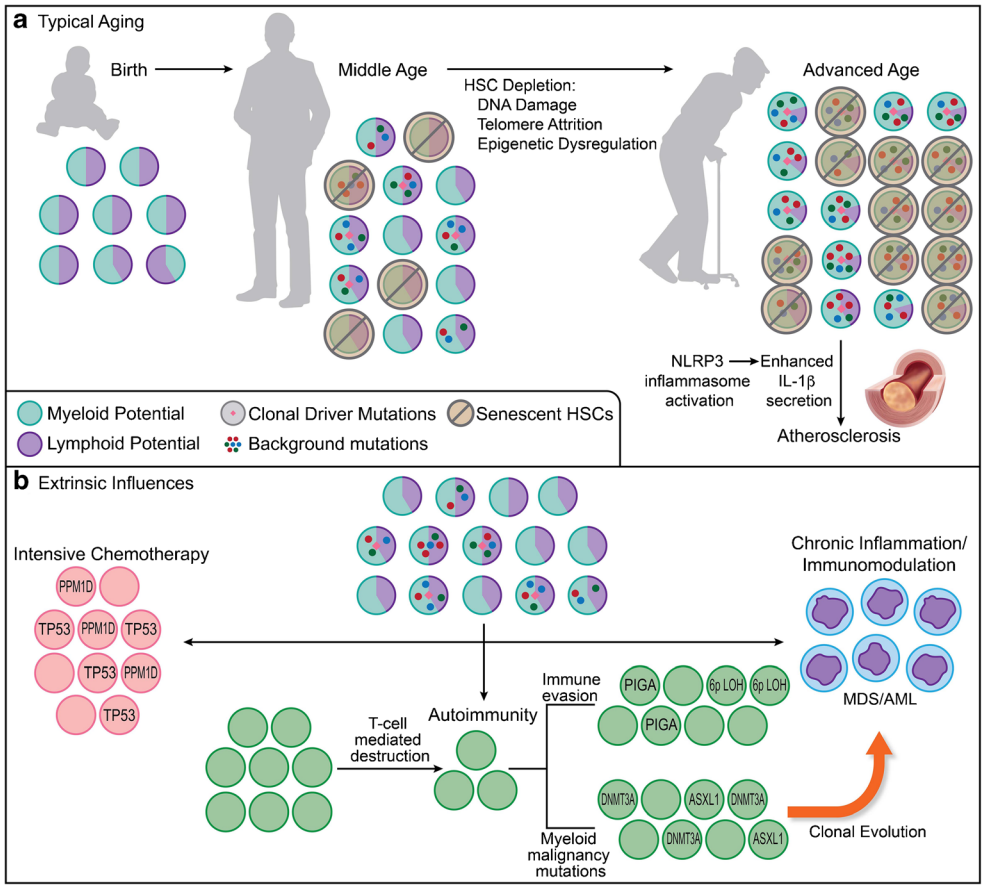

Fig. 1 Clonal hematopoiesis in relation to aging and selective pressures. **a** Young HSCs exhibit balanced differentiation potential. Normal aging leads to expansion of myeloid skewed HSCs that become senescent with time. By middle age, HSCs have accumulated random background mutations that do not impact cellular fitness. HSCs with driver mutations are detectable at middle age and may expand without biological consequence to sustain normal hematopoiesis in advanced age. Aging-associated somatic mutations like *TET2* can accelerate atherosclerosis by generating a pool of macrophages with enhanced atherogenic capabilities. **b** Extrinsic factors act on a polyclonal pool of HSCs that includes small subclones with diverse cell-intrinsic alterations and somatic mutations. Distinct alteration types are selected by specific cell-extrinsic influences. Chemoresistant clones with *TP53* or *PPM1D* mutations are preferentially expanded after cytotoxic chemotherapy. T-cell-mediated autoimmunity selects for clones capable of immune evasion or with a growth advantage conferred by myeloid malignancy-associated mutations. Chronic inflammation and immunomodulation increase the risk of developing clonal neoplasms like MDS/AML

represented at extremely low levels. However, when an HSC clonally expands, these ancestral passenger mutations are replicated along with the clone and can be used to identify its presence in WGS studies. Clonality identified by detecting a large number of passenger variants appears to be at least as common as CHIP defined by driver gene mutations. In one WGS study of more than 11,000 Icelanders, individuals with more subclonal somatic mutations, particularly over 250, were increasingly more prevalent with age, approaching 55% for those in their 80s [35••]. This majority may be an inevitable consequence of normal aging whereby functional HSC depletion fosters clonal expansion as a result of neutral drift [51]. Neutral drift argues that even when polyclonal HSCs have equal regenerative potential, stochastic turnover eventually leads to the persistence and expansion of only a few clones. Individuals with this type of clonal hematopoiesis lacking myeloid-malignancy-associated mutations were still at comparably increased risk of developing a hematologic malignancy and had higher rates of overall mortality [35••]. However, no significant associations were seen with cardiovascular disease, implying that specific mutations might be necessary for inflammatory plaque development as illustrated in recent studies.

