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CDX2 and IDH1/2: new potential players in ALL

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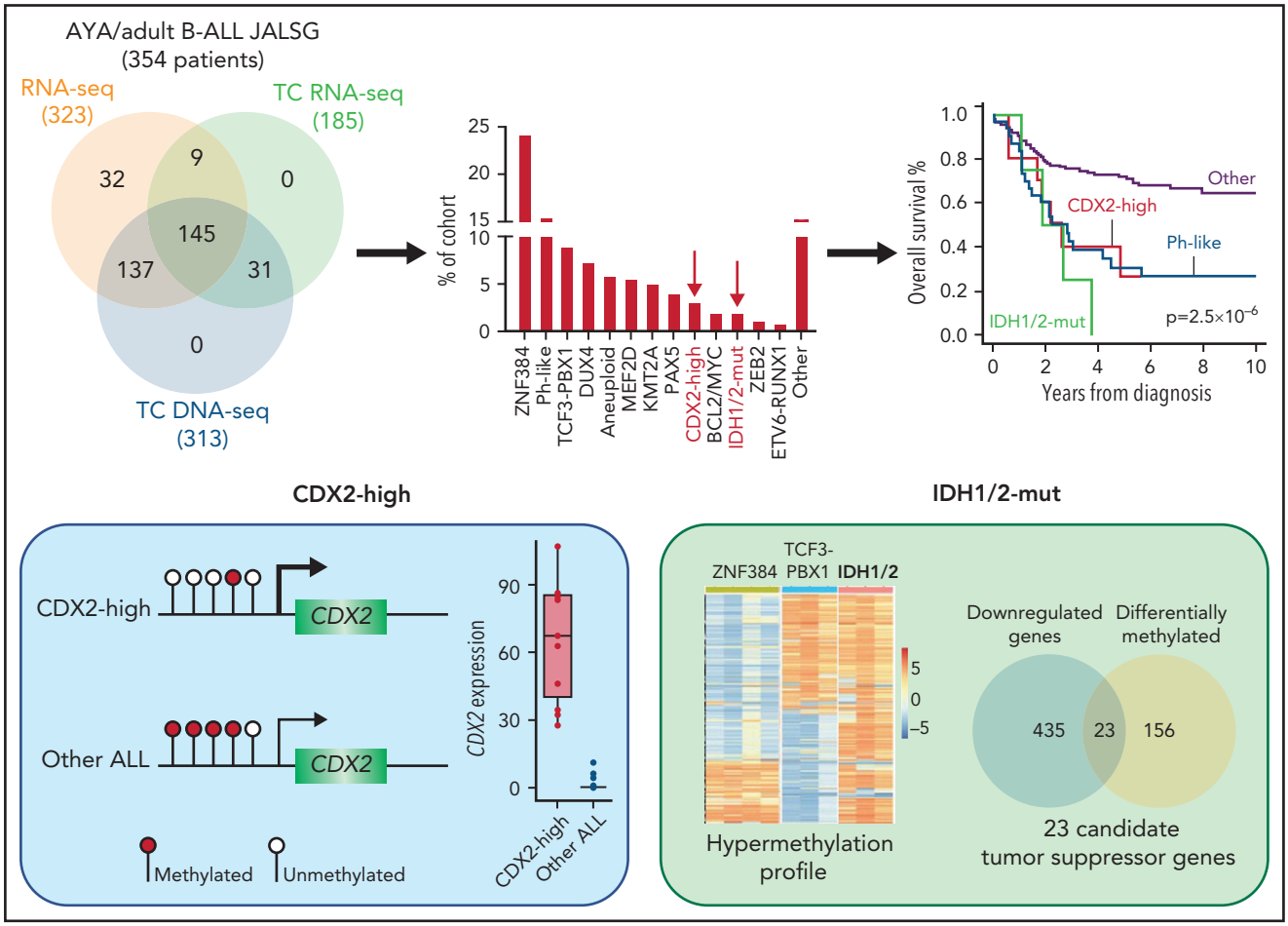
In this issue of *Blood*, Yasuda et al identify 2 putative subgroups of acute lymphoblastic leukemia (ALL) in adolescents and adults driven by high *CDX2* expression and *IDH1/2* mutations that are associated with poor prognosis.[1]

