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INVITED PERSPECTIVE

Biological implications of clonal hematopoiesis

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Adult hematological malignancies, such as acute myeloid leukemia, are thought to arise through the gradual acquisition of oncogenic mutations within long-lived hematopoietic stem cells (HSCs). Genomic analysis of peripheral blood DNA has recently identified leukemia-associated genetic mutations within otherwise healthy individuals, an observation that is strongly associated with age. These genetic mutations are often found at high frequency, suggesting dominance of a mutant HSC clone. Expansion of clones carrying other mutations not associated with leukemia or larger chromosomal deletions was also observed. This clinical observation has been termed clonal hematopoiesis, a condition associated with increased risk of both hematological malignancy and cardiovascular disease. Here, we discuss the identification of clonal hematopoiesis and its implications on human health, based on the May 2019 International Society for Experimental Hematology New Investigator Committee Webinar. Published by Elsevier Inc. on behalf of ISEH – Society for Hematology and Stem Cells.

Blood formation, or hematopoiesis, is essential for human health [1]. The blood and immune systems are responsible for providing the body with oxygen and nutrients, supporting wound healing, and fighting pathogens. These functions are carried out by the various cell types that constitute the hematopoietic system: red blood cells; platelets; innate immune cells (neutrophils, monocytes, etc.); and adaptive immune cells (T cells, B cells). To maintain hematopoietic system homeostasis, new blood cells must be constantly produced. Hematopoietic stem and progenitor cells (HSPCs) are responsible for maintaining homeostasis [1–3].

Within the HSPC compartment, multipotent hematopoietic stem cells (HSCs) are thought to progressively differentiate into lineage-restricted hematopoietic progenitor cells (HPCs), which act as transiently amplifying cells that generate the vast numbers of blood cells required to sustain the hematopoietic system [2,4]. Besides multipotency, HSCs also possess self-renewal capacity, the ability to multiply without differentiation [2,4]. Self-renewal and differentiation are highly regulated to sustain hematopoietic system homeostasis throughout life. In adults, HSCs (and HPCs) reside in the bone marrow, which represents a specialized microenvironment for hematopoiesis [5,6]. At steady state, HSCs are largely quiescent or dormant, but can enter the cell cycle in response to hematopoietic stress. As HSCs are a long-lived cell type, DNA mutations acquired by HSCs are propagated throughout the hematopoietic system by differentiation and across the HSC compartment through self-renewal [7,8].

Adult hematological malignancies, such as acute myeloid leukemia (AML), are thought to arise through the gradual acquisition of genetic mutations within long-lived HSCs [7–9]. Consistent with this idea, genomic analysis of peripheral blood DNA has recently identified leukemia-

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associated genetic mutations within otherwise healthy individuals, an observation that is strongly associated with age [10–12]. Expansion of clones carrying other mutations not classically associated with leukemia, as well as larger chromosomal deletions, were also observed. This phenomenon is known as clonal hematopoiesis (CH) and represents a risk factor for AML. Interestingly, the development of clonality in the hematopoietic system also increases risk of other diseases, notably atherosclerosis [10,13].

The biology and implications of CH were recently discussed in the May 2019 International Society for Experimental Hematology New Investigator Webinar, presented by Dr Sidd Jaiswal and Dr Liran Shlush and moderated by Dr Isabel Beerman. In their presentations, Dr. Jaiswal started by discussing the prevalence, mutational spectrum, and implications of CH of hematological malignancies and cardiovascular diseases. Dr Shlush then discussed the process of clonal evolution from CH to AML, and the challenges in separating CH from pre-AML and in predicting which individuals with CH will eventually develop AML. Here, we summarize the current state of understanding in CH and discuss the ideas presented in this Webinar, which can also be viewed online (https://www.iseh.org/news/451201/Webinar-Recording-Now-Available-Clonal-Implications.htm).

Identifying and defining clonal hematopoiesis

Before discussing the associations and implications of CH, it is important to understand how this clinical observation is defined. Given that CH is identified from allele frequencies, it is also worth remembering that its detection is dependent on the technology used. CH was initially inferred from the identification of skewing in X-inactivation [14] and, more recently, from next-generation sequencing (NGS) analysis. Importantly, the accuracy of detection by NGS depends on the depth of the sequencing, with higher depths allowing increased detection of lower-frequency alleles.

The prevalence and health implications of CH were initially identified from whole-exome sequencing (WES) association studies [10–12]. These initial studies were limited to detecting CH at a variant allele frequency (VAF) >3%. Shallower sequencing coverage, for example, in whole-genome sequencing, can also identify CH, but only where the VAF is >7% [15]. More recent targeted sequencing [16,17] and error-corrected sequencing [18] approaches have allowed identification of CH with VAFs of just >1% and >0.01%, respectively. Unsurprisingly, the prevalence of CH changes with the assay sensitivity and mutant allele frequency cutoff.

