IN THE SPOTLIGHT

Fingerprinting Acute Leukemia: DNA Methylation Profiling of B-Acute Lymphoblastic Leukemia

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Summary: In this issue of *Cancer Discovery*, Geng and colleagues report on their use of a combination of promoter cytosine methylation profiling with gene expression and ChIP sequencing to elucidate molecular signatures of adult B-acute lymphoblastic leukemia patient samples with *BCR–ABL1, E2A–PBX1,* and *MLL* rearrangements. The unique epigenetic and gene expression signatures of these clinically unfavorable B-ALL subtypes identify novel biomarkers and provide a strong rationale for repurposing existing therapies to treat these molecularly distinct diseases. *Cancer Discov; 2(11); 976–8. ©2012 AACR.*

Commentary on Geng et al., p. 1004 (4).

The advent of next-generation sequencing technology has provided cancer researchers with an unprecedented ability to characterize the molecular diversity of tumors. Whole-genome sequencing approaches have recently revealed a plethora of new genetic lesions with prognostic and therapeutic relevance (1). The contribution of epigenetic deregulation to leukemia has also gained increased attention. Certain molecular lesions associated with acute myelogenous leukemia (AML) have been shown to impart a hypermethylated gene signature, providing a rationale for the use of inhibitors that target aberrant methylation (2). In adult B-acute lymphoblastic leukemia (B-ALL), several molecular lesions are known to be associated with a poor prognosis, such as *BCR–ABL1, E2A–PBX1,* and *MLL* rearrangements (MLLr; ref. 3); however, the underlying molecular mechanisms are only partially understood.

In this issue of *Cancer Discovery*, Geng and colleagues (4) report on their comprehensive molecular analysis of a large cohort of adult B-ALL patients to identify novel biomarkers, improve risk stratification, and provide a rationale for targeted therapies. The authors combined promoter cytosine methylation and gene expression profiling in 215 adult patients newly diagnosed with B-ALL, focusing on 3 cytogenetically defined B-ALL subtypes associated with poor outcome (BCR–ABL1, MLLr, and E2A–PBX1) compared with normal pre-B cells. Certain DNA methylation signatures overlapped among the B-ALL subtypes, for instance, enrichment of MYC target genes in all 3 groups and BCL6 target genes in both *BCR–ABL1* and *MLLr* B-ALLs. In addition, compared with normal pre-B cells, the majority of gene promoters found to be differentially methylated are hypomethylated in all B-ALL subtypes, suggesting that dominant gene activation pathways are triggered by these fusion proteins. However, what was strikingly evident was that each subtype of B-ALL displayed a unique pattern of cytosine methylation and gene expression. Using predictive algorithms, the power of these unique epigenetic and gene expression signatures was confirmed by their individual ability to reclassify with 85% to 100% accuracy the BCR–ABL1, E2A–PBX1, or MLLr status of the B-ALL patient cohort.

In BCR–ABL-positive B-ALL, *interleukin (IL-2) cytokine receptor alpha chain (IL2RA)* was the most overexpressed and the second most hypomethylated gene, forming the core of a gene network that correlated gene expression and methylation. IL2RA encodes CD25 and is normally expressed in developing pre-B cells. Expanding the cohort to a total of 465 B-ALL patients, the authors conducted flow cytometry for CD25 expression and found that the majority of *BCR–ABL*-positive patients were also positive for CD25 and that *IL2RA* was indeed the most differentially expressed gene and hypomethylated. In addition, the *BCR–ABL*-positive patients with CD25 expression have significantly worse overall survival than the CD25-negative patients. These data suggest that CD25 expression has clinical relevance as a biomarker and might be a driving factor in the poor overall survival of *BCR–ABL*-positive B-ALL.

Recent studies have shown that CD25 expression in a large cohort of *de novo* patients with AML also confers inferior overall survival and serves as a novel prognostic biomarker (5). How BCR–ABL1 expression induces hypomethylation of the IL2RA promoter remains to be studied, but the current findings provide a rationale for developing therapies that focus on the IL2RA signaling network to improve survival of this subset of BCR–ABL-positive B-ALL patients.

Unlike BCR–ABL1, whose effect on DNA methylation is most likely to be indirect, given that it predominantly resides in the cytoplasm of leukemic cells (6), E2A–PBX1 and MLL-fusion proteins are DNA-binding transcriptional regulators capable of directly influencing gene expression. Therefore, in these subtypes of B-ALL, the authors carried out chromatin immunoprecipitation (ChIP) experiments to determine which of the core genes that were hypomethylated and overexpressed were also directly bound by these fusion proteins.

E2A–PBX1 is known to act as a transcriptional activator that cooperates with the histone acetyl transferase p300 (7).

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doi: 10.1158/2159-8290.CD-12-0435

ChIP sequencing (ChIP-seq) for E2A, PBX1, and p300 revealed that 96% of the putative E2A–PBX1 binding sites indeed overlap with p300; however, only 8% of these joint-binding sites were located at traditional promoters, with the majority instead located to intronic, distal, and intergenic regions. The limitation of looking only at the promoter DNA methylation is highlighted here where E2A–PBX1 promoter binding sites represent the minority of fusion protein–binding sites. Nevertheless, the hypomethylated and upregulated genes in the core signature of E2A–PBX1 B-ALL show strong correlation with the binding of E2A, PBX1, and p300, suggesting that this complex drives the epigenetic and transcriptional signature of these genes directly.

