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Comment on Han et al, page 2796, and comment on Fawal et al, page 2780

ALK-mediated oncogenesis: mechanisms that matter

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Two reports in this issue of *Blood* provide new insights into the lymphomagenic mechanisms used by ALK fusions, one highlighting the effects of loss of expression of the tyrosine phosphatase SHP1 that occurs in the majority of ALK-positive lymphomas and the other describing a heretofore unappreciated role for ALK fusions in the enhancement of mRNA stability.

Primary systemic anaplastic large cell lymphoma (ALCL) can be subdivided into 2 biologic subtypes based on the presence or absence of oncogenically transforming fusions of the anaplastic lymphoma kinase (ALK), a receptor tyrosine kinase truncated and fused to a variety of N-terminal, activating partner proteins—the most common chimeric form being nucleophosmin (NPM)–ALK—in these lymphomas.[1] The distinction between the so-called ALK-negative and ALK-positive ALCLs (the latter colloquially known as ALKomas) is relevant both from basic research and clinical standpoints. For example, a substantial body of basic research data suggest ALK-positive ALCLs to be "ALK addicts" that are exquisitely dependent upon the continuous growth-promoting cellular signals conferred by chimeric ALK proteins, making ALK a very attractive target for the development of directed therapies for future clinical use such as small molecules analogous to the BCR-ABL inhibitors imatinib and dasatinib that have revolutionized chronic myeloid leukemia treatment. Two articles published in this issue of *Blood* report important basic research data that help to further elucidate the downstream cellular signaling mechanisms that modulate oncogenic transformation by ALK fusions and that may ultimately permit identification of additional targets for therapeutic intervention in the clinic for ALK-positive ALCLs.

Han and colleagues report studies that follow up on earlier data from this group showing that almost all ALK-positive ALCLs experience silencing of the nonreceptor tyrosine phosphatase SHP1 due to methylation of the *SHP1* gene promoter. SHP1 phosphatase is normally abundant in hematopoietic cells, but is silenced in many hematologic cancers; the biologic importance of SHP1 is exemplified by the phenotype of so-called moth-eaten mice, in which absent or markedly reduced Shp1 expression causes abnormal myeloid cell development and function as well as a propensity for lymphomagenesis.[2] Using ALK-positive ALCL cell lines and primary tumor samples, Han et al show that reintroduction of *SHP1* expression into lymphoma cells that had silenced the gene produces marked reductions in the phosphorylation and activation of JAK3 (a known dephosphorylation target for SHP1) and STAT3 and down-regulation of the STAT3 transcriptional targets cyclin D3, MCL1, and BCL2.

Intriguingly, re-establishment of SHP1 expression in ALCL tumor cells also resulted in marked decreases in the levels of both JAK3 and NPM-ALK, an effect due to enhanced proteasome-mediated protein degradation by a yet-to-be defined mechanism. The biologic consequences of SHP1 re-expression were noteworthy: Karpas-299 and SU-DHL-1 ALCL cells experienced significant cell-cycle arrest and impaired growth upon introduction of exogenous SHP1. These data, as well as recent corroborating results from others,[3] demonstrate that SHP1 loss contributes to the growth of ALK-positive ALCLs by allowing enhanced, unregulated phosphorylation and activation of JAK3/STAT3 and by permitting increased levels of JAK3 and NPM-ALK protein expression due to decreased proteasome degradation. Taken collectively, these results provide impetus for additional preclinical studies to examine the feasibility of therapeutic efforts for the clinical management of ALCL (and other SHP1-silenced cancers) that are designed to reawaken SHP1 expression by the inhibition of *SHP1* promoter methylation.[4]

In a second ALK-related study, Fawal and colleagues report a novel oncogenic signaling mechanism—enhancement of mRNA stability by a fusion tyrosine kinase. These investigators discovered the physical interaction of NPM-ALK with AUF1/hnRNPD, one of the family of AU-binding proteins (AU-BPs) that regulates the half-lives of many mRNAs by directly interacting with A+U–rich elements (AREs) found in their 3′ untranslated regions.[5,6] AUF1/hnRNPD was shown to be hyperphosphorylated due to its association
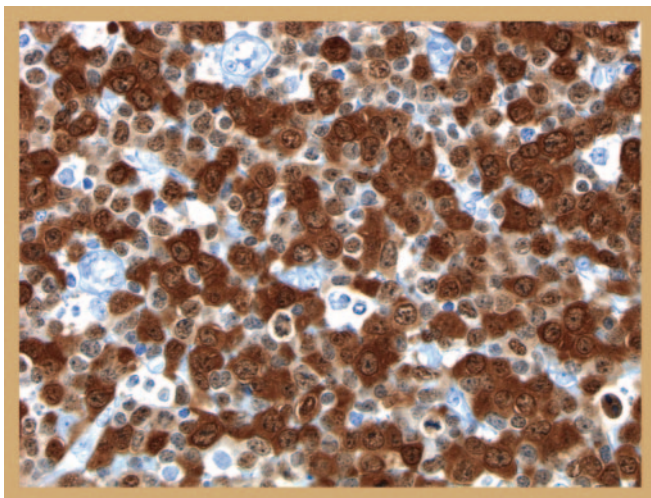
Anti-ALK immunostain of a typical NPM-ALK–positive anaplastic large-cell lymphoma. Illustration by Dr Mihaela Onciu, St Jude Children's Research Hospital.

with NPM-ALK, and this phosphorylation correlated with the increased stability of a number of mRNAs encoding critical growth-promoting proteins such as c-MYC and cyclins A2, B1, D1, and D3. Biologically, this posttranscriptional enhancement of mRNA stability was associated with an increased survival of cells in which transcription had been experimentally arrested by actinomycin D.

