Concise Review: Preleukemic Stem Cells: Molecular Biology and Clinical Implications of the Precursors to Leukemia Stem Cells

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Key Words. Acute myelogenous leukemia • Hematopoietic stem cells • Cancer stem cells • Stem/progenitor cell

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ABSTRACT

Recent experimental evidence has shown that acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) arise from transformed immature hematopoietic cells following the accumulation of multiple stepwise genetic and epigenetic changes in hematopoietic stem cells and committed progenitors. The series of transforming events initially gives rise to preleukemic stem cells (pre-LSC), preceding the formation of fully transformed leukemia stem cells (LSC). Despite the established use of poly-chemotherapy, relapse continues to be the most common cause of death in AML and MDS. The therapeutic elimination of all LSC, as well as pre-LSC, which provide a silent reservoir for the re-formation of LSC, will be essential for achieving lasting cures. Conventional sequencing and next-generation genome sequencing have allowed us to describe many of the recurrent mutations in the bulk cell populations in AML and MDS, and recent work has also focused on identifying the initial molecular changes contributing to leukemogenesis. Here we review recent and ongoing advances in understanding the roles of pre-LSC, and the aberrations that lead to pre-LSC formation and subsequent LSC transformation. STEM CELLS TRANSLATIONAL MEDICINE 2013;2: 143–150

INTRODUCTION

Healthy hematopoiesis is hierarchically organized, beginning with multipotent, quiescent stem cells that are able to perpetuate themselves through self-renewal and generate more mature, transit-amplifying progeny through differentiation. Recent experimental evidence has shown that acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) arise from transformed immature hematopoietic cells following the accumulation of multiple stepwise genetic and epigenetic changes in hematopoietic stem cells (HSC) and committed progenitors. The series of transforming events is thought to give rise to preleukemic stem cells (pre-LSC), including the initial leukemia cell-of-origin (LCO), preceding the formation of fully transformed leukemia stem cells (LSC) (illustrated in Fig. 1). Whereas pre-LSC consist of self-renewing hematopoietic stem and progenitor cells that can give rise to leukemia in vivo with variable latency upon gradual accumulation of additional genetic hits, LSC are functionally defined by their capacity to give rise to fully penetrant, short-onset leukemia in murine transplantation models.

Despite the established use of poly-chemotherapy and the application of newer agents that transiently reduce the tumor burden, relapse continues to be the most common cause of death in AML and MDS. Such failures demonstrate that the functional LSC compartment is not effectively eradicated by current regimens. However, the therapeutic elimination of all LSC, as well as pre-LSC, which provide a silent reservoir for the re-formation of LSC, will be essential for achieving lasting cures. Thus, the identification of specific LSC and pre-LSC populations, as well as the study of their molecular characteristics, is critical for understanding the genesis of leukemia and for developing strategies by which these cells can be eradicated.