Heterogeneity of Extrinsic Influences in Clonal Selection

The presence of somatic mutations alone does not appear to be sufficient to induce clonal expansion. Mutant clones can remain stable for years without clinical consequences in healthy individuals [46]. Additional cell-extrinsic factors may alter the bone marrow niche and drive the course of natural selection to favor clonal expansion. The influence of hematopoietic cell-extrinsic factors is best represented by specific patterns of

clonal hematopoiesis in the context of cytotoxicity, immune marrow failure, and normal aging (Table 1 and Fig. 1b).

Cytotoxic Stress When selective pressures change, the presence of small clones may become much more relevant as their fitness advantage becomes exaggerated or the mechanisms controlling their expansion are altered [52, 53]. Genotoxicity from chemotherapy positively selects for pre-existing HSC clones that are more resistant to apoptosis and tolerant of DNA damage, increasing the risk for leukemic transformation. Genetic analysis of paired antecedent and diagnostic samples from patients with therapy-related myeloid neoplasm (tMN) identified patient-matched *TP53* mutations many years before the development of tMN [54••, 55]. WGS at diagnosis and relapse from eight patients with AML demonstrated clear evidence of clonal evolution at relapse with new mutations in the founding clone and subclones [56]. These studies support the idea that pre-existing clones undergo clonal evolution under the selective pressure of chemotherapy and are not necessarily created by treatment-induced DNA damage.

Chemotherapy alters the genetic diversity of surviving HSCs by conferring a strong competitive advantage to cells harboring specific resistance mutations. Targeted sequencing of 86 genes with involvement in CHIP or myeloid malignancies in a large cohort of patients with non-Hodgkin lymphoma at the time of transplant revealed that mutations in *PPM1D* and *TP53* occurred at a higher frequency relative to CHIP in the general population [57••]. Because these mutations suppress activation of the DDR, clonal hematopoiesis in this context may inadvertently select for inherently resistant clones. Another study led by Jacoby et al. identified subclonal evolution at disease relapse following myeloablative or reduced-intensity allogeneic transplant in nine patients with MDS [58]. Somatic alterations in the founding clone persisted at post-transplant relapse in all nine patients. Notably, disease relapse was driven by subclonal expansion in seven patients, highlighting contributions of subclonal evolution to disease progression. Structural variants were present in the majority of subclones and detected as the only new genetic lesion in five patients at relapse, a finding that may be specific to the extreme selective pressures of transplant.

Finally, the persistence of clonal hematopoiesis in patients with AML in morphologic remission is common [59–64] and can be associated with an increased risk of relapse. Such clones often represent reversion to benign clonal hematopoiesis that may or may not be related to the founding clone. Those closely related to the founding clone may be subject to further clonal selection leading to subsequent disease relapse. Enhanced exome sequencing on paired diagnostic and remission samples from 15 patients with AML found that chemotherapy also provides a strong selection pressure on unrelated clones [65]. One third of these patients exhibited rapid clonal expansion of a non-leukemic hematopoietic population harboring age-associated mutations including *DNMT3A*, *TET2*, and *ASXL1* in the remission sample. Two of the five patients with unrelated clones went on to develop relapsed disease originating from the founding clone, not the clone present during remission. This study shows that seemingly innocuous age-associated HSC clones may acquire a proliferative advantage that enables expansion and persistence following chemotherapy exposure. However, the clinical significance of non-leukemic HSC expansion remains unclear and how to distinguish them reliably will be difficult in practice.