B-precursor ALL (B-ALL) is a genetically heterogeneous disease characterized by distinct founding alterations, including aneuploidy, chromosomal rearrangements and sequence mutations that are important for risk stratification.[2] Although ALL is less common in adolescents and young adults (AYAs) and adults than children, survival rates are markedly inferior (historically 30%-40% for adults compared with >85% for children), and the long-term prognosis for adults is poor.[2] In recent years, significant advancements have been made in treating both AYAs and adults because in part to the increased use of pediatric-inspired chemotherapy regimens[3] and the introduction of novel immune therapies, including monoclonal antibodies (eg, inotuzumab ozogamicin, blinatumomab) and chimeric antigen receptor T cells, which have shown remarkable activity in the relapsed/refractory setting and are now being tested in frontline trials.[4] Improvements in our understanding of the biological differences in ALL across the age spectrum have also helped to explain this discrepant prognosis. In the era of cytogenetic diagnostic assays, it was observed that the prevalence of genetic alterations associated with a favorable outcome decreased in adults (eg, hyperdiploid ALL), whereas those associated with a poor prognosis simultaneously increased (eg, the Philadelphia



An integrated analysis was performed on 354 adolescent and young adult (AYA) and adult patients with B-ALL enrolled in Japan Adult Leukemia Study Group (JALSG) trials. Genomic profiling included whole transcriptome sequencing (RNA-seq), targeted capture (TC) RNA-seq, and TC DNA sequencing. The authors identified 2 novel and distinct subgroups of B-ALL characterized by high *CDX2* expression (3.4%, CDX2-high) and *IDH1/2* mutations (1.9%, IDH1/2-mut) that were both associated with poor outcome. The promoter of *CDX2* was hypomethylated in the CDX2-high group compared with other ALL, leading to outlier high expression. The IDH1/2-mut group was associated with a hypermethylation profile that converged on 23 candidate tumor suppressor genes that may play a role in IDH1/2-induced leukemogenesis.

chromosome [Ph]).[2] More recent comprehensive next-generation sequencing has revealed multiple cytogenetically cryptic alterations, most commonly gene rearrangements, that constitute distinct ALL subgroups driven by distinct gene expression profiles.[5] These studies have further refined the genomic landscape of AYA and adult ALL and have identified new therapeutic targets, with the identification of subtypes associated with poor outcome, such as Ph-like ALL, but have also revealed subsets of adult ALL patients with a relatively good prognosis, such as those harboring rearrangement of *DUX4*.[6] They have also revealed new founding roles for genes previously considered support players in the development of ALL, including subgroups driven by *PAX5* (PAX5 P80R and PAX5alt).[5] Despite these new insights, a significant proportion of ALL remains genetically unclassified.

This study by Yasuda et al extends our knowledge of the genomic repertoire in ALL. They performed integrated whole transcriptomic and targeted DNA sequencing on a cohort of 354 Ph-negative B-ALL cases aged 15 to 64 years enrolled on consecutive Japan Adult Leukemia Study Group trials and classified them into 22 specific molecular subtypes (see figure). Two small but distinct clusters of patients were identified, characterized by high *CDX2* expression (3.4%, "CDX2-high") and *IDH1/2* mutations (1.9%, "IDH1/2-mut"). The frequency of both groups was significantly lower in children: 0.3% and 0%, respectively. Of clinical importance, both CDX2-high and IDH1/2-mut subgroups were independently associated with poor survival, similar to Ph-like ALL.

