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Human hematopoiesis: aging and leukemogenic risk

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Abstract

Purpose of review: Our understanding of the effects of aging on human hematopoiesis has advanced significantly in recent years, yet the full implications of these findings are not yet fully understood. This review summarizes these findings and discusses their implication as they relate to malignant hematopoiesis.

Recent findings: With human aging there is an impaired immune response, loss of HSC function, increase in clonal hematopoiesis and higher frequency of myeloid malignancies. While murine models have implicated abnormalities in DNA damage repair, autophagy, metabolism and epigenetics, studies in primary human specimens are more limited. The development of age-related clonal hematopoiesis and the risk associated with this is one of the major findings in the field of recent years. This is accompanied by changes in bone marrow stem and progenitor composition, changes in the epigenetic program of stem cells and an inflammatory milieu in the bone marrow. The precise consequences of these changes for the development of age-related malignancies are still unclear.

Summary: Advances in the field have begun to reveal the mechanisms driving human HSC loss of function with age. It will be critical to delineate between normal and malignant aging in order to better prevent age-associated myeloid malignancies.

Keywords

Aging; human hematopoiesis; clonal hematopoiesis; hematopoietic stem cells; HSC

Introduction

From childhood acute lymphoid leukemias to myeloid neoplasms in elderly individuals, many leukemias have a strong association with age. Yet, diseases such as Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML) remain relatively rare, with only about 20 in 100,000 people getting these diseases (1). These differences in incidence raise questions, such as why does aging predispose for specific forms of leukemia? Why don't age-related leukemias occur at higher frequency? And what are the differences between

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normal hematopoietic aging and malignant hematopoiesis? In this review we seek to review how much progress the field of human hematopoiesis has made in understanding normal aging and how this compares and predisposes for malignant disorders of the aged hematopoietic system.

The Aged Hemopoietic System

Impaired Immunity with normal aging—Aging is defined as progressive decline leading to an increased likelihood of death. In the hematopoietic system, aging is accompanied by alterations in the various lineages of the hematopoietic compartment. Among the functional consequences of this decline is an impaired immune response referred to as immunosenescence. The adaptive immune response is impaired to a greater degree than the innate response, due in part to altered production and function of lymphocytes with age. Thus, older individuals do not respond as vigorously to immune challenges as their younger counterparts. With age, there is an increase in the frequency of bacterial infections from species such as *Mycobacterium tuberculosis* and *Streptococcus pneumonia* and an increase in susceptibility to viral infections such as *herpes zoster* and influenza (2–6). Furthermore, the mortality rate for some of these infections is 2–3 times higher in the elderly compared to younger individuals (2–6). Prevention of infection in older people is further complicated because the elderly have a reduced response to vaccination (7).

Alteration of both the T- and B-cell lineages is observed with aging. In addition to normal thymus involution, which in humans is completed by puberty, aged T cells present several changes compared to their young counterpart. By measuring T-cell receptor beta (TCRB) sequences, *Qi et al.* reported a 2–5-fold decrease in the naïve T-cell repertoire with aging (8). Furthermore, aging was accompanied by an oligoclonal expansion of the naïve and memory CD8+ populations (8–10). This reduced diversity within the T-cell pool may limit an aged individual's response to newly encountered viruses. Like CD8+ cells, B-cells also show an increase in clonality with aging (11). B-cells isolated from human peripheral blood of aged donors show reduced class-switch recombination compared to those from younger donors (12). Additionally, antibodies produced by B-cells from elderly individuals have reduced specificity and affinity for their target antigens (12,13). Taken together, impairments in lymphocyte function contribute to loss of adaptive immunity with age. Finally, while multiple murine studies have shown that immunosenescence is also evident in the impairment of macrophage function, including impaired cytokine and chemokine production, and reduced generation of oxidative radicals and reactive oxygen intermediates comparable data in human macrophages is lacking (14–21).