Autoimmunity Immune-mediated HSC destruction by autoreactive T-cells, like that which occurs in aplastic anemia, creates chronic regenerative stress, and alters the bone marrow

Table 1 Mutation profiles of clonal hematopoiesis in different contexts

Clinical context	Enriched mutation profiles
Normal aging	
50–70 years	*DNMT3A*, *TET2*, *ASXL1*, *JAK2*, *TP53*
70+ years	*DNMT3A, TET2, ASXL1, TP53, SF3B1*, *SRSF2*, *JAK2*
Chemotherapy	*TP53*, *PPM1D*, *DNMT3A*
Aplastic anemia	
Immune escape	*PIGA*, *BCOR*, *BCORL1*, *HLA class I allele loss/inactivation*
Autoimmunity + aging	*DNMT3A*, *ASXL1*, *RUNX1*, *TP53*, *CSMD2*
Immunosenescence	*DNMT3A*, *TET2*, *ASXL1*
Clonal cytopenias of undetermined significance	
Predictive value 0.88–0.97	*SF3B1*, *SRSF2*, *U2AF1*, *JAK2*, *RUNX1*
Predictive value 0.29–0.57	Sole mutations in *DNMT3A*, *TET2*, or *ASXL1*
Myelodysplastic syndrome	*SF3B1*, *TET2*, *ASXL1*, *SRSF2*, *DNMT3A*, *RUNX1*
Acute myeloid leukemia	*FLT3*, *NPM1*, *DNMT3A*, *IDH1/2*, *NRAS*, *KRAS*

niche to induce clonal expansion. Under these selective pressures, the prevalence of clonal hematopoiesis is high approaching nearly 50% [66••, 67]. In contrast to genotoxicity, the autoimmune marrow environment of aplastic anemia imposes distinct selective pressures favoring evolution of cells into two discrete clonal patterns.

Mutations in *PIGA* and *BCOR/BCORL1*, mutations of human leukocyte antigen (HLA) alleles, and copy number-neutral loss of heterozygosity of chromosome arm 6p (6p CN-LOH) constitute a group of somatic events that are highly specific to aplastic anemia [68]. Inactivating mutations in *PIGA* result in loss of glycosylphosphatidylinositol-anchored surface proteins critical for immune recognition (and represents the underlying lesion in paroxysmal nocturnal hemoglobinuria) [69]. It has been hypothesized that *PIGA* mutations confer a strong selective advantage within an autoimmune environment via means of immune escape [70]. Likewise, HLA allele mutations and acquired 6p CN-LOH events (which functionally delete one HLA allele locus) may mediate immune escape through loss of HLA class I molecules required for antigen presentation [71]. The mechanisms by which *BCOR/BCORL1* mutations achieve clonal dominance are not yet understood as mutations in these genes have very different prognostic associations depending on their context [72].

Mutations commonly seen in other myeloid-malignancy-associated genes like *DNMT3A* and *ASXL1*, two epigenetic modifiers frequently mutated in CHIP, comprise the second pattern of clonal hematopoiesis seen in aplastic anemia [66••]. The frequency of this pattern increases with age while immune escape changes do not. This mechanism of clonal selection may share a common origin with aging as both conditions expose the bone marrow niche to regenerative demand from HSC depletion. Limited number of residual HSCs could prime the marrow microenvironment to select for clones with a proliferative advantage in their quest to repopulate the hematopoietic pool. Surprisingly, a subset of mutations enriched in age-related clonal hematopoiesis is affected at much lower frequencies in aplastic anemia. For example, *TET2* and *JAK2* are commonly mutated in age-related clonal hematopoiesis but rarely seen in aplastic anemia [73]. Such discordance may represent shared selective pressures of autoimmunity and aging in the preferential selection for certain clones and deserves further investigation.

Immunosenescence As we age, HSC attrition contributes to changes in adaptive and innate immunity that are responsible for the increased susceptibility to infections, malignancies, and autoimmune disorders seen in the elderly [74–76]. This aging of the immune system is collectively referred to as immunosenescence. Senescent immune remodeling is believed to result in the phenomenon known as "inflammaging," a state of chronic, low-grade inflammation driven by proinflammatory cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor among others [77]. Upregulation of the inflammatory response that occurs with age has been shown to reduce HSC self-renewal potential and result in myeloid skewing [78, 79], suggesting immunosenescence likely plays a key role in HSC aging. Pharmacologic inhibition of the mechanistic target of rapamycin has been shown to attenuate HSC aging and may be an intriguing option to restore functional and regenerative capacity, thereby improving immune protection [80].

Senescent cells in the bone marrow niche produce proinflammatory cytokines that can lead to accelerated HSC aging [81]. Aberrant inflammatory signaling supports myeloid differentiation and establishes a positive feedback mechanism whereby myeloid skewed HSCs generate proinflammatory mediators to promote further myelopoiesis [82]. In this manner, age-associated inflammatory dysregulation of the bone marrow niche may disrupt the HSC pool and select for clones more adept at self-renewal. Such clones are favored through their ability to withstand chronic regenerative stress and escape immune surveillance from a senescent hematopoietic system. This may explain the high incidence of age-related clonal hematopoiesis defined by mutations in the epigenetic modifiers *DNMT3A* and *TET2*.