Although still a work in progress, recent research has improved our understanding of the molecular pathways and the temporal and spatial order of events involved in the transformation process. According to the current molecular pathogenetic model of AML, at least two molecular events are required for the development of malignant cells [1]. One causes a differentiation arrest, and the second confers proliferative properties to the cells, leading to the formation of a pool of LSC that in turn gives rise to a hierarchy of functionally heterogeneous bulk tumor cells [2]. In addition, some disease alleles also increase stem cell self-renewal, further contributing to leukemogenesis and to leukemia stem
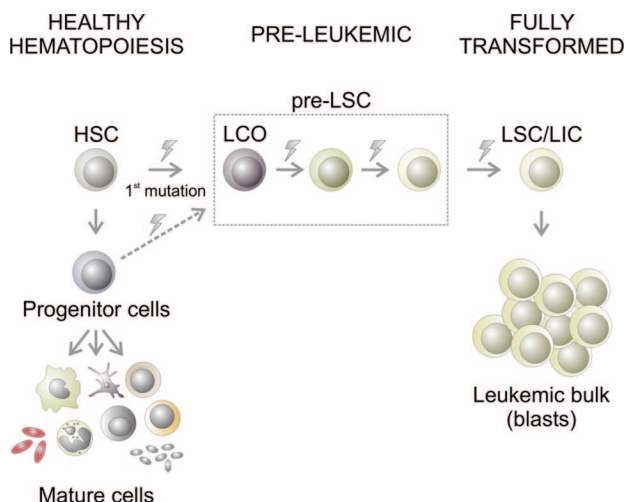
Figure 1. Multistep transformation of leukemia stem cells. During healthy hematopoiesis (displayed on the left), HSC give rise to committed progenitor cells that further differentiate into all mature blood cell types. Leukemia arises from hematopoietic cells that have progressively acquired genetic and/or epigenetic modifications (represented by lightning bolt symbols) and ultimately form self-renewing LSC, also known as LIC, that sustain the disease. An initial ("founding") mutation in HSC or progenitor cells originates the LCO. During the preleukemic stage, the LCO and subsequent preleukemic stem cell stages (pre-LSC) progressively acquire further aberrations and finally generate fully transformed LSC/LIC, which are functionally defined by their ability to initiate disease upon transplantation. These aberrations, illustrated by lightning bolt symbols, include mutations or deregulation of transcription factors, epigenetic factors, metabolic factors, and proteins involved in signal transduction and cell cycle regulation. LSC/LIC are self-renewing and contain a full set of genetic and epigenetic changes that lead to blocked hematopoietic differentiation and the accumulation of dysfunctional leukemic blasts that form the bulk of the tumor cells but are not capable of initiating/maintaining the disease on their own. Abbreviations: HSC, hematopoietic stem cell; LCO, leukemia cell-of-origin; LIC, leukemia-initiating cell; LSC, leukemia stem cell.

cell maintenance. Although conventional sequencing and next-generation genome sequencing of blast populations have allowed us to describe many of these recurrent mutations, our understanding of the initial genetic changes of the pre-LSC contributing to leukemogenesis remains limited. Here we review recent and ongoing advances in understanding the roles of pre-LSC, and the changes that lead to pre-LSC formation and subsequent LSC transformation.

INSIGHTS OBTAINED FROM GENETIC MODELING

As a constantly increasing number of recurrent mutations have been identified in myeloid malignancies, murine models of disease alleles found in human AML patients have been used to investigate how early disruptions in the hematopoietic stem cell compartment contribute to leukemogenesis. Models of leukemogenesis include transplantation models in which leukemia oncogenes are ectopically expressed, as well as transgenic mice carrying mutations or mimicking other molecular changes found in human leukemia. Murine models also offer the opportunity to understand the effects of combining recurring mutations with epigenetic and transcriptional changes and, importantly, allow for specific investigation of preleukemic stem and progenitor cell populations.

Transcription Factors

The process of leukemogenesis is understood to begin at the level of hematopoietic stem and progenitor cells (HSPC). The involvement of several transcriptional regulators that govern normal hematopoietic differentiation and stem cell maintenance in leukemogenesis is suggested by their frequent mutation or altered regulation in patients [3–5]. Properties of increased self-renewal, blocked differentiation, reduced programmed cell death, and altered signaling and metabolism play a role in clonal pre-LSC development and promote the acquisition of subsequent genetic alterations by the pre-LSC, leading in a stepwise fashion to complete transformation to LSC and the full leukemic phenotype.

Mutational profiling of paired diagnosis and relapse bone marrow samples from patients with AML has attempted to identify the molecular drivers of leukemogenesis. Modeling such changes in mice has offered insight into which molecular changes effect development of pre-LSC. For example, there is strong evidence that mutations in CCAAT/enhancer-binding protein-α (*CEBPA*) may be early events in the generation of leukemic clones. In contrast to mutations in genes such as fms-related tyrosine kinase 3 (*FLT3),* the rat sarcoma genes (*RAS*), or stem cell growth factor receptor (*KIT*), the majority of patients carrying *CEBPA* mutations undergoing relapse of AML display the same mutations in both *CEBPA* alleles as they did before achieving complete remission [6–8]. A biallelic knockin mouse model of the most common *CEBPA* mutation in AML results in myeloid progenitors with increased self-renewal capacity and mice uniformly progress to a transplantable AML. Moreover, these *CEBPA* mutations also induce pre-LSC development characterized by a cell-intrinsic, premalignant expansion of the HSC and multipotent progenitor (MPP) compartments induced by loss of quiescence prior to overt leukemia development [8, 9]. *CEBPA* is not alone: other mutations such as the fusion oncogenes *AML1* (acute myeloid leukemia-1)-*ETO* (eight-twenty-one corepressor) and *MLL* (mixed lineage leukemia)-*AF9* (ALL1-fused gene from chromosome 9 protein) similarly cause an increase in HSC numbers followed by accumulation of abnormal myeloid progenitors [10, 11]. *CBFB* (core binding factor subunit-β)-*SMMHC* (smooth muscle myosin heavy chain) mutations, in contrast, have been shown to decrease HSC repopulating activity while leading to the accumulation of abnormal myeloid progenitors [12]. These studies demonstrate how preleukemic mutations affecting key transcription factors may allow HSPC to evade normal homeostatic control of their numbers and survival and form pre-LSC primed for acquisition of further changes that contribute to the subsequent generation of fully transformed LSC.