Cytopenias and clonal hematopoiesis—With age, there is an increase in the rate of isolated cytopenias. Specifically, aged individuals have increased rates of anemia. *Guralnik et al.* analyzed data from 39,695 Americans and determined that 10% of men and 11% of women over 65 years old are anemic, with the frequency increasing to greater than 20% in people over 85 years old (22). Notably, while many of the incidences of anemia in the elderly are due to nutritional deficiency, up to 33% of the cases have unknown causes (22,23). Currently, cytopenias of unknown causes (such as anemia) that cannot be classified as a hematological disorder or myelodysplastic syndrome (MDS) are categorized as

idiopathic cytopenia of undetermined significance or ICUS (24). A recent prospective study by *Malcovati et al.* profiled 154 patients with ICUS (median age=53) and found that 25% went on to develop a myeloid neoplasm (25). Analysis of somatic mutations in these individuals revealed that 36% of patients with isolated cytopenias carry one or more mutations, and can therefore be better classified as having clonal cytopenia of undetermined significance or CCUS (24,26). The epigenetic modifiers *TET2*, *ASXL1*, and *DNMT3a* were some of the genes most frequently mutated in these individuals with CCUS (median age=68), who tended to be older than those with ICUS alone (25).

A number of recent studies have shown that this phenotype of clonal hematopoiesis, in which one progenitor gives rise to a disproportionately high number of mature cells, is quite frequent with normal aging (27–31). *Jaiswal et al.*, used whole exome sequencing on peripheral blood DNA from 17,182 individuals ranging from 20–108 years old and found a low incidence of mutations in people <40 years old, with an increase in the frequency of mutations with age. Amongst individuals aged 70–79, 9.5% had a clonal mutation, with the majority of variants occurring in *DNMT3A*, *TET2*, and *ASXL1* (28). These findings have been recapitulated in a number of other studies, and the phenomena dubbed as age related clonal hematopoiesis (ARCH) or clonal hematopoiesis of indeterminate potential (CHIP) (27–32). It is postulated that the incidence of CHIP is even higher than initially reported due to the moderate sequencing depths of the initial studies, and could be greater than 50% by the age of 85 depending on both the sequencing platform and the analytical approach applied (27,30,31,33). While individuals with CHIP do not have any diagnosable hematological ailment, they have 11 times the risk of developing a hematological cancer, although the rate of progression is low (0.5–1% per year) (28). However, people with CHIP have twice the risk of developing coronary heart disease compared to those who do not have CHIP (29). This may be attributable to increased inflammation and loss of immune effector cell function, as CHIP mutations are found in mature granulocytes and lymphocytes (34). Thus, while CHIP is fairly common with age, and does not pose an immediate hematologic risk, it is still of clinical significance and it is recommended that these individuals be monitored more closely.

Myeloid malignancies in aged individuals—Advanced age is associated with an increased incidence of myeloid malignancies. Whereas more than 50% of acute lymphoid leukemias are seen in children and young adults, acute myeloid leukemia (AML) is the most common acute leukemia in adults, and the average age at diagnosis for AML patients is 68 years old (1,35). Cytogenetic abnormalities and mutations in *NRAS*, *FLT3*, *TP53*, and the epigenetic modifiers *DNMT3A*, *TET2*, *ASXL1*, and *IDH1/2* are frequent in AML. Chemotherapy is the standard treatment for AML, however older individuals have higher rates of treatment-related mortality and a poorer prognosis compared to people diagnosed at a younger age (36). Another age-related myeloid malignancy is myelodysplastic syndromes (MDS). In the United States, the incidence of MDS in people under 40 years old is 1 case per 100,000 individuals, but this rate increases to 20 in 100,000 in people aged 70–79 years old (37). Ultimately, this disease progresses to bone marrow failure or AML. Mutations in over 40 genes have been observed in MDS, with the spliceosome components *SRSF2,*

SF3B1, and *U2AF1*, and epigenetic modifiers *DNMT3A*, *TET2, ASXL1* being the most often mutated (38).

