SEQ-ing the genetic constellation of acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is a heterogeneous cancer driven by a constellation of diverse recurrent genetic aberrations. The ease to sample bone marrow allows easy access to the cancer cells and enables deep exploration of the genetics that drive ALL. Naturally, with every new genetic tool, the genetic constellation of ALL is often the first frontier to be explored. These deep explorations result in a detailed map of the genetic constellation of ALL (Figure 1) which is the basis of World Health Organization classification of tumors of hematopoietic and lymphoid tissues.

From the 1960s, when karyotyping and chromosomal banding were established, investigators embarked on this 60-year journey of discovery. This discovery started with abnormal whole chromosome copy numbers termed aneuploidy. Excess chromosomes >50, also known as hyperdiploidy, was the most common driver (Figure 1). Translocations, where bits of chromosomes were aberrantly fused, led to the discovery of Philadelphia (Ph) chromosome t(9;22)/*BCR::ABL1* and t(1;19)/*TCF3::PBX1*. Translocations which do not change the banding patterns like t(12;21)/*ETV6::RUNX1* took a while longer before yielding to discovery.

Paralleling this discovery is better treatment. With better treatment, investigators found that these genetic drivers are prognostic i.e., they predict the risk of relapse. This prognostic value of genetic subtypes gave birth to genetic risk stratification and eventually genetically driven treatment like addition of imatinib and dasatinib for Ph ALL. However, the difficulty to karyotype lymphoblasts and the need for many different diagnostic platforms, like multiple fluorescence *in situ* hybridization (FISH) probes, limited widespread use of genetic stratification.

In the 2000s, gene arrays enticingly promised a single platform to interrogate genetic drivers of ALL. Gene expression microarrays, which measure the expression levels of tens of thousands genes at the same time, allowed the discovery of the "novel" subtype[1] (later found to be the *DUX4* subtype) and the Ph-like subtype.[2] Using single nucleotide polymorphism (SNP) arrays which simultaneously genotype hundreds of thousands of SNP, deletion of a segment in pseudoautosomal region 1 (PAR1) next to *CRLF2* was identified.[3]

In late 2010s, transcriptomic sequencing (RNA-seq) promised another revolution. With RNA-seq, we can study both genetic expression profiles and the sequences of mRNA. Together, RNA-seq allowed us to identify gene rearrangements, karyotype, gene expression patterns as well as sequence mutations. Using RNA-seq of leukemic blasts, Gu *et al.* elegantly showed that >90% of ALL patients can be assigned to a specific genetic subtype.[4] We and others have tried to implement RNA-seq in clinical practice.[5,6] With standardization of RNA-seq library preparations and affordable sequencing services, perhaps the most significant obstacle remaining was bioinformatics analysis. In this issue of Haematologica, Hu *et al*.[7] shared the Molecular Diagnosis of ALL (MD-ALL), an integrated analysis software for ALL subtype classification using RNA-seq. Using published RNA-seq data, they carefully selected the feature genes responsible for each subtype distinction, constructed machine learning models to perform gene expression analysis, and combined gene expression and genomic alterations to classify ALL subtypes. MD-ALL advanced the bioinformatics analysis for RNA-seq-based ALL classification by addressing three key areas:

i) a reliable reference dataset. Hu *et al*. assembled an RNA-seq dataset with 2,955 ALL cases around the world, representing more than 20 subtypes from both children and adult patients.

ii) standardization of gene expression analysis. With different analysis methods or features used, gene expression defined subtypes can be variable. For example, the *BCR::ABL1*-like subtype defined by European researchers have minor variations compared to the Ph-like subtype defined by St. Jude investigators.[2,3] Hu *et al*. tested the different feature selection methods and streamlined gene expression analysis

