these 19 genes accounted for 81% of all mutations detected. The most frequently mutated genes were NRAS, Kras, PAX5, and Janus kinases in > 10% of patients each. When these mutations were combined with copy number alterations, the following 4 known cancer signaling pathways—B-cell development and differentiation, Ras signaling, JAK/STAT signaling, and the TP53/RB1 tumor suppressor—were involved in 68%, 54%, 11%, and 54% of cases, respectively. In a subset of cases, multiple genes from the 4 individual signaling pathways were mutated. More surprising was the finding that the mutations of the Ras signaling pathway were not mutually exclusive; 5 patients had multiple mutations of NRAS or Kras. These data suggest a strong selection for mutations within these signaling pathways and, as not all genes in these pathways were sequenced, these results likely provide an underestimate of the mutation rates in high-risk BCP-ALL. With the exception of the B-cell development and differentiation pathways, the frequency of alterations was higher in this high-risk patient cohort than unselected BCP-ALL patients.9,12 There was a striking difference in frequency of mutations within the 4 major pathways between the ROSE cluster groups. For example, virtually all patients in the R8 subgroup, which is associated with a high incidence of relapse, had mutations in the B-cell development and differentiation pathways. They had a higher frequency of mutations in TP53/RB1 and JAK pathways, while the rate of Ras mutations was lower than other subgroups. Notably, each ROSE cluster group was also characterized by distinct patterns of copy number alterations. IKZF1 deletions and rearrangements leading to CRLF2 overexpression were particularly prevalent and significantly associated with cluster group R8. Collectively, these findings indicate that the genetic profile, as defined by the differential gene expression patterns and genomic aberrations, contributes to patient outcome.

In addition to these mutations in key pathways, inactivating mutations were observed in other noncanonical pathways, including ETV6 and CREBBP. Using a similar targeted sequencing approach, authors from this paper by Zhang et al have recently reported the strong association between mutations in the histone acetyltransferase, CREBBP, and relapsed BCP-ALL.11 These findings endorse the value of comprehensive evaluation of sequence alterations toward yielding additional biologic insights into this disease.

This study has extended the prior knowledge of the genetics of high-risk childhood BCP-ALL. It has begun to decipher the interrelationships between different genetic abnormalities and place them into clinical context.

Sequencing of the entire coding genome is likely to add to these findings, while the study of unselected patient cohorts is required to fully establish the clinical relevance. In the meantime, this demonstration that the genetic basis of high-risk BCP-ALL is truly multifactorial has highlighted potential novel therapeutic approaches, for example, targeting of the Ras signaling pathway.

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

REFERENCES


Comment on Balusu et al page 3096

NPM1-mutated AML: targeting by disassembling

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In this issue of Blood, Balusu and colleagues provide preclinical evidences that targeting nucleophosmin (NPM1) induces differentiation and death of acute myeloid leukemia (AML) cells harboring NPM1 mutations. These findings establish the rationale for novel treatment strategies in this large subgroup of AML.

NPM1 is one of the most abundant proteins in the nucleus. Despite its nuclear localization, NPM1 physiologically shuttles constantly across various cell compartments (nucleolus, nucleoplasm, cytoplasm) and this traffic is critical for most of its functions, including regulation of ribosome biogenesis and control of centrosome duplication. NPM1 also interacts with the tumor suppressor p14ARF and p53, and influences the cellular apoptotic response, although its exact role in this pathway still remains poorly understood. Mutations involving the NPM1 gene are the most frequent molecular alteration in AML with normal karyotype, accounting for ~ 60% of cases (ie, one-third of adult AML). As a consequence of these mutations, the NPM1 mutant intracellular traffic is altered leading to its aberrant accumulation in the...
cytoplasm of leukemic cells.4 Through formation of heterodimers with the wild-type NPM1 protein, the leukemic mutants also dislocate the normal nucleophosmin in the cytoplasm (see figure).4

Because of its distinctive molecular, pathologic, and clinical characteristics,5 NPM1-mutated AML has been included as a provisional entity in the 2008 World Health Organization classification of lympho-hemopoietic neoplasms. AML with mutated NPM1 is generally characterized by good response to induction chemotherapy3 and favorable prognosis (in the absence of a concomitant FLT3-ITD mutation).5 However, a significant number of cases with NPM1-mutated AML still show poor outcome, especially those associated with FLT3-ITD mutation and elderly patients. Therefore, there is the need for exploring new treatment strategies.

Intervening on the abnormal traffic of the NPM1 mutant appears a difficult task.4 A more concrete possibility in NPM1-mutated AML is to interfere with the function of residual wild-type NPM1 encoded by the normal allele. NPM1 mutations in AML are always heterozygous,4 thus NPM1-mutated AML retains in the nucleolus a certain amount of wild-type NPM1 protein.4 This finding and the observation that complete deletion of NPM1 gene in knockout mice leads to death during embryogenesis2 strongly suggests that maintaining wild-type NPM1 in the nucleolus may be required for leukemic cell survival.4,6

Therefore, there is a rationale for interfering with wild-type NPM1 to enhance the propensity of NPM1-mutated AML cells to die or to be killed by chemotherapeutic drugs.3 Balusu et al explore this interesting issue.