Studies in both mouse and human LSC and preleukemic HSPC have shown that in addition to recurrent mutations, disturbed gene expression not only causes deregulation of homeostatic hematopoiesis but may contribute to leukemogenesis. These factors comprise key transcriptional regulators that are crucial for maintaining basic cell functions and allowing for a sufficient life span of pre-LSC to acquire multiple further hits for full transformation. For example, in a murine model of AML caused by 80% reduced levels of the transcription factor PU.1 (*Spi1*), the course of disease includes a preleukemic phase with an accumulation of immature myelomonocytic cells in the marrow, eventually followed by a leukemic phase with high numbers of malignant cells in both the blood and marrow [13]. Progressive

inactivation of transcription factor jun-B (*Junb*) in a murine model deregulates cell-cycle machinery and increases the proliferation of long-term repopulating HSC (LT-HSC) by destabilizing a complex network of genes and pathways that normally limit myeloid differentiation leading to the development of a myeloproliferative disorder [14, 15]. In another murine model, GATA-binding protein 1 (*Gata1*) knockdown leads to the formation of a disease resembling MDS after a long latency, and, importantly, some remaining GATA1 activity (∼5%) seems to be required for MDS formation, as *Gata1*-null mice have a normal life expectancy [16]. These models, and the paucity of null mutations or deletions in transcription-factor encoding genes in patients, highlight the importance of remaining residual levels of key regulators, as opposed to complete deficiency. A minimal remaining level may be required for maintaining basic cell functions and allowing for a sufficient life span of pre-LSC to acquire multiple further hits for full transformation.

Genome-wide transcriptional profiling of pre-LSC prior to the onset of AML has delineated transcriptional pathways that are functionally essential for formation of LSC that arise later during the course of disease. For instance, animals with low PU.1 have definable preleukemic HSC and develop AML only in combination with subsequently deregulated cofactors such as *JunB* and after a fairly long latency (median, 6 months) [17]. Downregulation of *JUNB* expression, correlating with low *PU.1* expression, has also been found in the HSC compartment of patients with AML [17]. This strategy of pre-LSC gene expression profiling also led to the discovery of the nonclustered homeobox transcription factor H2.0-like homeobox (*Hlx*), which is upregulated in PU.1 low pre-LSC. HLX was found to block the differentiation of HSC and lead to formation and accumulation of abnormal myeloid progenitors with unlimited serial clonogenicity [18]. Importantly, *HLX* upregulation appears to be an early lesion and by itself seems not sufficient for full LSC transformation [18].

These models, together with other genetic studies in mice, have supported the idea of a network of genes that regulate HSC numbers, proliferative activity, resistance to apoptosis, and retention in the bone marrow niche [19] and thereby contribute to formation and maintenance of pre-LSC populations. Other important mediators of these processes include cell-cycle regulators, such as the D cyclins; transcription factors, such as growth factor-independent 1 transcription repressor (*Gfi1*), *homeobox B4* (*HoxB4*), GATA-binding protein 2 (*Gata2*), and homeobox A9 (*HoxA9*); and extrinsic regulatory pathways, including S-phase kinase-associated protein 2 (*Skp2*), *notch* (*Notch*), *transforming growth factor-β* (*Tgfb*), and wingless-type MMTV integration site family members (*Wnt*), which control HSC self-renewal and proliferation [15, 20–28].

