Preleukemic stem cells: leave it or not?

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Abstract

Acute myeloid leukemia (AML) has been shown to undergo multiple acquired mutations in hematopoietic cell lineages over years before becoming clinically apparent. The early stage of AML (before it becomes clinically recognizable) may be characterized by acquisition of some, but not all, leukemia-related somatic mutations in hematopoietic stem cells (HSCs). The pathological roles of these mutations remain puzzling. These HSCs have been termed as preleukemic HSCs. However, those frequent acquired somatic mutations are also found in healthy aging adults, namely, “age-related clonal hematopoiesis.” Multiple studies have demonstrated that the preleukemic HSCs survive through chemotherapy and then contribute to the relapse and the development of de novo AML. Whether preleukemic HSCs should be targeted or whether a preventive therapy should be considered for those individuals remains to be determined. This article aims to shed light on this special subject and to discuss the roles of preleukemic HSCs in leukemogenesis.

Keywords: Acute myeloid leukemia, Age-related clonal hematopoiesis, Clonal evolution, Preleukemic stem cells

BACKGROUND

The term “preleukemia” was first used in the 1940s and 1950s to differentiate a preleukemic phase of acute leukemia from acute leukemia on the basis of clinical manifestation, peripheral blood cell count, and tissue studies.\textsuperscript{1–3} This preleukemic phase later came to be known as myelodysplastic syndrome (MDS),\textsuperscript{4–6} which is considered as a different entity from leukemia based on the present concept. The concept of preleukemia has undergone an evolution over the past decades due to technological advances, including research on X chromosome inactivation and the development of cytogenetics and deep nucleotide sequencing. Preleukemia might represent a condition in which a subpopulation of stem cells harbors some specific mutations but retains normal differentiation potential, which can eventually give rise to leukemia. These cells are called preleukemic hematopoietic stem cells or progenitor cells (pre-L-HSCs or pre-L-HSPCs) depending on the stringency of the stem cell definition.\textsuperscript{7–9} The preleukemic mutations are restricted not only in hematopoietic stem or progenitor cells but also in various mature cells such as B and T lymphocytes, mature monocytes, macrophages, and natural killer cells.\textsuperscript{9,10} The preleukemic mutations occur in the mature population either by gaining the mutations originally or by acquiring from the differentiation of the HSCs or HSPCs. If the preleukemic mutations happened in the mature blood cells population, in most of the cases they exhaust before the leukemia.\textsuperscript{11,12}

The full repertoire of recurrent mutations in leukemia can be uncovered on account of the increasing efficiency and decreasing cost of next-generation sequencing.\textsuperscript{13,14} Recurrent molecular genetic abnormalities such as DNA methyltransferase 3A (DNMT3A) and ten-eleven translocated 2 (TET2), additional sex comb-like (ASXL1), CAT enhancer binding protein Alpha (CEBPA), FMS-related tyrosine kinase 3 (FLT3), nucleophosmin (NPM1), runt-related transcription factor 1 (RUNX1), and isocitrate dehydrogenase 1/2 (IDH1/2)\textsuperscript{15} have been utilized for the risk stratification,\textsuperscript{16} prognostic evaluation,\textsuperscript{17,18} and also guidance of therapy.\textsuperscript{19} NPM1, RUNX1, FLT3-ITD, and IDH1/IDH2 could be used with monitoring minimal residual disease in AML by next-generation sequencing.\textsuperscript{20,21} However, there are still some equivocal recurrent gene mutations harbored in the clonal expansion of hematopoietic cells observed in normal aging individuals, which is termed as age-related clonal hematopoiesis (ARCH) (model A in Fig. 1). Clonal hematopoiesis with somatic mutations has been observed in 10% of persons older than 65 years of age, but in only 1% of those younger than 30 years of age.\textsuperscript{22}

