inferior survival. In multivariate analysis, *SF3B1* mutation maintained an independent adverse effect on overall survival of relapsed/refractory patients, along with del(17p)/*TP53* mutation and complex karyotype. The unfavorable effect of *SF3B1* mutation could be related to the generation of alternatively spliced transcripts.

Sequencing technologies have dramatically improved insights into the mutational landscape of CLL, and the number of recurrently mutated genes identified is growing. Many mutated genes in this and other studies have shown a prognostic effect on the outcomes of patients with CLL. However, the identification, reproducibility, and validation of the clinical effect of novel gene mutations require large series of cases uniformly treated and analyzed. The sample size was not sufficient in this study to achieve adequate power to demonstrate a predictive impact of *IKZF3* mutations on response to lenalidomide or to better clarify the clinical significance of other recurrent mutated genes. Larger prospective studies are therefore needed to elevate the multitude of novel mutations identifiable with the always advancing technologies from the research to the clinical setting.

There is also a growing need for data from studies exploring the effects of the genetic background on the outcomes of patients included in clinical trials comparing chemoimmunotherapy with targeted agents. These studies could better define the best treatment option for patients with a given genetic profile. Overall, large, cooperative studies applying next-generation sequencing techniques with standardized methods in uniformly treated or untreated patients are needed to rigorously ascertain the role of each or multiple gene mutations in the pathogenesis, outcome, and treatment management of patients with CLL.

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MYELOID NEOPLASIA

Comment on Shima et al, page 1833

A fellowship of Ring1 maintains AML stem cells

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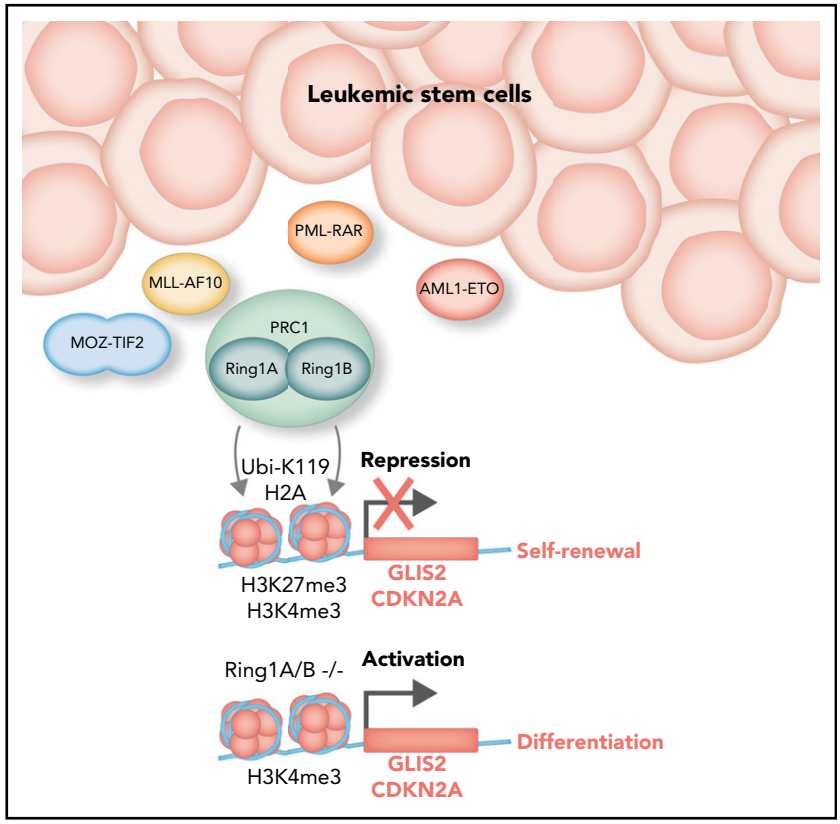
Polycomb repressive complex 1 (PRC1) and PRC2 are transcriptional repressors that function as key regulators of the self-renewal and differentiation (cell lineage specification) pathways in stem cells. In this issue of *Blood*, Shima et al[1] report that 2 ubiquitin ligases (Ring1A and Ring1B), which are key components of PRC1 complexes, are essential for the establishment and maintenance of acute myeloid leukemia (AML) in mouse models.

AMLs frequently harbor nonrandom, recurrent chromosomal translocations involving fusions of genes encoding transcription factors, chromatin (epigenetic) regulators, or signaling proteins. The resultant oncogenic fusion proteins such as MLL-AF10, AML1-ETO, or MOZ-TIF2 can subvert normal self-renewal and differentiation pathways and promote the proliferation of leukemia-initiating cells and their progeny. Revealing the mode of action of leukemic fusion proteins and the processes that underpin them is important to advance new strategies to treat AML.