Epigenetic Factors

Although the regulation of hematopoietic stem cell self-renewal and lineage differentiation by specific transcription factors has been well-characterized, our understanding of the epigenetic processes upstream of these factors is still in its infancy. DNA cytosine methylation is an epigenetic modification that has an important role in chromatin organization and in regulating gene expression [29, 30]. Significant changes in methylation patterns have been observed when comparing different stages of murine hematopoietic cells [31, 32], indicating that methylation patterns may effect important transcriptional changes during differentiation. Knockout of the maintenance DNA methyltransferase

1 (*Dnmt1*), for example, has shown that DNA methylation protects HSC from myeloerythroid restriction [33, 34].

Recurring mutations in a number of important epigenetic mediators, including regulators of DNA cytosine methylation, have been identified in patients with myeloid malignancies, and recent studies have shown that aberrant DNA cytosine methylation is a hallmark of patients with AML and MDS [35–38]. Genetic modeling of individual epigenetic factors involved in AML pathogenesis has shown that the majority affect the HSC compartment and alter hematopoietic differentiation, in some cases leading to myelodysplasia, stem cell expansion, or other preleukemic conditions. These include suspected loss-of-function or dominant interfering mutations such as DNA methyltransferase 3α (*DNMT3A*), tet methylcytosine dioxygenase 2 (*TET2*), enhancer of zeste homolog 2 (*EZH2*), and additional sex combs like 1 and 2 (*ASXL1/2*), as well as neomorphic mutations, such as isocitrate dehydrogenase 1 and 2 (*IDH1/2*) [36, 39–53].

Loss of DNMT3A impedes HSC differentiation, expanding HSC numbers in the bone marrow [54]. Gene expression analysis showed that *DNMT3A* loss leads to upregulated expression of HSC multipotency genes and lower expression of genes with a known role in HSC differentiation [54]. However, despite these preleukemic features, no development of overt leukemia was observed in a *Dnmt3a* knockout model. Alterations that impair the catalytic activity of TET2, which hydroxylates 5mC, lead to increased stem cell self-renewal and progressive stem cell expansion, ultimately causing a myeloproliferative disease-like disorder [55]. Again, no acute leukemias were observed in this model. Likewise, mutations of the IDH1 enzyme, which produces a new metabolite (2-hydroxyglutarate) that competes with α-ketoglutarate to impair the catalytic activity of dioxygenases, including TET2, lead to increased numbers of early hematopoietic progenitors and dysfunctional hematopoiesis, although, like TET2, these mutations do not lead to overt leukemia [56]. Loss of ASXL1 was recently shown to cooperate with *NRASG12D* mutations in promoting the development of AML, an effect that is mediated through a loss of polycomb repressive complex 2 (PRC2)-mediated gene repression [57]. The *MLL-AF9* fusion oncogene was recently reported to require the histone demethylase lysine (K)-specific demethylase 1A (KDM1A) to maintain functional LSC [58]. Together, these findings suggest that these commonly mutated epigenetic factors work in concert to protect HSC from aberrant expansion and myeloid transformation, and that their respective mutations lead to alterations in DNA methylation patterns and other epigenetic modifications in HSC and consequent expansions of pre-LSC populations.

Metabolic Factors

Recent data from various murine models have begun to clarify roles for molecular changes in metabolic pathways in leukemogenesis. Disruption of metabolic pathways in HSC may play a role in the formation of pre-LSC and transformation to LSC. Recent models have shown that deletion of several negative regulators of mammalian target of rapamycin (mTORC1) signaling, including phosphatase and tensin homolog (*Pten*), tuberous sclerosis 1 (*Tsc1*), promyelocytic leukemia (*Pml*), and F-box and WD repeat domain containing 7 (*Fbxw7*), result in similar stem cell phenotypes characterized by HSC hyperproliferation and subsequent exhaustion and defective repopulating potential [59–64]. Inactivation of PTEN, a negative regulator of the phosphatidylinositol

3-kinase-Akt pathway, for example, causes short-term expansion but long-term decline of HSC, and mice with *Pten*-mutant bone marrow develop a myeloproliferative disorder [60]. Similarly, deficiency of the tumor suppressor PML induces cycling of HSC and results in short-term increased but long-term reduced HSC repopulation ability [63]. Although these and other regulators of metabolic pathways in HSC, such as hypoxia inducible factor 1, α-subunit (*Hif-1a*) [65], and the liver kinase B1 (*Lkb1*) [59, 66, 67], have been shown to alter the size of the hematopoietic stem cell compartment in mouse genetic models, the role of changes in their expression or mutations in the genes encoding these factors in LSC or pre-LSC of patients with myeloid malignancies is still largely unclear.