With use of a VAF cutoff >0.01%, CH is essentially ubiquitous within the human population from middle age onward [18] and, therefore, lacks prognostic value. To develop a clinically relevant definition, CH of indeterminant potential (CHIP) was defined as the detection of genetic mutations in leukemia-associated genes within the peripheral blood with a VAF >2% [19]. This includes the most common genetic mutations in CH (*DNMT3A*, *TET2*, and *ASXL1*) [10–12]. CHIP therefore represents a subset of cases of CH.

Clonal hematopoiesis during aging

Aging is associated with increased mutational burden in many cell types, and although HSCs are protected by cell intrinsic properties (such as efflux activity and low metabolic rate) and are largely quiescent, these cells also accumulate DNA damage with age [20,21]. Human HSPCs are estimated to develop 1.3 ± 0.2 exonic mutations per decade of life, and thus it can be estimated that by age 50, an individual would accumulate an average of five coding mutations within each HSPC [22]. The gradual acquisition of mutations in HSPCs with aging has also been observed by two other studies and allowed inferences about lineage relationships and population dynamics in human hematopoiesis [23,24]. Although most of these mutations will likely be neutral, a mutation driving either a selective advantage or disadvantage of an HSPC clone will be disproportionally represented and could drive CH.

The association between CH and aging has long been known, with early evidence for age-related CH coming from studies of X-chromosome inactivation throughout age [14]. The presence of somatic mutations in *TET2* in individuals without hematological disease was also observed in a small cohort of elderly individuals [25]. With the advent of more accessible high-throughput sequencing, several groups used large-cohort studies to identify the accumulation of specific mutations in the blood of healthy aged individuals at surprisingly high prevalence and, as discussed later, a strong association with adverse outcomes [9–12]. Although the most common mutations are in epigenetic regulators *DNMT3A, TET2*, and *ASXL1*, mutations were also found in genes regulating DNA damage (*TP53, PPM1D*), RNA splicing (*SRSF2, SF3B1*), signaling (*JAK2*), and other epigenetic regulators (*BCORL1*). Interestingly, although murine models harboring mutations in *Tet2* and *Dmnt3a* reproduce many phenotypes of CH [26,27], these mutations have not been reported as common in aged mouse blood.

Although CH does not appear to alter hematopoiesis, aging itself is associated with changes in hematopoiesis [21]. This includes alterations in lineage composition, decreased regenerative potential, and significant increases in myelogenous disease. Aging is associated with increased red cell distribution width (RDW), and this increased heterogeneity of red cell size is also associated with increased mortality [28]. When increased RDW was combined with the presence of mutations associated with CH, there was an even greater exacerbation of the hazard ratio [10,29].

Taken together, these two age-associated phenotypes may be hallmarks of potentially negative clinical outcomes.

Clonal hematopoiesis and hematological diseases

CH presents in healthy individuals and is not considered a disease state. However, it is a significant risk factor for future hematological malignancies such as AML [10–12] and is therefore often considered a preleukemic state. Even before the identification of CH from sequencing studies, preleukemic HSCs had been identified in cases of AML [7,9]. From clonal analysis, preleukemic HSCs were identified as a subset of HSCs that contained only one (or a subset) of the genetic mutations seen in the leukemic blasts [7,9]. This provided the basis for the clonal evolution model of AML from HSCs. In comparison with other preleukemic states such as myelodysplastic syndrome (MDS), CH tends to have only a single detectable genetic mutation, while multiple genetic mutations are observed in the peripheral blood of MDS patients [19,29].

It is worth remembering that CH is only a risk factor for AML, and just a fraction (~10%) of CH cases will progress to AML. In part this may be due to the presentation of CH in the elderly, in whom other diseases may cause mortality before CH progresses to a hematological disease. Through analysis of very large patient cohorts, it has been possible to further stratify CH in terms of AML risk. For example, larger clone sizes increase the risk of AML [16]. Additionally, certain genetic mutations are stronger predictors of AML progression, including *SRSF2, TP53*, and *U2AF1* [16]. *RUNX1, IDH1*, and *IDH2* mutations were similarly predictive, although they exhibited longer latency. Interestingly, although most abundant, *DNMT3A* and *TET2* mutations were weaker predictors of AML [16].

Analysis of genetic mutations associated with CH in murine models have suggested that a number of mutations are associated with enhanced HSC self-renewal or larger HSC pools, such as *TET2* [26], *DNMT3A* [27], and *ASXL1* [30]. However, the mechanism for other recurrent mutations is currently less clear. Regardless of the exact molecular mechanism, it appears mutations arise in HSCs that clonally expand and eventually give rise to a significant fraction of the peripheral blood. Evidence for the acquisition of these "early" mutations within functional HSCs comes from the identification of the same leukemic mutations within T cells in patients with AML [9]. These results suggest that these genetic mutations do not inhibit HSC multilineage potential. By contrast, "later" mutations such as those in *NPM1, FLT3*, and *CEBPA* are observed only in the myeloid leukemic blasts and are not seen in the HSC compartment or lymphoid lineages, implicating a role in driving preleukemic HSC differentiation or their acquisition in a downstream myeloid cell type [8,9].