Importantly, genes that are mutated with normal aging in individuals with CHIP are frequently mutated in MDS and AML, and the overwhelming frequency of mutations in DNMT3A and TET2 in CHIP are not compatible with a model of random mutations across the genome with aging, a fact that is being studied in depth by multiple groups. Moreover, the mutations themselves are not always those seen in myeloid malignancies, such as those in *DNMT3A* for which the majority (83%) of mutations in CHIP do not occur at the R882 residue as most frequently seen in AML (28), indicating possibly a different potential for malignant progression of the different CHIP mutations. Mutations associated with CHIP likely provide a selective advantage for aged hematopoietic stem cells (HSC), aiding them in surviving amongst the increased genotoxic stress and aged bone marrow milieu. Modeling of the *Dnmt3a* and *Tet2* mutations in mice has shown that these mutations led to an expansion of the stem cell pool and in the case of *Tet2*, a block in differentiation (39–41). Yet given that clonal hematopoiesis seems almost an inevitable feature of aging, it is clearly not the sole age-associated factor contributing the development of myeloid malignancies.

Aged HSC and LSC

Loss of HSC function with age—Much of what is known about HSC aging has been derived from murine studies, and while strain-to-strain variations have been observed, it is clear that aged murine HSC have altered reconstitution and lineage potential (42). Even though the frequency of HSC in the bone marrow increases with age (43,44), their function declines. Aged HSC have decreased homing ability with increased self-renewal under transplant conditions, impairing their ability to differentiate and reconstitute the marrow long-term (43,45) (46–48). Notably, while the ability of lymphoid biased HSCs (Ly-HSCs) or myeloid-based (My-HSCs) to generate their respective linages does not change with age, the composition of the HSC pool does. With age, there is an increase in the frequency of My-HSCs, and over a 50-fold increase in platelet biased HSCs, with a decrease in Ly-HSCs and Bal-HSCs (49–51). However, total numbers of Ly-HSC may not be reduced with age (52). Intriguingly, the more times that an HSC has divided, the less multipotent and more skewed towards myeloid differentiation it becomes, and aged Ly-HSC have an increase in myeloid gene expression, suggesting that epigenetic alterations may be at play in HSCs becoming less potent with age (53,54).

Whether human HSCs have the same functional impairments with age is unclear. HSCs from aged donors have reduced transplantation success in bone marrow transplants, suggesting there is loss of function with age (55). Similar to what has been observed in mice, several groups have shown that there is an increase in HSC frequency in human bone marrow with age (56–58). However, xenotransplant studies of young and aged human HSC into NSG immunocompromised mice and *in vitro* experiments have generated conflicting results, that are not always in concordance with the aged murine HSC phenotype. Analysis of bone marrow from xenografts has shown both an age-associated increase in the myeloid/lymphoid ratio from mice transplanted with HSC (Lin^-, $CD34^+$, $CD38^-$, $CD90^+$, $CD45RA^-$) as well as a decreased myeloid output from a less purified aged ($CD34^+$, $CD38^-$) cell type.

Similarly, contradictory findings using stromal co-culture experiments have been observed. Using a very pure Lin^{-}, $CD34^{+}$, $CD38^{-}$, $CD90^{+}$, $CD45RA^{-}$ population with AC6.2.1 stromal cells, *Pang et al.* demonstrated a significant myeloid bias with age, while *Nilsson et al.* found no significant difference when culturing highly purified hemopoietic Lin^{-}, $CD34^{+}$, $CD90^{+}$, $CD45RA^{-}$,$CD123^{low}$ with MS5 stroma cells (56–58). Unexpectedly, no decrease in HSC engraftment or donor chimerism was observed in transplanted aged human HSC compared to young (56,57). Additionally, in contrast to murine HSC, no significant difference has been observed in colony forming ability *in vitro* with human HSC aging (56).

Numerous intrinsic alterations contribute to murine HSC loss of function. These changes are associated with altered metabolism, decreased activation of autophagy, impaired proteostasis, accumulation of DNA damage, loss of polarity, and epigenetic remodeling. Due to the technical limitation of working with human samples, it is unknown if many of these phenotypes are maintained in human HSC. However, much has been learned about how epigenetic alterations contribute to aged HSC loss of function, which will be later discussed.

Aged HSC versus LSC: Like their normal counterparts, Leukemic Stem Cells (LSC) are highly quiescent cells capable of self-renewal and long-term engraftment in immunodeficient mice (59,60). These malignant progenitors have been observed in both the age-associated malignancies MDS and AML (59–63). Given the therapeutic challenges for treatment of these diseases, it is beneficial to delineate between normal aged HSC and LSC biology.