Cellular Stages of Transformation

A lingering question in the understanding of leukemogenesis is which cellular stages during hematopoiesis acquire the molecular hits leading to formation of pre-LSC and, ultimately, complete transformation to LSC. The initial changes can occur at the level of the HSC, and then their downstream progeny achieve additional mutations until achievement of full leukemic transformation. Alternatively, it is possible that the initial mutations could confer inappropriate self-renewal on downstream progenitor cells and thereby give them the necessary life span to acquire further transforming mutations.

Using mouse genetic modeling, at least two approaches to this question have been taken: to analyze models of leukemogenesis and dissect which cellular population(s) the (transplantable) LSC reside in, and to specifically introduce molecular changes in defined HSC or progenitor compartments and assess whether LSC formation occurs. The examination of different models of myeloid malignancies has provided evidence that in principle both HSC and committed progenitor types can be leukemia cells-of-origin that give rise to LSC, depending on the specific disease allele. For instance, studies using retroviral expression of the *MLL-ENL* (mixed-lineage leukemia translocated 1) and *MOZ* (monocytic leukemia zinc finger)-*TIF2* (transcriptional intermediary factor 2) fusion genes have shown that both HSC and committed myeloid progenitor cells can be transformed by these fusion oncogenes, although with higher efficiency in HSC populations [68, 69]. In a series of tissue-specific knockout models of *Junb*, it was shown that loss of JUNB in the HSC compartment is required for leukemia initiation and that *Junb* deletion in granulocyte/monocyte progenitors (GMP) or downstream progeny only resulted in a transient myelomonocytic expansion [14, 15, 70]. Other studies have shown that retrovirally introduced *MLL-AF9* can transform both early HSC and committed myeloid progenitors; however, in an *MLL-AF9* transgenic model, where *MLL-AF9* is expressed at more physiologic gene dosages, the HSC population, but not the more committed GMP population, was able to be transformed [11, 71, 72]. These findings indicate that the gene dosages in some of the retroviral studies may have allowed for activation of target genes in populations that may not naturally be a receptive cellular environment for transformation. Interestingly, in the *CEBPA* knockout model, despite preleukemic expansion and elevated cycling activity of the HSC and MPP populations, the LSC responsible for sustaining AML did not exhibit features of HSC or MPP [9]. These findings indicate that pre-LSC can reside in different cellular compartments than LSC and that the final genetic or epigenetic changes that lead to generation of fully transformed and transplantable LSC may occur in more committed myeloid progenitors. Further systematic combinatorial modeling of different disease alleles in future studies will provide more insight into the exact temporal and spatial order of events during multistep transformation of LSC.

Of note, although mouse genetic models have provided important insight into cellular stages that can contribute to leukemogenesis, they cannot definitively answer the question of which cell types actually contribute to leukemogenesis and what characterizes the naturally occurring series of events in human leukemia formation. The analysis of purified stem and progenitor populations from patients with AML and MDS seems to suggest that HSC harbor the leukemia cells-of-origin in many subtypes of these diseases, whereas multipotent or further committed progenitors harbor the majority of fully transformed LSC.

INSIGHTS OBTAINED FROM THE STUDY OF HUMAN LEUKEMIAS

Advanced cell sorting techniques and the use of xeno-transplantation models have improved our ability to isolate and study LSC in human AML. However, only recently, studies have begun to attempt to define the human pre-LSC compartment including the LCO, which harbor the founding molecular changes that put in motion the process of leukemogenesis. Understanding the pre-LSC and attempting to distinguish pre-LSC from their healthy neighbors have both prognostic and therapeutic implications for the clinic.