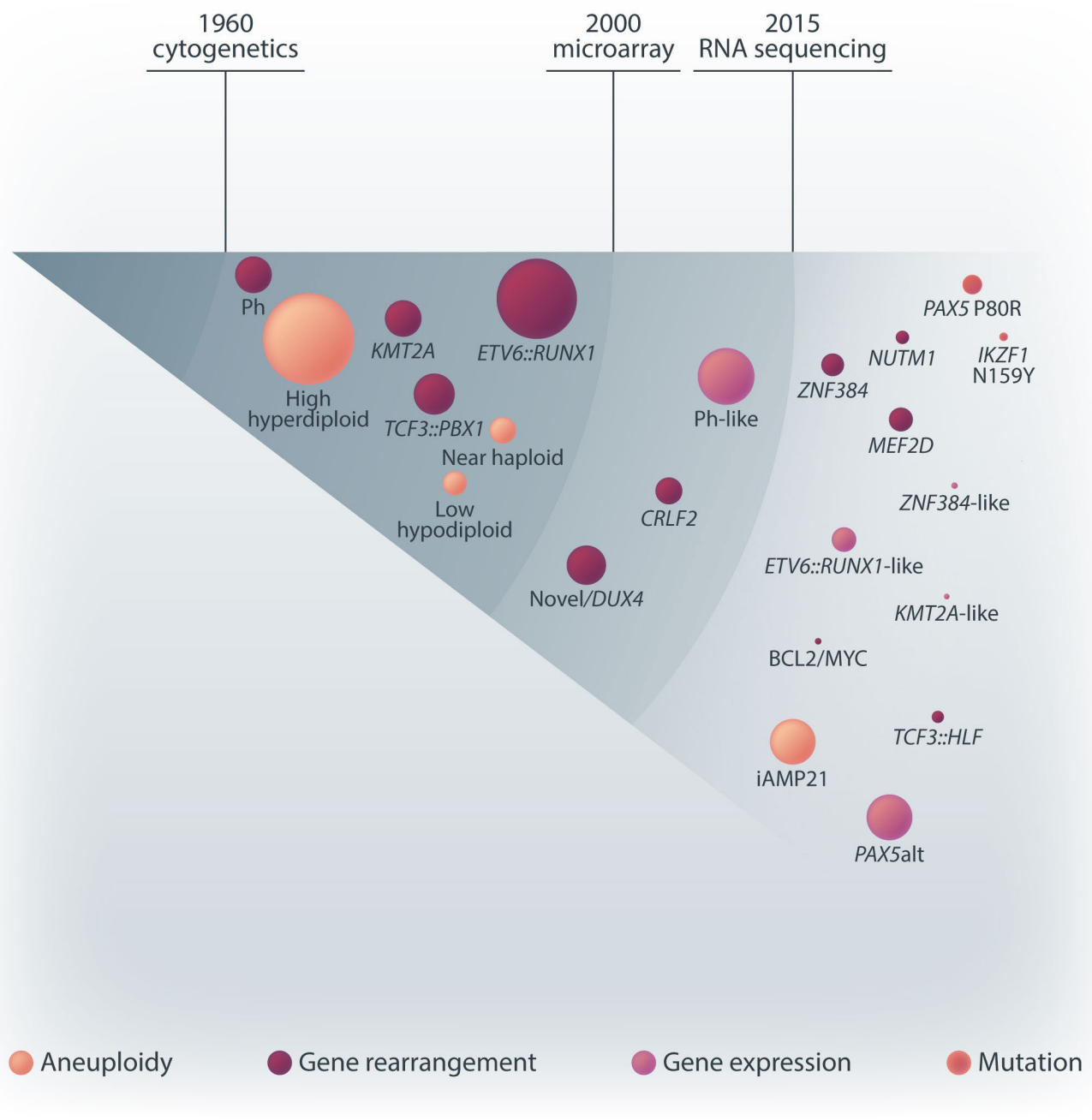

Figure 1. A brief history of acute lymphoblastic leukemia subtype classification. This figure summarizes the main technologies available and the subtypes discovered during different time periods. Sizes of the circles indicate approximate relative frequencies of acute lymphoblastic leukemia subtypes in children.

using multiple machine learning methods. This enhanced reproducibility and robustness for clinical use.

iii) integration of multiple types of information into a final call. Though majority of cases can be uniquely assigned to a subtype, multiple genetic events may appear together. For example, high hyperdiploidy can occur with *BCR::ABL1* fusion, and low hypodiploidy with *TP53* mutations. A decision-making workflow is implemented in MD-ALL.

The recent International Consensus Classification of acute lymphoblastic leukemia/lymphoma included nearly 30 subtypes. Efforts like MD-ALL are important for clinical use of the newly discovered subtypes, particularly in resource-constrained settings.

ALL subtypes have distinct sensitivity patterns to commonly used chemotherapy agents,[8] targeted therapy, and even to immune therapy.[9] How to integrate these subtypes into risk stratification or treatment protocols need further investigations. For example, the *DUX4* subtype, despite poorer end of induction minimal residual disease (hence treated with intense treatment), have excellent outcomes. Yet, de-intensification for this favorable subtype needs to be done cautiously. On the other hand, intensifying therapy or use of novel treatment for newly discovered unfavorable subtypes, such as *TCF3::HLF* and *MEF2D*, is necessary. In additional, targeted or immune therapy could be used for certain subtypes, e.g., *ABL1* inhibitors and blinatumomab for Ph ALL creating a chemotherapy free regimen is exciting.[10]

We are on the cusp of a brave new world of ALL: better understanding of the biological basis of each genetic subtype and better ways to treat them. With better and more ways to treat ALL, exploration of the genetic constellation of ALL is no longer an academic exercise, it transforms care.

Disclosures

No conflicts of interest to disclose.

Contributions

Both authors wrote, reviewed and approved the manuscript.

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