It is becoming clear that evolution of human acute myeloid leukemia (AML) is a multistep process that gradually transforms a normal hematopoietic cell into a dysregulated self-renewing leukemic stem cell (LSC). Lapidot et al\textsuperscript{23} identified for the first time an AML-initiating cell by transplanting the CD34+CD38−subset cells of fresh bone marrow, fresh peripheral blood cells, or banked samples from AML patients into severe combined immune-deficient (SCID) mice. These leukemia-initiating cells result in a pattern of dissemination and leukemic cell morphology...
similar to those observed in the original patients and were immunophenotyped as CD34+CD38-. The authors therefore concluded that AML was initiated by HSCs that had undergone requisite steps. Later, Weissman\textsuperscript{24} proposed a hypothetical model for myeloid leukemia progression stressing that multiple independent genetic and epigenetic changes must occur to drive a normal HSC or hematopoietic progenitor cell to an LSC depending on a specific type of myeloid leukemia. Nonetheless, because the self-renewal feature is shared between tissue stem cells and cancer or tumor stem cells, normal stem cells must be directly or indirectly involved in cancer development.\textsuperscript{25} In fact, accumulating evidence has now indicated that there is a latent phase or preclinical phase in which there exist HSCs harboring some, but not all, of the leukemia-associated mutations before AML onset.\textsuperscript{26,27} However, the duration and sequential events of the progression from normal HSCs to leukemic cells are poorly understood.

**PRELEUKEMIC MUTATIONS**

The most prevalent preleukemic mutations lie in \textit{DNMT3A} and \textit{TET2} genes (Table 1), but intriguingly they are also of the

**Table 1**

| Gene name | Proportion of pre-AML cases with events (author/year) | Abelson et al (2018)\textsuperscript{43} | Evidence for preleukemia (author/year) |
|-----------|------------------------------------------------------|----------------------------------------|----------------------------------------|
| DNMT3A    | 37%                                                  | 37%                                    | Corces-Zimmerman (2014)\textsuperscript{50} |
| SRSF2     | 7%                                                   | 12%                                    | Hahn et al (2015)\textsuperscript{49} |
| TET2      | 25%                                                  | 24%                                    | Shlush et al (2014)\textsuperscript{9} |
| ASXL1     | 3%                                                   | 10%                                    | Abelson et al (2018)\textsuperscript{43} |
| U2AF1     | 4%                                                   | 7%                                     | Jan et al (2012)\textsuperscript{7} |
| SF3B1     | 6%                                                   | 2%                                     | Corces-Zimmerman (2014)\textsuperscript{50} |
| IDH1      | 2%                                                   | 2%                                     | Shlush et al (2014)\textsuperscript{9} |
| IDH2      | 6%                                                   | 4%                                     | Jan et al (2012)\textsuperscript{7} |
| SMC1A     | –                                                    | –                                      | Corces-Zimmerman (2014)\textsuperscript{50} |
| CTCF      | –                                                    | –                                      | Jan et al (2012)\textsuperscript{7} |
highest frequency in other nonhematological malignancy cases and in the blood cells of normal aging individuals compared with other preleukemic mutations and ARCH-related genetic alterations.29,30 These discoveries are in line with the myeloid lineage bias and higher incidence of AML in the elderly population, although the majority of those carrying the mutations will not eventually progress to AML. Because the HSCs bearing these common mutations undergo clonal expansion,29 these mutations may be relevant for initiating the clonal expansion process and serve as a “first hit” or “early hit” for the hematological malignancies.22,30 Other prevalent mutant genes such as FLT3 and NPM1 are thought to be late events31,32 in the timing of the mutation events. Somatic mutations of the TET2 gene were reported not only in leukemia but also in MDS and malignant lymphoma.33–35 which suggests a critical role for TET2 in hematopoiesis. Conditional deletion of TET2 in the hematopoietic compartment in vivo demonstrated that the loss of TET2 enhances repopulating activity in competitive reconstitution assays and leads to progressive myeloid proliferation.44 In addition, TET2 haploinsufficiency confers increasing self-renewal to stem/progenitor cells and to extramedullary hematopoiesis.45 DNMT3A mutations are observed in myeloid malignancies, including myeloproliferative neoplasms,36 MDS,46 T-cell lymphoma,39 and AML. However, the mechanisms by which DNMT3A mutations contribute to leukemogenesis have yet to be fully elucidated. Loss of function of DNMT3A induces mature myeloid and myeloid progenitor expansion in vivo, increases self-renewal of hematopoietic cells, and results in liver-specific myeloid proliferation and extramedullary hematopoiesis.40 The similar phenotypes of DNMT3A and TET2 when silenced or when loss of function mutations is present indicate that they may alter HSPCs’ self-renewal capacity and myeloid lineage expansion bias through some similar pathways after the epigenetic disruption.