Clonal hematopoiesis and inflammatory diseases

A strong correlation between CH and reduced overall survival was clear in the initial WES association studies [10–12]. However, hematological malignancies alone could not account for this reduced survival. Instead, the cause of death was associated with cardiovascular disease, with CH carriers having a nearly doubled risk of coronary heart disease and a four-times-greater risk of myocardial infarction [13]. Of note, the three most common genes in CH (*DNMT3A, TET2*, and *AXSL1*) confer similar risk to develop coronary heart disease, a risk that also correlates with clone size [13].

Atherosclerosis is largely a disease of chronic inflammation, which initiates with elevated levels of low-density lipoprotein (LDL) cholesterol or other atherogenic glycoproteins in the peripheral blood, causing damage in the vascular endothelium. A damaged endothelium leads to the recruitment of monocytes (and other immune cells) to those areas, which then enter the vascular wall, differentiate into macrophages, and become inflammatory. Increased secretion of pro-inflammatory cytokines and chemokines by these macrophages subsequently leads to the recruitment of more monocytes/macrophages, resulting in the formation of atherosclerotic plaques [31].

To understand why individuals with CH have a higher risk of cardiovascular disease, two independent studies investigated the effect of *TET2* mutation in atherosclerosis [13,32]. These studies used a bone marrow transplantation strategy to generate an atherosclerosis-prone LDL receptor (*Ldlr*) gene-deficient mouse with *Tet2*-induced CH. $Ldlr^{-/-}$ mice fed a high-cholesterol diet and transplanted with *Tet2*-deficient bone marrow cells developed larger atherosclerotic lesions, comparing with mice receiving wild-type bone marrow, despite having overall normal blood cell parameters. Importantly, gene expression analysis of wild-type and *Tet2*-deficient macrophages treated with LDL/cholesterol [13] or LPS/IFNg [32] revealed increased expression genes associated with inflammation. These included known pro-inflammatory cytokine genes, such as *Il1b* and *Il6*, and other chemokine genes, such as *Cxcl1, Cxcl2,* and *Cxcl3*. Similar inflammatory activation has also been observed in human patient-derived *TET2*-mutant macrophages [33]. Together these studies indicate that *Tet2* deficiency accelerates atherosclerosis by generating a pool of macrophages with increased pro-inflammatory and subsequently increased pro-atherosclerotic activity.

Future directions

From association studies to mechanistic studies

Although genetic association and longitudinal studies using large human cohorts have been maximized to uncover the prevalence and clinical implications of CH, mechanistic studies have been more difficult. As

described above, a mechanistic basis for the association between *Tet2* mutations and atherosclerosis has been recently identified using elegant mouse models [13,32]. However, there are still open questions regarding the interactions between CH and inflammation during aging, particularly for other recurrent mutations. Additionally, although certain recurrent mutations in CH, such as *Tet2*, cause enhanced HSC self-renewal in mice models [26,34], the mechanisms underlying other recurrent mutations have not yet been described. While mice models represent a useful and tractable model system to study hematopoiesis, the functions of mouse and human genes are not always fully conserved. Further, mice live only ~2.5 years, limiting the study of slow-developing CH. Additional models are therefore necessary, such as nonhuman primate models, to study CH in longer-lived and more representative models.

Factors influencing clonal outgrowth

It will also be important to understand not only the intrinsic consequences of these recurrent mutations in HSCs, but also the role of the aging bone marrow niche on HSC clones and clonal expansion. Several alterations in the composition of aged bone marrow occur, including a significant increase in adipocyte frequency and senescence of mesenchymal stem cells. It is unknown if or how the changes in the niche environment could affect CH but these cannot be excluded as potential factors that influence the clonal selection of HSPCs.

Clinical implications of clonal hematopoiesis

As a state that does not alter hematopoietic parameters, CH is currently identified through NGS assays, which are still not routinely performed in the clinic. Additionally, when very low VAF cutoffs are used (0.01%), CH is essentially ubiquitous from middle age onward; its prognostic relevance is not clear. However, the recent demonstration that certain clone sizes and genetic mutations, such as those within *SRSF2, TP53*, and *U2AF1*, are more strongly associated with leukemic progression [16] suggest that screening and preventative treatment options should be considered in certain cases.

Conclusions

Although the first suggestions of age-related CH were reported more than 20 years ago, it is only in the last 5 years that the clinical implications of CH have become appreciated. This recent progress has been driven by technological advances, particularly NGS, and large-cohort genomic association studies that have become affordable because of reduced sequencing costs. Since the discovery that CH is a risk factor for hematological and cardiovascular diseases, there has been intense research interest in this predisease state. We look forward to the next steps in this rapidly progressing field, particularly efforts to understand the underlying mechanisms, and progress toward the application of our knowledge to develop clinical strategies to prevent hematological and cardiovascular diseases.

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