It has been demonstrated that preleukemic mutations are present in the self-renewing HSC after chemotherapy and achievement of long-term complete remissions, suggesting that residual HSC in leukemia patients are preleukemic [73–75]. In fact, very early studies examining X-chromosome-linked glucose-6-phosphate dehydrogenase of de novo AML cells and hematopoietic cells during remission had found that cells can possess identical inactivation patterns [76–79]. This observation indicated that AML cells and normal hematopoietic cells in remission could be derived from a dominant HSPC clone that is itself not fully malignant—thus fulfilling the criteria of a pre-LSC. Studies of t(8,21)-positive AML have demonstrated that patients in long-term complete remission contain apparently normal HSC that produce *AML1-ETO* transcripts [73, 74]. Similar observations were made in chronic myeloid leukemia [80]. The presence of such cells in patients in long-term remission implies that HSC that carry a clonotypic marker of the leukemic clone but are functionally normal and produce healthy progeny are persisting pre-LSC. Recent studies using genome-wide sequencing in de novo AML patients and at the time of relapse have suggested that subclones of the primary clone at initial diagnosis can drive the relapse [81], and clonal analysis revealed the coexistence of "*n* minus 1" and "*n* minus 2" subclones (with *n* defined as the number of mutations in the fully transformed clone) [82], providing additional evidence for the existence of relevant pre-LSC populations in AML.

One approach to understanding the identity of and characterizing pre-LSC in human AML has been the transcriptional analysis of purified stem cells. It was found that fractionated phenotypic LT-HSC from AML patients (distinct from LSC-enriched populations, which have a short-term HSC/MPP immunophenotype) show deregulated transcription of key regulators such as *PU.1* and *JUNB* in comparison with LT-HSC from healthy controls [17]. These findings demonstrated that transcription is disturbed already in very early HSC (i.e., at a pre-LSC level) in AML and that

it is possible, in principle, to delineate the molecular events that distinguish pre-LSC from LSC and from HSC from healthy control persons. Further studies compared more comprehensive expression profiles of normal HSC with those of LSC, and pathways such as Wnt signaling, MAP kinase signaling, and adherens junctions and phenotypic markers such as CD32 and CD25 were shown to distinguish healthy HSC from LSC [83–85]. Owing to the recent discovery that LSC can be contained within different cellular compartments and at relatively low frequencies [86], transcriptional analysis strategies have been further expanded. To identify genes dysregulated early on in AML, we have recently used a strategy of parallel transcriptional profiling of multiple, highly fractionated stem and progenitor populations in individual AML patients and compared them with the respective control populations from age-matched healthy individuals [87]. This led to the identification of a relatively small number of genes that were commonly deregulated in all examined compartments and which may thus be characteristic of early pre-LSC. For instance, we found that the protein interleukin-1 receptor accessory protein (IL1RAP) is overexpressed specifically on the surface of HSC of AML and high-risk MDS patients, that IL1RAP is functionally relevant for the clonogenicity of AML cells, and that high *IL1RAP* expression is independently associated with poor overall survival of AML patients [87].

Evidence at the genetic level for an involvement of the stem cell compartment in disease initiation in AML was initially obtained by cytogenetic analysis of subpopulations of CD34$^+$ cells [88, 89]. Important insight has also come from the study of MDS, which frequently progresses to AML. In MDS patients with a 5q deletion, almost all CD34$^+$CD38$^-$ cells appeared to be involved in the 5q-deleted clone [90], and similar findings were made in MDS with trisomy 8 (+8) aberrations [91]. We have recently found in MDS patients with monosomy 7 (−7) or monosomy 20, and in AML with −7, that the majority of Lin$^-$CD34$^+$CD38$^-$ early HSC carry the clonotypic aberrations [87, 92]. Recently, genome-wide sequencing of different types of AML cases and of healthy HSC has demonstrated how different molecular events at a genetic level act as initiating mutations in different forms of AML. Comparison of the genome of patients with *PML-RARA* (retinoic acid receptor-α) with genomes of other AML patients (FAB M1, normal karyotype AML) suggested that mutations in nucleophosmin (*NPM1*), *DNMT3A*, and *IDH1* may act as major initiating mutations in M1 AML [82]. Recent work has also compared de novo AML and patient-matched residual nonleukemic HSC, to distinguish "early," persisting mutations present in residual HSC from "late" mutations absent from residual HSC and only present in leukemic cells [75]. Applying exome sequencing and single-cell assays of residual HSC, the authors mapped the clonal evolution of the AML genomes of patients with *FLT3-ITD*-positive AML, identifying founding mutations in pre-LSC (e.g., *TET2* mutations). Notably, the *FLT3-ITD* mutation was absent in residual HSC, indicating that it was acquired at later stages during LSC transformation [75]. In another recent report, examination of myeloid skewing in normal elderly individuals revealed the existence of clonal somatic mutations, including TET2, in the hematopoietic compartment without overt hematopoietic malignancies [93].

