Myeloid Pre-Leukemia and Leukemia of Down Syndrome

David Cruz Hernandez1,2, Paresh Vyas1,2

1 MRC MHU, BRC Hematology Theme, Oxford Biomedical Research Centre, Oxford Centre for Hematology, WIMM, Radcliffe Department of Medicine, University of Oxford, Oxford OX3 9DU, UK; 2 Department of Hematology, Oxford University Hospitals NHS Trust, Oxford OX3 7LE, UK

Take-home messages:
- Maturation arrest is a hallmark of myeloid leukemia and remains largely unexplored at the molecular level.
- ML-DS is an excellent model to conduct mechanistic studies due to its genetic simplicity to explore maturation arrest and mutation cooperativity.

Introduction

A hallmark of blood cancers is the gradual acquisition of genetic or epigenetic changes that progressively disrupts normal hematopoiesis and eventually leads to full transformation. Therefore, most leukemias are preceded by a pre-leukemic phase. For example, Myeloid Leukemia of Down Syndrome (ML-DS) is an acute leukemia with megakaryoblastic and erythroid features that clonally evolves from a preleukemic condition referred as Transient Abnormal Myelopoiesis or TAM. The pathogenesis of ML-DS is well characterized and consists of 3 key temporally separated stages.1 First, partial or complete trisomy 21 (T21) alters hematopoietic stem cell (HSC) homeostasis in fetal liver. Second, somatic mutations acquired during fetal life in the key transcription factor GATA1 cooperate with T21 and result in the preleukemic myeloproliferative disorder known as Transient Abnormal Myelopoiesis, TAM. Third, a single allele mutation in genes encoding the cohesin complex, epigenetic regulators or in the JAK family kinases transforms the TAM clone to full-blown leukemia. Due to its genetic simplicity, ML-DS and its precursor clonal disease TAM, will serve as a disease model to define the molecular mechanism leading to maturation arrest (Fig. 1).

Current state of the art

Trisomy 21 alters HSC homeostasis

The initiating genetic alteration in the multi-step model of ML-DS is an extra copy of chromosome 21 (T21) in fetal hematopoietic stem cells. T21 causes expansion of the immunophenotypically defined fetal-liver HSC compartment with skewed cellular differentiation toward the erythroid/megakaryocytic lineage, and conversely severe impairment of B-lymphopoiesis and reduced granulocyte-monocyte output.2 A cell-autonomous gene imbalance due to T21 might be responsible for perturbation of fetal hematopoiesis. In the few cases where ML-DS develops from partial T21 suggest that the leukemic risk is confined to an 8.5Mb region on chromosome 213 where key hematopoietic genes lie, such as RUNX1, ETS2, ERG, Dyrk1A. Unfortunately, it is challenging to identify gene expression differences between aneuploid and disomic cells mainly due to modest differences that can be masked by patient variability. The molecular mechanism by which T21 alters HSC homeostasis and predisposes to ML-DS remains largely unexplored.

Aberrant transcription factor function due to GATA1s

The second step on the evolution of ML-DS requires mutations in the X-chromosome encoded erythro-megakaryocyte transcription factor GATA1. In normal GATA1-expressing cells, 2 GATA1 isoforms are detected; a full-length 414 amino-acid (aa) protein (GATA1fl) and an N-terminal truncated 331-aa protein, GATA1s. Somatic GATA1 mutations identified in TAM and ML-DS abrogate GATA1fl production but leave GATA1s expression intact.4 Experimental evidence from murine modes5,6,7 as well as from patient-derived induced pluripotent cells,8 demonstrate that exclusive expression of GATA1s promotes megakaryopoiesis at the expense of erythropoiesis. At the molecular level, Gata1s fails to repress Gata2 and Runx1 transcription despite both Gata1 and Gata1s having similar chromatin occupancy at both loci.9 GATA2 and RUNX1 expression are required for hematopoietic stem and progenitor cells (HSPCs) to balance self-renewal and differentiation.
but their expression is normally down-regulated during terminal differentiation.\(^9\)\(^\sim\)
Moreover, RUNX1 has been shown to play a fundamental role during lineage fate decisions at the erythroid/megakaryocytic branching point by repressing KLF1 transcription, an erythroid specific transcription factor and promoting a megakaryocytic, FLI1-driven, gene expression program.\(^1\)\(^2\) However, since similar GATA1 mutations are not leukemogenic in the absence of trisomy 21, the key constitutional abnormality present in all patients with Down syndrome, the interaction between trisomy 21 and GATA1s is clearly of importance. It is still not clear if GATA1s inability to repress at least two key transcription factors, GATA2 and RUNX1, cooperates with trisomy 21 to induce cellular maturation delay.