Chen et al. has shown that polyclonal myeloid-derived suppressor cells (MDSCs) are markedly expanded in the bone marrow of patients with lower-risk MDS [83]. MDSCs also accumulate with age and their frequency may be influenced by the inflammaging environment observed in older individuals [84], suggesting that senescence-dependent changes driving MDSC expansion may play an important role in clonal selection. MDSCs produce and secrete the alarmin S100A9, which functions to promote progenitor cell death and autocrine-reinforced MDSC activation [83]. Levels of S100A9 increase with age and trigger a feed-forward cycle that sustains chronic inflammation [85]. Binding of S100A9 to both CD33 and toll-like receptor 4 initiates NLRP3 inflammasome assembly, leading to nuclear factor-kappa B (NF-κB)-induced transcriptional priming and proinflammatory cytokine production, including interleukin-1β (IL-1β) and interleukin-18 [86••]. Additionally, S100A9 amplifies inflammatory cascades and itself activates NF-κB [87, 88]. Unexpectedly, generation of IL-1β appears to facilitate clonally induced atherogenesis, potentially explaining the clinical risk associated with clonal hematopoiesis.

Clonal Hematopoiesis: a Normal Consequence of Aging?

The presence of mutations in myeloid-malignancy-associated genes raises the important question of whether

CHIP explicitly represents a preleukemic state. Several seminal studies have shown CHIP to be associated with a small but increased risk of hematologic malignancies, analogous to other asymptomatic premalignant conditions such as monoclonal gammopathy of undetermined significance and monoclonal B-cell lymphocytosis [46, 47]. However, a closer look at these two population-based studies reveals the risk of developing a hematologic malignancy to be nearly negligible in individuals without antecedent candidate gene mutations. Of more than 12,000 individuals screened in both studies, approximately 0.5% of the participants developed a hematologic malignancy, of which only one third were found to have a candidate driver mutation. Malignancy risk was associated with clonal abundance suggesting that the size of the clone is more relevant than its mere presence.

The development of error-corrected sequencing has enabled researchers to detect somatic variants two orders of magnitude lower than standard next-generation sequencing (NGS). Young et al. applied this ultrasensitive technique to study the prevalence of clonal hematopoiesis in healthy middle-aged women [89••]. Remarkably, they observed clonal hematopoiesis, frequently harboring mutations in *DNMT3A* and *TET2*, in 95% of individuals studied. The clones were very small with a median VAF of 0.0024 and many remained stable over a 10-year period. As described earlier, Zink et al. employed a whole-genome approach to discover that more macroscopic clonal hematopoiesis is very common in the elderly [35••]. These findings challenge previous studies in which clonal hematopoiesis was identified in only 5% of middle-aged individuals [46–48] and suggests that clonal hematopoiesis is a near universal feature of aging and not necessarily a pathogenic state.

Its ubiquity with age has led to suggestions that clonal expansion might represent a compensatory mechanism that sustains normal hematopoiesis in advanced age when most HSCs have diminished proliferative potential. This is supported by work in murine models demonstrating that the two most commonly mutated genes in clonal hematopoiesis, *DNMT3A* and *TET2*, enhance HSC self-renewal [21, 90]. However, it is important to recognize mouse models do not necessarily recapitulate CHIP in humans. Phenotypic analysis of *TET2*-deficient mice was reminiscent of human chronic myelomonocytic leukemia [21]. The observation of a 115-year-old woman with a single clone carrying 450 somatic mutations that had supported normal blood production throughout her long life is an extreme example of how mutations can bestow HSCs with enough self-renewal capacity to prolong their lifespan [91]. Surprisingly, she never developed any malignant hematopoietic disorder and instead died from metastatic gastric cancer. Even with such a highly mutated clone, this case highlights there is much to be learned about the relationship between clonal hematopoiesis and risk for malignant transformation.