These data are consistent with recent results from a number of investigators indicating that the mRNA-binding properties of AU-BPs including AUF1/hnRNPD can be modified by various posttranslational modifications such as ubiquitinylation, methylation, and phosphorylation. The observations of Fawal et al show that NPM-ALK increases the stability of otherwise short-lived mRNAs, thus contributing to enhanced proliferation, survival, and oncogenesis. Now established for NPM-ALK, it will be important to examine other oncogenic kinases and AU-BPs for a similar functional interplay; if identified, such a mechanistic relationship could represent an important, heretofore unrecognized, paradigm underlying oncogenic transformation in not only hematopoietic but perhaps other malignancies as well. ■

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Comment on Pollard et al, page 2764

FLT3: the root of the problem

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Most AML patients with FLT3/ITD mutations have poor outcomes. This article by Pollard and colleagues suggests that the explanation, not surprisingly, may be found at the root of the problem, the leukemia stem cell.

Acute myeloid leukemia (AML) is organized into a hierarchy that in some respects parallels normal hematopoiesis.[1] Primitive hematopoietic precursor cells acquire mutations that eventually result in transformation to leukemia. The exact stem or progenitor cell that eventually becomes the leukemia stem cell probably varies from case to case, but the more primitive it is, the less curable the leukemia is likely to be.

FLT3/internal tandem duplication (ITD) mutations have emerged as the most common molecular abnormality in AML, and only a minority of patients harboring these mutations can be cured.[2] It has been postulated that FLT3 mutations are relatively late hits in leukemogenesis because they are not by themselves capable of inducing transformation and are occasionally lost (or acquired) at relapse.[3,4] Nonetheless, previous work using nonobese diabetic/severe combined immunodeficient (NOD/SCID) engraftment and polymerase chain reaction (PCR) assays of CD34+/CD38− fractions of AML samples suggests that FLT3 mutations are present in leukemia stem cells.[5] The findings of Pollard and colleagues, in this issue of *Blood*, offer another opportunity to understand how FLT3 mutations fit into the stem cell paradigm of leukemia.

These authors examined the FLT3 mutation status of 24 diagnostic AML samples from pediatric patients harboring FLT3/ITD mutations. Their purpose was to determine if the FLT3 mutations were present in mature versus immature myeloid cell populations and whether this affected the clinical outcome. They sorted the samples into CD34+/CD33− (more primitive) or CD34+/CD33+ (less primitive) progenitor cell fractions and then used those cells to generate granulocyte-macrophage colony-forming units (CFU-GMs) and erythroid burst-forming units (BFU-Es) in colony assays. Using patient-specific FLT3 primers, PCR was used to determine mutation status of the sorted cells and of CFU-GMs and BFU-Es plucked from the colony plates. They were able to detect the FLT3 mutations in the more differentiated CD34+/CD33+ cells from all 24 of the samples. However, 5 of the 24 samples had no detectable mutation in the less mature CD34+/CD33− cells or in any of the colonies. In other words, the early progenitor cells of these 5 patients lacked the FLT3 mutations. The other 19 patients did harbor the mutations within the CD34+/CD33− fraction, and in some cases the mutations were present in both CFU-GM and BFU-E colonies, implying derivation from a precursor with bilineage potential. Strikingly, the 5 patients in whom the mutation was present in only the more differentiated cell fraction had
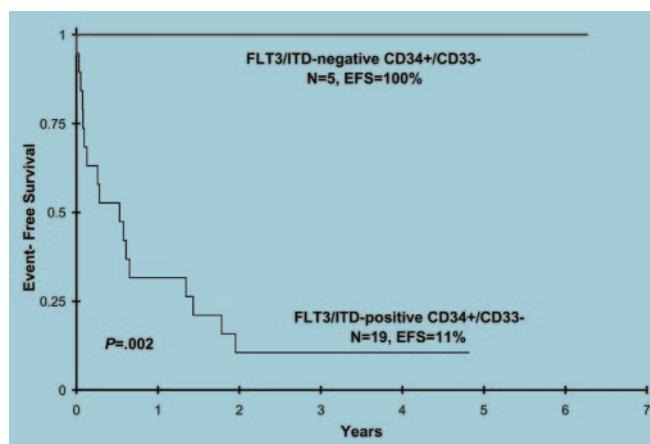
Event-free survival for patients with and without FLT3/ITD detection in CD34+/CD33− progenitor cells. See the complete figure in the article beginning on page 2764.