**Altered chromatin accessibility and activated signaling in ML-DS**

Finally, transformation of TAM to ML-DS is most likely due to a single allele mutation in genes encoding the cohesin complex (47%), the JAK family kinases, MPL, KIT (48%) or epigenetic regulators such as KANSL1, EZH2, and SUZ12 (36%).\(^7\)\(^2\) This mutation frequency in ML-DS samples illustrates that transformation occurs by cooperation between aberrant transcription factor function due to exclusive GATA1s expression and activated signaling and/or deregulation of epigenetic process. With the advent of gene editing technologies such as CRISPR/Cas9, it becomes possible to functionally annotate the genetic variants during transformation of TAM to ML-DS. A murine model that closely resembles Gata1s driven pre-leukemia served as a platform on which to interrogate predicted loss-of-function variants required to induce frank leukemia.\(^1\)\(^2\) In this murine model of frank leukemia, a similar frequency of variants inducing activated signaling (32%) were observed. There was also an over representation of mutations in epigenetic regulators (40%). Surprisingly, there was a reduced mutational frequency in cohesin complex (5%), a four-protein ring-shaped structure necessary to bring distant regulatory units in close proximity to promoters through DNA-loop formation. This tells us that cohesin mutations might not be easily studied in murine tissue because of the difference in chromosome architecture between species. Moreover, it suggests that trisomy 21 background might be required to produce the high cohesin mutation frequency observed in ML-DS.

**Future perspectives**

Transformation of human preleukemia to leukemia is not entirely understood. ML-DS and its precursor disease TAM are genetically simple models where to study the transforming genetic and epigenetic changes to define maturation arrest at the molecular level. Despite our understanding of role of GATA1 and the mutation landscape in ML-DS, there are many questions left unexplored. Why do GATA1 mutant progenitor cells have a remarkable proliferative advantage during T21 fetal life? Why are N’ terminally truncated GATA1 mutations virtually absent from adult leukemia? Does the function of GATA1 changes according to ontology? Why there is a higher frequency of mutations in the cohesin complex in ML-DS than in adult acute myeloid leukemia? Nonetheless, with the mutational landscape of TAM and ML-DS defined as well as understanding of ML-DS pathogenesis new concept will emerge if mechanistic studies are performed on oncogenic cooperativity. A full understanding of oncogenic cooperation is necessary to prevent progression of pre-leukemia to transformed leukemia.

**References**


Korbel JO et al, shows that the increase risk for myeloid leukemia in Down syndrome individuals is confined to 8.35 megabases in chromosome 21.

The genetic alteration that promote acute megakaryoblastic leukemia in Down syndrome remained elusive until Wechsler J et al, demonstrated that leukemic cells ML-DS contain mutation in the GATA1 locus that lead to loss of the GATA1 full-length isoform.


Zhe Li et al, developed the first mouse model of exclusive expression of the short isoform of Gata1. This model demonstrates and enhanced proliferation of the megakaryocyte lineage and conversely the erythroid lineage is markedly diminished.


Labuhn et al provide the most comprehensive functional interrogation of the genetic variants of pre-leukemia and leukemia in ML-DS.