Clonal Cytopenias of Undetermined Significance

Clonal hematopoiesis in normal individuals with small clones is typically benign. But when more abundant candidate driver mutations are identified in individuals with cytopenias, the predicted probability of developing a myeloid malignancy increases markedly. Patients with unexplained cytopenias who do not meet the criteria for established myeloid neoplasms but who harbor somatic mutations indicative of clonal hematopoiesis have been described as having clonal cytopenias of undetermined significance (CCUS) [22, 92••]. Contrary to the name, a large prospective study of 683 patients led by Malcovati et al. has shown that individuals with CCUS have a 14-fold higher rate of malignant progression than those with no evidence of clonality, approaching 100% probability over 10 years [93••]. In this clinical context, clonal hematopoiesis appears prognostic for the development of MDS and AML. Larger clones with VAFs ≥ 10% and detection of ≥ 2 somatic driver mutations were associated with greater risk. Specific mutation profiles were noted to have varying predictive values for myeloid neoplasm. In particular, mutations in RNA spliceosome genes, *JAK2*, and *RUNX1* had high predictive values irrespective of co-occurring mutations whereas solitary mutations in common CHIP-associated genes like *DNMT3A*, *TET2*, and *ASXL1* showed lower risk of progression. This latter finding echoes earlier sentiments that presence of aging-associated mutations alone is inadequate for malignant progression. Regardless, the significance of clonal hematopoiesis in a cytopenic population is quite profound and should not be considered a manifestation of normal aging.

Clones and Cardiovascular Mortality

While the absolute risks of progression to cancer is low in healthy individuals, clonal hematopoiesis was unexpectedly found to be associated with an increased rate of cardiovascular mortality. The presence of CHIP nearly doubled the risk for coronary heart disease and was comparable to traditional atherosclerotic risk factors such as hypertension and dyslipidemia [94••]. Two recent studies elegantly demonstrated that atherosclerosis-prone mice engrafted with *TET2*-deficient cells had larger and more rapid plaque formation than did mice that had received non-mutated HSCs [94••, 95••]. Walsh et al. also identified enhanced NLRP3 inflammasome-mediated IL-1β production as a major mediator of atherosclerosis via endothelial cell activation and monocyte recruitment to plaques [95••]. Treatment with a specific NLRP3 inhibitor was able to completely abrogate IL-1β secretion in LPS/IFN-γ/ATP-primed *TET2*-deficient macrophages. The finding that *TET2* deficiency positively regulates atherogenic inflammation

suggests that clonal hematopoiesis and cardiovascular disease may share pathophysiologic consequences of aging.

CHIP may well be a critical mediator driving the inflammatory hypothesis of atherosclerosis. Canakinumab, a humanized monoclonal antibody against IL-1β, was shown to independently lower the rate of recurrent cardiovascular events in the CANTOS trial [96]. It would be keen to determine whether the individuals in this trial had CHIP and if canakinumab was able to influence clone size alongside clinical outcomes. In this regard, clonal hematopoiesis may be a modifiable risk factor for atherosclerosis and other age-related diseases. For now, no specific management guidelines exist for patients found to have CHIP, many of whom are diagnosed incidentally. Given what we currently know, it seems prudent to advise age-appropriate screening for coronary heart disease in this select cohort.

Conclusion

Clonal hematopoiesis was initially postulated to be an infrequent condition that exhibited clear age dependency. Technological advances in NGS and bioinformatics have shown us that somatic mutations in genes associated with hematologic malignancies are almost universally detected in the blood of otherwise healthy middle-aged individuals. Cell-intrinsic mechanisms of HSC aging contribute to clonal dominance as a result of HSC senescence and depletion that arise from failure to maintain genetic and transcriptional fidelity during cell division. Extrinsic influences such as cytotoxic chemotherapy and autoimmunity can exploit the vulnerabilities of aging hematopoiesis and alter the bone marrow niche to provide selective advantages that nurture specific mutant clones. Normal aging appears to exert selective pressures favoring clones with enhanced self-renewal. Perhaps clonal hematopoiesis represents a double-edged sword that simultaneously poses health risks but also sustains hematopoiesis in a manner compatible with modern increases in human longevity.

The current era of precision medicine has made molecular diagnostics a routine part of cancer care. With increasing integration of genomic testing into conventional clinical care, we recognize the need for greater awareness and evidence-based guidelines to inform best practices. However, several important questions will need to be explored before broad screening for CHIP can be implemented. It remains to be seen if normal aging processes can accurately mimic the more stringent cell-extrinsic stressors that regulate HSC fate in pathological conditions associated with abnormal aging. Less severe exposures like smoking, infection, chronic inflammation, immunomodulatory drugs, and diet may modify the risk or timing of clonal hematopoiesis to alter one's predisposition for disease and mortality. But for now, the clinical implications of CHIP remain unclear and must be interpreted with caution as we continue to discover more about its natural history. A better understanding of clonal HSC contribution in aging hematopoiesis will help us identify key drivers of clonality and separate them from their consequences to better facilitate healthy aging.

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Compliance with Ethical Standards

Conflict of Interest Soo J. Park declares that she has no conflict of interest. Rafael Bejar has received research funding from Celgene and Takeda and served as an advisor to Celgene, Genoptix, AbbVie, and Astex.

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