Using the human Ontario Cancer Institute (OCI)/AML3 cell line (known to harbor NPM1 gene mutation), the authors show that knocking down NPM1 induced differentiation, growth inhibition, and increased apoptosis more specifically in NPM1-mutated AML cells than in other cell lines not carrying the NPM1 mutation. These events were associated with activation of the p53 tumor suppressor pathway and down-regulation of HOX A9 and Meis1 genes, which are known to be up-regulated in NPM1-mutated AML4 and likely contribute to leukemogenesis. Knockdown of NPM1 also abolished lethal AML phenotype induced by OCI-AML3 cells in the immunocompromised NOD/SCID mice.1 Balusu et al also provide evidence that down-regulation of NPM1 acts in synergy with other drugs, such as all-trans retinoic acid (ATRA) and cytarabine, in inducing cell apoptosis.1 Interestingly, ATRA itself was previously reported to induce in vitro apoptosis in NPM1-mutated AML cells in association with down-regulation of NPM1 mutant protein.2 However, it remains uncertain whether the addition of ATRA to induction chemotherapy improves survival in NPM1-mutated AML patients.

Balusu and colleagues also explored the effects of a small molecule (NSC348884) that is able to interfere with NPM1 oligomerization. They showed that inhibition of the NPM1-NPM1 interaction induced apoptosis and sensitized OCI-AML3 and primary NPM1-mutated AML cells to ATRA without affecting NPM1 protein levels. By contrast, AML or normal CD34+/H11001 progenitor cells not carrying NPM1 mutation were relatively unaffected.

The underlying mechanisms of these pharmacologic effects are still unknown. Interfering with the levels or the oligomerization status of NPM1 may influence its capability to properly build up the nucleolus in NPM1-mutated AML cells (see figure). This feature is related to the fact that wild-type NPM1 (and other proteins, such as nucleolin) act as “hub” proteins (ie, capable to interact with many other proteins) that serve as building blocks for nucleolar assembly.8 Interestingly, ablation
of either NPM1 or nucleolin by RNA interference is sufficient to disrupt the nucleolar structure. Similarly, the anticancer peptide CIGB-300 leads to nucleolar disassembly and apoptosis, most likely through its capability to bind NPM1.10

Because AML cells carrying the NPM1 mutation are depleted of wild-type NPM1 protein in their nucleolus pool (because of haploinsufficiency and cytoplasmic dislocation through formation of heterodimers with the mutant), they are more sensitive than cells with germ line NPM1 gene (containing a full dose of wild-type NPM1) to drugs that affect the levels and oligomerization status of NPM1 and lead to disruption of the nucleolar structure (see figure).5 Thus, tuning the dose of these drugs could become a strategy for targeting leukemic cells harboring NPM1 mutations more selectively than other leukemic or normal cells.

Conflict-of-interest disclosure: B.F. applied for a patent on the clinical use of NPM1 mutants. M.P.M. declares no competing financial interests.

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Comment on Gong et al, page 3163

Warfarin pharmacogenetics meets clinical use

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The FDA in their revised approval of the warfarin prescribing information of 2007 noted the relevance of genetic testing for VKORC1 and CYP2C9 variants. Yet there is still a lack of prospective clinical trials using genetic testing, underscoring the difficulty of a pharmacogenetics-based initiation dosing strategy. In this issue of Blood, Gong and colleagues provide evidence for the clinical use of a newly established warfarin pharmacogenetics-initiation protocol (WRAPID).1

A vision to harness genomic research for effective therapy and improved health care has been recently discussed in a key article by Green and colleagues.2 They predict that, with few exceptions, translation of genomic information to clinical practice will be implemented only beyond 2020. One major barrier is the lack of research activities addressing the clinical use of genetic tests. The study by Gong and colleagues provides a good example of how a prospective evaluation of a novel pharmacogenetics-initiation protocol for warfarin therapy can improve safety and efficacy of drug therapy, using recently proposed translational research guidelines.1

Warfarin has been used for > 60 years as an oral anticoagulant and has been shown to impressively reduce the risk of stroke in patients with atrial fibrillation. Although other oral anticoagulants (eg, dabigatran,4 rivaroxaban,5 apixaban) are beginning to emerge as potential alternatives, warfarin will continue to be an important drug. Warfarin over-anticoagulation leads to a substantial increased risk for bleeding complications, as recently corroborated by a nationally representative public health surveillance of adverse drug reactions and a cross-sectional survey of outpatient medical visits. Here, warfarin was listed as 1 of the 10 most commonly implicated drugs in older adults for emergency department visits for adverse drug events.3

The impact of genetic variants in the vitamin K epoxide reductase complex subunit 1 (VKORC1) and the drug metabolizing enzyme cytochrome P450 2C9 (CYP2C9) has been well established by several retrospective and prospective studies. The dosing requirements of warfarin are unpredictable and highly variable (up to 20-fold).4 Variants in both VKORC1, which recycles the oxidized form to the reduced hydroquinone form of vitamin K1, and CYP2C9, which predominantly catalyzes (S)-warfarin to the 7-hydroxylated form as the major metabolite, determine the requirement of warfarin dosing. Although there have been previous attempts at pharmacogenetics-based initiation of warfarin dosing, the work by Gong et al for the first time employs VKORC1- and CYP2C9-dependent loading doses in patients treated for atrial fibrillation and venous thromboembolism. Because the dosing regimen includes loading and maintenance doses of warfarin, systematic pharmacokinetic and pharmacodynamic factors have been taken into consideration for the application of a novel standardized loading dose algorithm (WRAPID) in a prospective cohort study of outpatients (see figure).

In addition to VKORC1 and CYP2C9, other candidate genes such as CYP2C19, CYP2C19, CYP4F2, and apolipoprotein E, microsomal epoxide hydrolase, protein C, calumenin, y-glutamyl carboxylase, and orosomucoid 1/2 have been targeted to optimize warfarin dosing, with inconsistent results. Although the contribution of CYP4F2 could not be excluded with certainty in the present study, it is important to note that, after warfarin initiation based on the WRAPID algorithm with the incorporation of VKORC1 and CYP2C9 genotypes only, no significant effects have been observed on the time required to reach the first international normalized ratios (INRs) within the therapeutic range of 2.0-3.0 and on the time spent in therapeutic range or
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