The Shima et al study focused on MOZ-TIF2, which results from translocations involving the *KAT6A* and *NCOA2* genes, encoding the histone acetyltransferase MOZ and the nuclear receptor coactivator TIF2, respectively. This resultant expressed protein contains the *N*-terminal half of MOZ comprising a histone acetyltransferase domain and several histone recognition domains fused to the C-terminal portion of TIF2, a region that contains domains for binding other epigenetic regulators such as the CBP/p300 acetyltransferases. Expression of MOZ-TIF2 protein in bone marrow progenitors is sufficient to induce leukemia in mice, revealing that the oncogenic fusion protein has a causal role in leukemogenesis.[2] However, the modes of action of the MOZ-TIF2 and other leukemic fusion proteins (eg, MLL-AF10 and AML1-ETO) are complex, as they can promote changes in histone modifications and impact the stability or recruitment of other epigenetic regulators to chromatin, and thus produce substantial changes in gene expression. For example, MOZ-TIF2 interacts with hematopoietic transcription factors, including AML1/Runx1 and Pu.1,[3] as well as chromatin regulators such as CBP/p300, KDM4C, PRMT1, and the ING/BRPF1/EAF6 complex.[4-6] This complexity of function potentially exacerbates



Schematic representation of the action of Ring1A and Ring1B proteins in promoting the maintenance of AML cells derived from MOZ-TIF2, MLL-AF10, PML-RAR, or AML1-ETO fusion proteins. Ring1 proteins associated with PRC1 complexes catalyze the ubiquitylation of lysine K119 of histone H2A (H2AK119-Ubi). This modification, in addition to the H3K27me3, contributes to repression of genes, including *GLIS2* and *CDKN2A*, promoting the self-renewal of leukemia-initiating cells. Because these normally bivalent promoters also contain the activating chromatin modification H3K4me3, inactivation of Ring1 results in derepression of *GLIS2*, *CDKN2A*, and other genes, promoting differentiation.

the challenge of developing bespoke therapies for AMLs driven by different translocation events.

The study by Shima et al, combined with several other reports,[7,8] now reveals the importance of the Ring1A and Ring1B ubiquitin ligases in facilitating the maintenance and survival of leukemia-initiating cells derived from several different oncogenic fusions (see figure). Ring1A and Ring1B are core components of PRC1 Polycomb complexes that repress genes that promote differentiation. A major function of Ring1 (the collective term for the Ring1A and Ring1B proteins) is to add a ubiquitin polypeptide to lysine residue K119 of the histone H2A. This event can be facilitated by PRC2-mediated deposition of H3K27me3, a repressive mark that can be recognized by other components of PRC1. The trimethylation of H3K27, together with H2AK119-Ub, is believed to tether RNA polymerase II complexes on regions that also contain active marks such as H3K4me3, and therefore, such "bivalent" promoters may be poised for expression in response to differentiation signals. Shima et al showed that conditional inactivation of Ring1 results in differentiation of the MOZ-TIF2–induced leukemic cells into mature myeloid cells. As a consequence, the loss of H2AK119-Ubi releases the brake on expression of a cohort of differentiation promoting genes that includes the cell-cycle regulator *CDKN2A*.

CDKN2A is a tumor suppressor gene that encodes the INK4A/ARF proteins (also termed p16 and p19), and many studies have shown that repression of this gene by Polycomb complexes and other regulators is vital to maintain the self-renewal capacity of stem cells. Interestingly, both wild-type MOZ and MOZ-TIF2 play a role in the repression of *CDKN2A* to inhibit senescence in normal and leukemic stem cells, respectively,[9,10] although the mechanism is not fully elucidated. Consistent with other studies,[7] inactivation of Ring1A/B resulted in derepression of *CDKN2A*, accompanied by concomitant loss of PRC1-mediated H2A ubiquitylation and H3K27me3, which is catalyzed by PRC2. However, using combined knockout experiments, these studies revealed that inactivation of *CDKN2A* gene by genetic ablation was not sufficient by itself to restore fusion protein-dependent leukemogenicity,[1,7] suggesting other targets are important in this process.

In attempts to identify other players, Shima et al turned to transcriptomic profiling to identify genes that are derepressed by Ring1 inactivation and identified a strong upregulation of *GLIS2*, which encodes a Krüppel-like zinc-finger protein. *GLIS2* is involved in reciprocal translocations with *CBFA2T3/ETO2* gene in pediatric acute megakaryoblastic leukemia patients, and Shima et al provide further evidence for a role for *GLIS2* in the differentiation of myeloid progenitors. However, enforced overexpression of GLIS2 in MOZ-TIF2–transformed cells only delayed rather than blocked AML progression, and similarly, simultaneous inactivation of GLIS2 and Ring1 did not rescue MOZ-TIF2–induced leukemogenesis. However, the inactivation of GLIS2 in addition to Ring1 preserved a population of AML-derived granulocyte-macrophage progenitors. These results support a role for *GLIS2* in myeloid differentiation but suggest that *GLIS2* and *CDKN2A* are not the only Ring1 targets whose suppression facilitates leukemogenesis.