In the case of MLL-fusion proteins, more than 60 translocation partners involving MLL have been reported (8). The role that each translocation partner may play in disease and the distinct molecular functions of MLL fusions are still unclear. Although there are particular translocations that show lineage specificity and hence lineage-associated gene expression patterns (e.g., *MLL–AF4* in ALL and *MLL–AF9* in AML), there seems to be a core gene expression profile found in all *MLL*-rearranged human leukemias independent of lineage or fusion partner. One such dominant gene signature typical of all *MLL*-rearranged leukemias is the high expression of multiple HOX family genes (8). Given that all leukemogenic MLL-fusion proteins retain the CXXC domain, which binds to nonmethylated CpG DNA sites (9), it is possible that the MLL-fusion proteins directly regulate a subset of these genes by virtue of this motif independently of the fusion partner. In addition, the authors suggest that *MLLr* cases may impart a common DNA methylation profile because many of the MLL-fusion partners, such as AF4, ENL, and AF9, form part of a transcriptional elongation complex that can either directly or indirectly recruit DOTL1 histone 3 lysine 79 (H3K79) methyltransferase (10). This may, in part, explain why the authors found no significant differences in the DNA methylation and gene expression patterns when they compared *MLL–AF4, MLL–ENL, MLL–AF9,* and *MLL–EPS15* cases of *MLLr* B-ALL. It is possible, however, that the statistical power was too low to assess the contribution of the underrepresented fusion partners in this analysis.

When all MLLr-positive B-ALLs were compared with MLLr-negative B-ALL cases in the cohort, a unique signature was observed, with *FLT3* and *BCL6* among a core set of genes that were hypomethylated and overexpressed. Furthermore, by conducting ChIP-seq, the authors confirmed binding overlap for MLL, AF4, and H3K79me2 at the promoters of *FLT3* and *BCL6*, implicating both genes as direct targets of MLL–AF4. FLT3 and BCL6 are well-known oncoproteins in leukemia and lymphoma, respectively, and both are highly sought after targets for therapeutic intervention. Several FLT3 inhibitors have been developed and successfully used in the treatment of *FLT3*-mutated AML (11). Other groups have shown that FLT3 is highly expressed in *MLLr* leukemias (12), which in combination with the current study makes FLT3 an attractive therapeutic target in this B-ALL subtype. It also seems feasible that targeting BCL6 in MLLr B-ALL may be an effective therapeutic strategy given that inhibitors of BCL6 have been developed for the treatment of BCL6-positive B-cell lymphomas. The peptide inhibitor RI-BPI (retroinverso BCL6 peptide inhibitor) blocks the transcriptional repressor activity of BCL6 by inhibiting binding with SMRT (silencing mediator for retinoid and thyroid hormone receptor) corepressors (13). When the authors tested this inhibitor *in vitro*, they found that it specifically causes apoptosis, loss of cell viability, and a block in colony-forming capacity of MLLr-positive B-ALL cells.

Repurposing existing drugs for the treatment of different diseases is part of a new initiative by the NIH to speed up the translation of research findings into new treatment regimens. On the basis of the molecular characterization presented by Geng and colleagues (4), the fact that IL-2 receptor (14), FLT3 (11), and BCL6 inhibitors have been approved for use in clinical trials for the treatment of leukemia and lymphoma makes their potential use for treating B-ALL an imperative. Beyond the scope of the current study is the impact of genes aberrantly hypermethylated and underexpressed in B-ALL. Hypermethylated gene signatures may be targeted therapeutically by U.S. Food and Drug Administration–approved DNA methyltransferase and histone deacetylase inhibitors but further molecular studies need to be done to understand which aberrantly silenced genes would need to be reactivated to provide a beneficial outcome to B-ALL patients.

The current findings show the validity of combining epigenomic approaches with cytogenetic and gene expression profiling to identify novel therapeutic targets and biomarkers and improve risk stratification for patients with B-ALL. These findings also provide a striking example of how a specific molecular lesion within a phenotypically similar group of tumors can impart unique disease characteristics. These results form the groundwork for implementing selective therapies to treat patients with B-ALL. They also highlight how we can use the current sequencing technologies to diagnose and treat the heterogeneity of lineage-specific tumors, bringing us another step closer to personalizing medicine.

Disclosure of Potential Conflicts of Interest

L. Cimmino is supported by the National Health and Medical Research Council Overseas Biomedical Research Fellowship. I. Aifantis is a Howard Hughes Medical Institute Early Career Scientist.

Acknowledgments

The authors thank members of the Aifantis Lab for discussions and comments.

Grant Support

The Laboratory is supported by the NIH (RO1CA133379, RO1CA105129, RO1CA149655, and RO1GM088847), the William Lawrence and Blanche Hughes Foundation, the Leukemia & Lymphoma Society, The V Foundation for Cancer Research, and by a Feinberg Lymphoma Grant.

Published online November 12, 2012.

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