PRELEUKEMIC STEM CELLS AND RELAPSE

Shlush et al9 utilized the deep targeted sequencing of 103 commonly mutated leukemia genes on blast cells of AML patients and normal T-cells for genetic comparison. Interestingly, 70.5% of the patients harboring DNMT3A mutation in blast cells were also found to have DNMT3A mutation in T-cells, while NPM1c mutation did not present in the case. These data revealed a sequential order of the mutations in these patients, indicating that DNMT3A mutation is likely to be a very early mutation hit to the common progenitor of myeloid cells and lymphocytes, whereas NPM1c mutation may serve as a sequentially later hit that confers the disease (model B in Fig. 1). They later demonstrated that these mutant ancestral cells in nonleukemic hematopoietic cell populations from DNMT3A+/NPM1c+ AML patients still have multilineage differentiation capacity (model C in Fig. 1), and that preleukemic HSCs survive through chemotherapy and somehow undergo clonal advantages acting as a reservoir for relapsed disease (model D in Fig. 1). Another study carried out by Corces-Zimmerman et al10 illustrated a stepwise pattern of mutation acquisition based on a genotyping assay for all recurrent mutations of colonies derived from a single HSC. Consistently, the earliest founding mutations occur in “landscaping” genes that are involved in global regulation of gene expression through epigenetic mechanisms, whereas the “proliferative” gene mutations occur later. In addition, the authors, using bone marrow samples from remission and relapse, found that preleukemic HSCs survive through chemotherapy and persist in remission, and proposed a model for mechanisms of AML relapse, which consists of four patterns: (a) the primary disease clone is refractory to treatment; (b) growth of a major clone at diagnosis; (c) a minor clone at diagnosis outcompetes the others; (d) new clones arise from preleukemic HSC.

By following the variant allele frequency of leukemic-associated clones and hematopoietic clones unrelated to the initial AML (termed as a “rising” clone) at diagnosis, after induction therapy, relapse, and salvage therapy, those preexisting nonleukemic hematopoietic clones harboring a mutation such as TP53, TET2, DNMT3A, and ASXL1 had rapid expansion.41 Comparing the stable and slow-clonal hematopoiesis of indeterminate potential in healthy adults over decades, this expansion rate is very high. Whether this phenomenon is caused by compensation for the contraction of HSCs or survival of the fittest mutant clones via evolutionary selection via microenvironment after chemotherapy remains unclear. These findings provide a new perspective toward current leukemia chemotherapy and minimal residual disease monitoring, since these strategies largely aim at eliminating and monitoring the major mutant clone, whereas ignoring the subset of minor/other clones that might contribute to relapse. However, AML is a complex heterogeneous malignancy; thus, more large-scale and longer follow-up studies are needed to determine whether other mutant clones should be necessarily targeted.