In summary, PRC1 Polycomb complexes appear to be key guardians of normal hematopoietic stem cell populations, in addition to being vital to the propagation of AML arising from different recurrent chromosomal translocations. The advances reviewed here show that Ring1 ubiquitin ligase activity is essential to these processes,[1,7] perhaps identifying a potential Achilles' heel to target in AML-initiating cells.

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

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MYELOID NEOPLASIA

Comment on Berger et al, page 1846

Ups and downs of CHIP

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In this issue of *Blood*, Berger et al present a longitudinal study of the clonal trajectory of preleukemic mutations in patients who developed therapy-related myeloid neoplasms (t-MNs) after autologous stem-cell transplantation. The authors show that t-MN driver mutations are often detectable as clonal hematopoiesis of indeterminate potential (CHIP) many years before patients develop t-MNs, and they demonstrate complicated patterns of clonal expansion and evolution over time, leading to the development of t-MNs.[1]

t-MNs are one of the most devastating complications from cytotoxic chemotherapy and ionizing radiation therapy. Although overall incidence of t-MNs is <10% among patients with cancer, t-MNs often end with fatal outcomes.[2] When a patient develops a t-MN, we as oncologists struggle to share the bad news with the patient, who has already fought or been fighting the primary malignancy. It is hard to imagine how difficult it must be for the patient. Because current treatment modalities are ineffective in curing t-MNs, there is a real-world unmet need to predict and prevent t-MNs before their occurrence.

Recently, several studies identified that preleukemic mutations or chromosomal copy number alterations were detectable in the blood samples of patients with cancer before treatment.[3-7] Detection of preleukemic clonal hematopoiesis was associated with an increased risk of t-MNs. Similarly, preleukemic mutations were also detectable in autologous stem-cell apheresis samples, which was associated with significantly increased risk of t-MNs and non–t-MN–related mortality in patients with lymphoma.[8] The study by Berger et al, which accompanies this commentary, provides data on how these preleukemic mutations evolve over time, particularly in response to cytotoxic chemotherapies or hematopoietic growth factors.

The authors describe clonal kinetics of preleukemic mutations in 7 patients who developed t-MNs by analyzing multiple sequential blood or marrow samples taken before the t-MN development. Although the number of patients studied here was too small to derive meaningful patterns, the heterogeneous clinical courses of the 7 patients generated interesting questions about how clonal hematopoiesis behaves in response to external agents. In 1 patient, clonal expansion of *SMC1A* along with an increase in mean corpuscular volume followed treatment with danazole and erythropoietin. What do we know about clonal hematopoiesis and response to hematopoietic growth factors? In another patient, a *TP53*-mutated clone gradually increased while acquiring another *TP53* mutation during thalidomide and cyclophosphamide treatments. What is the response of clonal hematopoiesis to immunomodulatory imide drugs? In some patients, the authors observed clear expansion of preleukemic mutations under the selective pressure of chemotherapy, whereas there were small independent clones that remained stable. Why did some clones remain stable or disappear while others expanded under the pressure of chemotherapy? Ultimately, this leads to a more clinically relevant question: which clones have a high risk of developing into t-MNs, and which will remain stable for a long time? Longitudinal analyses of clonal hematopoiesis in a large number of patients as well as biological studies that address mechanisms of transformation from clonal hematopoiesis to t-MNs are needed to answer these questions.

There were several other findings that were noteworthy in the report by Berger et al. First, preleukemic mutations were also detectable in a T-cell fraction in 1 patient. Although this needs to be verified in larger cohort, it suggests that CHIP originates at early hematopoietic stem-cell or progenitor stages, which has been previously suggested by other studies.[9,10] Second, the authors concluded that lymphoma did not arise from CHIP, because the mutational landscape was completely different. Third, p53-overexpressed cells were detectable in marrow by immunohistochemistry in patients with *TP53*-mutated CHIP. Because mutations are detected "digitally," if these cells truly represent *TP53*-mutated CHIP, immunohistochemistry may help visualization of CHIP in marrow. Lastly, the authors found that t-MNs had higher numbers of mutations compared with de novo MDS but did not have a specific mutation signature; however, interpretation of these findings requires caution, because the data were derived from whole-exome sequencing, and they contradict previous findings.[7] Nonetheless, the study by Berger et al has helped advance our understanding of how CHIP behaves during chemotherapy and contributes to the development of t-MNs.

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