The authors demonstrate outlier allele specific expression of *CDX2* compared with all other ALL and normal lymphocytes that was associated with hypomethylation at the *CDX2* promoter and recurrent gain of chromosome 1q. Expression of CDX2 at the protein level was confirmed in matched diagnosis and relapse samples, suggesting its importance in both the development and maintenance of leukemia. CDX2 is a caudal-related homeobox transcription factor involved in early embryogenesis and hematopoietic development, although its expression in adult tissues is restricted to the intestine.[7] Although previous studies have demonstrated moderate levels of *CDX2* expression in acute myeloid and lymphoid leukemia,[8] this is the first study to demonstrate by whole transcriptome sequencing that outlier high expression constitutes a distinct ALL subgroup. Interestingly, in contrast to its role in normal hematopoietic development, CDX2-induced leukemogenesis is not driven by HOX genes in ALL but may involve different mechanisms including the insulin-like growth factor-1 receptor pathway, which also provides a potential therapeutic target.

The IDH1/2-mut subgroup was characterized by clonal *IDH1* R132C and *IDH2* R140Q mutations in diagnosis samples that persisted through to relapse in cases with available material. *IDH1/2* genes are known initiating events in AML and brain tumors, but their role in ALL is less clear. The distinct gene expression signature, including upregulation of genes associated with mitochondrial function, and a paucity of additional genomic alterations in this new subgroup suggest that *IDH1/2* may also be founding events in ALL. In AML, mutations in *IDH1/2* are associated with increased methylation.[9] Likewise, the IDH1/2-mut ALL group harbored a hypermethylation profile compared with other ALL, suggesting a new mechanism for lymphoid leukemogenesis, whereby aberrant methylation may lead to silencing of critical genes important for B-cell development. Accordingly, by combining DNA methylation and gene expression data, the authors identified 23 candidate tumor suppressor genes whose downregulation may play a role in IDH1/2-mut ALL. Functional experiments are required to validate these findings.

This paper lays the foundation for future studies focused on understanding the importance of CDX2 and IDH1/2 in the development of ALL, and significant work is required to demonstrate their role as initiating or founding events. For CDX2-high, the genomic mechanism responsible for outlier *CDX2* expression and additional genomic alterations that are required for leukemogenesis are still unknown. Comprehensive whole genome sequencing or long-read sequencing platforms will be helpful in illuminating these underlying mechanisms. Understanding the biology of these subgroups and functional contribution of CDX2 and IDH1/2 to leukemogenesis is also a critical question that remains to be explored. Furthermore, ~15% of AYA and adult ALL cases in this study remained uncharacterized; we must continue to push the boundaries of genomic technologies and algorithms to uncover all subtype-defining alterations in ALL.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Yasuda T, Sanada M, Kawazu M, et al. Two novel high-risk adult B-cell acute lymphoblastic leukemia subtypes with high expression of *CDX2* and *IDH1/2* mutations. *Blood*. 2022;139(12):1850-1862.
2. Roberts KG. Genetics and prognosis of ALL in children vs adults. *Hematology Am Soc Hematol Educ Program*. 2018;2018:137-145.
3. Stock W, Luger SM, Advani AS, et al. A pediatric regimen for older adolescents and young adults with acute lymphoblastic leukemia: results of CALGB 10403. *Blood*. 2019;133(14):1548-1559.
4. Phelan KW, Advani AS. Novel therapies in acute lymphoblastic leukemia. *Curr Hematol Malig Rep*. 2018;13(4):289-299.
5. Gu Z, Churchman ML, Roberts KG, et al. PAX5-driven subtypes of B-progenitor acute lymphoblastic leukemia. *Nat Genet*. 2019;51(2):296-307.
6. Paietta E, Roberts KG, Wang V, et al. Molecular classification improves risk assessment in adult BCR-ABL1-negative B-ALL. *Blood*. 2021;138(11):948-958.
7. Darvishi M, Mashati P, Khosravi A. The clinical significance of CDX2 in leukemia: a new perspective for leukemia research. *Leuk Res*. 2018;72:45-51.
8. Riedt T, Ebinger M, Salih HR, et al. Aberrant expression of the homeobox gene CDX2 in pediatric acute lymphoblastic leukemia. *Blood*. 2009;113(17):4049-4051.
9. Figueroa ME, Abdel-Wahab O, Lu C, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell*. 2010;18(6):553-567.

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