PREDICTION VALUES OF PRELEUKEMIC MUTATIONS FOR AML

AML, as the name suggests, is thought to be a rapid, almost unpredictable pathological process, but the appreciation of the leukemia-associated mutations in the stage of preleukemia has provided insight regarding the prediction of the course of the disease. Recent studies tried to identify the role of recurrent mutant genes in normal aging individuals and their impact on risk in preceding overt leukemia development. Desai et al42 investigated such mutations in 212 aging women with a median of 9.6 years before their diagnosis of AML and 212 age-matched controls. Mutations in TP53, IDH1/2, and spliceosome subunit genes (SRSF2, SF3B1, and U2AF1), as well as a higher variant allele frequency (>10%) and the presence of a higher number of variants in DNMT3A and TET2 are shown to be associated with increasing odds of developing AML. Preleukemia cases also demonstrated greater clonal complexity than controls. TP53 and IDH1/2 mutations conferred a significantly shorter latency period to AML, which indicated that these are the late events in AML development. Combining sequencing, clinical records, and machine learning, Abelson et al43 provided two distinct models for the prediction of de novo AML, one of which was based on gene mutations with sensitivity of 41.9% and specificity of 95.7%, and the other was based on routinely documented clinical information, such as red cell distribution width (RDW), absolute monocyte counts, and red blood cell counts, with a sensitivity of 25.7% and an overall specificity of 98.2%. For the genetic prediction models, mutations in TP53, U2AF1, and SRSF2 contributed to a large effect size on progression to AML. Conversely, mutations in DNMT3A and TET2, which were found to be the most frequent in both preleukemia groups and controls in line with the aforementioned previous studies, contribute little to the disease. Thus, a more comprehensive and accurate perspective may be gained if genetic and clinical data are combined in a single model. Collectively, these studies provided a proof-of-concept of preleukemia and also implied the feasibility of differentiating individuals at high risk of AML from
normal aging individuals. Whether the prediction models work well awaits more evidence from large population-based epidemiological survey. Since the notable differences in mutational landscapes between ARCH and preleukemia might lead to high-risk AML, early intervention of preleukemic stem cell evolution seems to be an attractive preventative strategy.

**CONCLUDING REMARKS AND FUTURE DIRECTIONS**

The studies identifying and tracing the preleukemic HSC in leukemia established it as an important scientific and clinical entity, as it demonstrates a compelling link with relapse and early prediction of AML. However, our understanding of the preleukemic stem cell is still in its infancy. Based on the aforementioned findings, early events often involve mutations of epigenetic regulatory genes during leukemia evolution and those mutations frequently occurs in aging adults. This raises several questions: How can we distinguish the benign mutant entity from the malignant one? Is LSC the direct consequence of a pre-HSC? How much does epigenetic alteration contribute to disease progression? Abelson et al’s recent study provided a preliminary answer by models in the prediction of AML based on specific mutations in HSCs and clinical data. Interestingly, the most common preleukemic and ARCH-related mutations in DNMT3A and TET2 confer low risk of AML progression, whereas TP53 and some spliceosome genes such as U2AF1 and SRSF2 have a larger effect. These models provide putative targets for therapeutic intervention, and their predictive value awaits validation in prospective studies with larger sample sizes.

For many leukemia patients, the current therapeutic strategy mainly aims at eliminating the major clones and ignoring the clones that harbor putative relapse mutations. Should we intervene in such situations before clinical appearance of AML or other type of blood cancer, and how? There is an urgent need for further work to determine whether intervention during the preleukemic phase is feasible, safe, beneficial, and ethical. The mechanisms underlying the evolutionary trajectory from the first somatic mutation to the eventual development of cancer and the role of chronological mutation order in the process remain to be further defined. How does this mutant cell, the preleukemic HSC, acquire survival advantages outcompeting the normal HSCs? Is this the result of adaptation to the aging microenvironment? More clonal evolution studies relevant to leukemogenesis will serve to answer these questions, and the emerging techniques of single cell sequencing analysis and deep machine learning certainly offer new exciting opportunities to further interrogate many burning issues around preleukemic stem cells.

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