Attempts have been made to identify core transcriptional signatures of LSC that can be used prognostically. Recently, it has been shown that an LSC-specific transcriptional signature and a core transcriptional program shared by LSC and healthy HSC is an independent predictor of AML patient survival [85, 94]. Correla-

tive evidence has supported the idea that leukemic stem cell frequencies and properties may be prognostic in AML, in particular for the minimal residual (stem cell) disease and relapse setting, linking clinical outcome with either the capacity for a sample to be xenografted or surface expression of LSC-linked markers [92, 95–97]. Other studies have shown that malignant stem cells persist in MDS with 5q− in complete remission [98]. Additionally, the specific expanded compartment has also been linked to prognosis. In MDS, for example, high-risk patients exhibit expansion of the phenotypic LT-HSC and GMP compartments, whereas lower-risk patients demonstrate expansion of the phenotypic common myeloid progenitor compartment [92]. Furthermore, expansion of the phenotypic HSC compartment can precede clinical relapse in MDS by several months [92]. These findings, together with the eventual progression of MDS to AML in approximately 30% of cases, suggest that aberrant MDS stem cells may be a pre-LSC stage with regards to AML.

Interesting insights into the progression of pre-LSC have come from studies of inherited and congenital disorders that predispose individuals to hematopoietic malignancies. In such cases, the first "hit" or cancer-initiating step may be the constitutional genetic abnormality itself. In Down syndrome, Fanconi anemia, and familial platelet disorder, for example, patients are predisposed to myeloid malignancies, indicating such predisposing genetic hits are necessary but insufficient for the development of malignancy. In Down syndrome, including in cases of mosaic Down syndrome, up to 10% of neonates develop a trisomy 21 positive transient myeloproliferative disorder (TMD) that resolves without therapeutic intervention. Between years 1 and 4 of life, in approximately 20% of these children, these TMD clones, which can be considered residual pre-LSC, acquire additional postnatal genetic events leading to acute megakaryoblastic leukemia characterized not only by trisomy 21 but also additional complex cytogenetic abnormalities [99–101]. Fanconi anemia (FA) is a genetic disease characterized by genomic instability, bone marrow failure caused by cell cycle arrest, and a predisposition to malignancies, including MDS and AML. In response to stem cell exhaustion in FA, there is pressure for clonal evolution of hematopoiesis in FA patients, resulting, interestingly, in some cells undergoing spontaneous reverse mutation, leading to somatic mosaicism and allowing genetically corrected cells to repopulate the bone marrow. Strikingly, however, such reversion is not capable of protecting against transformation to MDS and leukemia, and "nonreverted" HSC still clonally evolve to leukemia [102–104]. Such findings indicate that the congenitally aberrant HSC of patients with such disorders are actually pre-LSC, "predisposed" to the progression of further steps leading to MDS/AML.

Conclusion

Data obtained from both mouse and human AML and MDS have provided evidence for the existence of functionally relevant pre-leukemic stem cell populations, and mouse genetic models have shown that these pre-LSC are starting points for the multistep pathogenesis of AML and formation of fully transformed LSC. Data from patients with AML and MDS indicate the persistence of pre-LSC in remission and strongly suggest their involvement in minimal residual disease that can cause relapse. Further refined

sorting strategies and separation of pre-LSC (including the leukemia cells-of-origin), LSC, and normal HSC and progenitor populations, combined with current genome-wide sequencing technologies and functional testing in the context of clinical studies for the treatment of patients with AML and MDS, will be instrumental to target all relevant (pre-)LSC populations for both diagnostic and therapeutic purposes. Further combinatorial genetic modeling will be helpful to determine early and late aberrations in the pathogenesis of AML and may thus ultimately allow for more specific targeting of pre-LSC to achieve more long-term remissions and possibly a lasting cure of the disease.

AUTHOR CONTRIBUTIONS

A.P. and L.B.: manuscript writing; U.S.: manuscript writing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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