Like HSC, the majority of LSC express the CD34 antigen and lack CD38. Using flow cytometry to define the stem cell compartment as Lin-, CD34+, CD38−, $CD90^{+}$, $CD45RA^{-}$, *Pang et al.* did not find any difference in HSC frequency in low-risk MDS compared to age-matched controls. Moreover, by tracing the MDS monosomy 7 abnormality within the HSC compartment to distinguish malignant from normal stem cells, they demonstrated that LSC expand at expense to the normal HSC. In contrast to low-risk MDS, in high-risk MDS, *Will et al.* demonstrated that there is an increased frequency of LSC compared to aged-matched controls. In addition, altered frequencies of CMP, GMP, and MEP have also been observed, contributing to the idea that other progenitors contribute to MDS pathogenesis (61,63).

Perhaps the most important time at which a distinction needs to be made between LSC and normal aged HSC is after completion of therapy for malignant disorders, when a determination of persistent disease vs. complete remission needs to be made. Since many of the mutations in clonal hematopoiesis and those seen in myeloid malignancies are the same, a distinction between minimal residual disease and reversal to clonal hematopoiesis is of the utmost importance since it would signify the difference between requiring further treatment or not. While prior knowledge of the existence of pre-leukemic clonal hematopoiesis would be ideal in order to make this distinction, this is not always available. In the latter scenario, the presence of mutations frequently seen in clonal hematopoiesis along with the absence of overt leukemia should be evaluated as a possible return to clonal hematopoiesis and closely monitored before a decision to move forward with additional therapy is made.

Epigenetic changes with aging vs leukemia

HSC epigenetic changes with aging—Given that epigenetic modifiers are mutated with aging and that aberrant modification of cytosine bases and histone tails is found in age-related MDS and AML, a large focus of the field has been centered in understanding how epigenetic regulation becomes impaired with aging. Earlier studies of DNA methylation (mC) in aged human hematopoietic cells showed defined methylation changes in bulk mononuclear cells (MNC) and hypomethylation of differentiation associated genes in CD34+ cells (64,65). Recently, our group assessed mC in a more purified population of HSC enriched (HSCe) CD34+, CD38−, Lineage- cells and found focal changes that develop with aging at genes associated with WNT, cadherin, and cell-adhesion pathways. These changes included both gains and losses of mC (66). Similarly, methylation changes have also been observed in murine long-term HSC (LT-HSC) (67,68). Notably, *Soraas et al.,* who measured mC using a multi-tissue “epigenetic-clock” in patients following allogeneic HSC transplant from donors of different ages, found that at least some of these epigenetic alterations may be cell intrinsic (69).

In contrast to mC, more widespread histone modification alterations have been observed with human HSC aging. Using single-cell mass spectrometry, *Cheung et al* demonstrated that many chromatin marks increase with HSC aging, including H3K4me3, H3K36me3, and H3K27me3 while there is reduced H3K27ac and H3K9ac with aging (70). Our group studied the activating histone modifications H3K4me1, H3K4me3, and H3K27ac along with the repressive mark H3K27me3, in human HSCe with aging using chromatin immunoprecipitation with sequencing (ChIP-seq) and identified thousands of focal histone modification alterations with HSCe aging. Prominent amongst these changes was a switch from bivalency to repression at promoters associated with WNT signaling and developmental genes. Additionally, a reduction of H3K27ac was observed at active promoters and enhancers associated with immune and cancer related pathways supporting the idea that age-associated immune impairment and increased risk of myeloid leukemias may be epigenetically encoded at regulatory regions at the stem cell level. Importantly, aged HSCe alterations are accompanied by transcriptional deregulation of hematopoietic transcription factors such as *KLF6*, *BCL6*, *RUNX3* and *HIF1A* as well as epigenetic modifiers, including *KAT7*, *SETD1A*, and *KDM2A* (66). Notably, work by *Khokhar et al.* and *Keenan et al.* has shown that downregulation of the histone acetyltransferase *Kat6b* or the histone methyltransferases *Suv39h1* and *Suv39h2,* respectively, in mice, results in aging phenotypes such as increased myeloid differentiation (71,72) Furthermore, one of the genes most downregulated in both human and murine HSCe aging is the nuclear lamina associated gene, LMNA, a gene involved in the nuclear organization of heterochromatin and which when mutated results in a form of progeria (73)(66). However, whether age-related downregulation of LMNA contributes to altered chromatin accessibility or wide scale chromatin remodeling with age is unknown.

LSC epigenetic changes compared to aging—Given the technical limitations, the epigenome of human LSC has not been characterized in depth. However, studies using bulk populations of cells have shown that aberrant epigenetic modifications are recurrently observed in MDS and AML. Leukemic blasts have vast alteration of DNA methylation

compared to normal hematopoietic cells (74–77). Furthermore, clinically relevant subsets of AML patients can be identified solely based on their methylation profile(76). Initial studies of DNA methylation utilized promoter-based assays and found hypermethylation of promoters, including those involved in WNT signaling (75–77). However, a recent study that utilized a more genome-wide approach, found that gene neighborhood (region 2–50kb from the TSS or TES) methylation predicted AML epigenetic subtype better than promoter methylation, and that there is significant hypomethylation of enhancers (78). Thus, DNA methylation abnormalities may lead to activation of enhancer elements and their target genes in AML. In addition to the extensive methylation alterations seen in MDS, AML, and CMML, mutations of DNMT3A, TET2, and IDH1/2, enzymes in the DNA methylation pathways, are frequently found in patients with these diseases (79–86).

While less is known about how histone modifications are altered in primary myeloid leukemias, enhancer deregulation is an emerging feature of myeloid neoplasms. For example, in patients with inv(3)/t(3;3) AML, chromosomal rearrangement of the *GATA2* enhancer causes an enhancer translocation and the abnormal expression of the proto-oncogene *EVI1*, and downregulation of *GATA2* (87,88). Additionally, enhancers and chromatin accessibility can be used to identify subsets of AML (89,90).

As epigenetic abnormalities are observed in both normal aging and age-dependent leukemias, identifying the epigenetic features that may predispose or contribute to malignancy will be of therapeutic potential. Using unsupervised clustering, regions with altered mC in both aged HSCe and AML blasts compared to young HSCe were identified. These sites were annotated to transcription factors such as *HOXC4/6*, *KLF6*, and *RUNX1*. Similarly, 4,582 enhancers have reduced H3K27ac in both aged HSCe and AML blasts. These enhancers were associated with cell adhesion, immune cell activation, and myeloid differentiation and included genes such as *MEIS1*, *HES1* and *RXRA (66)*. However, determining which of these many age-associated events actually predispose to leukemia, and how they may do so, has yet to been experimentally demonstrated.

Future Directions

While a lot of progress has been made in the last decade into better understanding functional and molecular changes that occur in the human hematopoietic system with aging, limitations in the experimental approaches have meant that the field has lagged behind progress made in model organisms. However, technical advances in recent years now allow for proteomic, metabolomic and genomic studies in highly purified populations and even at the single cell level. Likewise, advances in genome-editing technologies now allow for gene and epigenome editing of primary human hematopoietic cells. It is expected that these advances, paired with appropriate murine and xenograft models, will allow us to address many of the unanswered questions about aging human hematopoiesis. Questions about the role of metabolism changes in the aging phenotype, clonal competition and selection in the context of CHIP, or how the aging bone marrow microenvironment influences this process will all be at the forefront of research in upcoming years. Finally, the ultimate goal of the field is to translate these observations into potential interventions that can slow functional decline with aging and even prevent the development of malignant transformation.

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* Of special interest

** Of outstanding interest

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Key points:

- Normal aging results in HSC dysfunction, impaired immunity, isolated cytopenias, increased risk of hematopoietic malignancies.
- Clonal hematopoiesis and altered epigenetic programming increase in frequency with normal aging and may predispose to malignant transformation.
- Normal aged HSC and LSC share many features and distinguishing between the two has important clinical implications for follow-up of patients with malignancies.