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Can Treating the SYK Cell Cure Leukemia?

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Targeted therapy has remained elusive for most acute myeloid leukemia patients. In this issue of *Cancer Cell*, Stegmaier and colleagues identify SYK as a key mediator of the differentiation block seen in this disease. Their data suggest that SYK inhibition may be an effective therapy for a significant proportion of AMLs.

Despite significant advances in the treatment of many hematopoietic malignancies, the treatment of acute myeloid leukemia (AML) remains a significant challenge, with current cytotoxic therapies producing overall cure rates of less than 50%. From a biological perspective, AML is known to be a heterogeneous disease composed of different genetic subtypes with distinct clinical features and responses to contemporary therapies. Recent results of detailed molecular studies aimed at defining the underlying pathogenesis of AML have yielded important insights into a number of the AML genetic subtypes. Moreover, these studies have directly impacted the development or application of new therapies targeted to the underlying genetic alterations or biological features of the leukemic cells. These advances have included the use of all-*trans* retinoic acid and arsenic for the treatment of acute promyelocytic leukemia, the recent development and assessment of FLT3 inhibitors for the treatment of patients with activating mutations in this receptor tyrosine kinase, and the use of gemtuzumab, a calicheamicin-conjugated anti-CD33 monoclonal antibody, for relapse. Despite these and other advances, however, we still lack a full understanding of the complement of genetic and epigenetic alterations that drive the malignant growth of AML blast. As a result, the identification of key alterations against which new therapeutic agents can be developed remains extremely difficult.

In this issue, an important way forward is revealed through an innovative study by Stegmaier and her colleagues, in which they outline an experimental approach that culminates in the unexpected finding that inhibition of the spleen tyrosine kinase, SYK, leads to differentiation and impaired growth in a high percentage of AML cell lines and primary patient samples (Hahn et al., 2009). Thus, although previous work suggested a role for this kinase primarily in B and T cells, mast cells, and macrophages, the present results implicate SYK as a new rational target for the treatment of AML.

These surprising results are based on a series of studies by this group that began back in 2004 with their development of a novel gene expression-based high-throughput small-molecule screening approach that used gene expression profiling as a surrogate for a desired biological state (Stegmaier et al., 2004). In their initial study, they demonstrated that 4,5-dianilinophthalimide (DAPH1) induced differentiation of the myeloid leukemia cell line HL60. Since DAPH1 was previously shown to inhibit the kinase activity of the epidermal growth factor receptor (EGFR), they next tested the Food and Drug Administration (FDA)-approved EGFR inhibitor gefitinib (Iressa) and demonstrated that it also was capable of inducing differentiation and inhibiting cell viability in several AML cell lines and, importantly, in a small number of primary AML patient samples (Stegmaier et al., 2005). These studies were rapidly confirmed by others and extended to a second FDA-approved EGFR inhibitor, erlotinib (Tarceva), which was shown to be slightly more potent than gefitinib at inducing differentiation and inhibiting cell viability (Boehrer et al., 2008). In addition, two patients with AML and concomitant non-small-cell lung cancer were reported to have complete remissions of their AML after receiving erlotinib intended for treating their lung cancer (Chan and Pilichowska, 2007; Pitini et al., 2008). Since AML cells do not express EGFR or the related receptor tyrosine kinase ERBB2, these data implicate an as-yet-uncharacterized AML kinase as the target of gefitinib/erlotinib. The most highly expressed tyrosine kinases in AML blasts include FLT3, KIT, INSR, CSF1R, JAK1, FYN, CSK, HCK, and SYK (Tomasson et al., 2008). However, with the exception of FLT3, KIT, CSF1, and JAK1, none of the other kinases are the target of activating mutations in AML (Loriaux et al., 2008; Tomasson et al., 2008). Moreover, none of these kinases bind in vitro to gefitinib or erlotinib (Fabian et al., 2005). Thus, the specific AML kinase(s) inhibited by gefitinib/erlotinib remained unknown.

To explore the mechanism by which EGFR kinase inhibitors induce differentiation of myeloid leukemia cells, Stegmaier used a combination of proteomic and RNAi-based screening methodologies (Figure 1). Using anti-phosphotyrosine immunoaffinity purification of digested cellular proteins, they found that after gefitinib treatment of HL60 cells, the SYK cytoplasmic tyrosine kinase was among the most highly dephosphorylated proteins detected, raising the possibility that SYK was the elusive kinase. To complement this approach, they then performed a forward genetic screen on HL60 cells with a kinome-targeted lentiviral-based shRNA library, coupled with a high-throughput gene expression-based readout for differentiation. Remarkably, this orthogonal approach identified a shRNA against SYK as one of the best inducers of differentiation. These initial results were confirmed on other leukemia cell lines and primary AML patient samples through the use of additional SYK shRNAs, as well as the SYK kinase inhibitor R406. Moreover, SYK was found to be highly expressed and phosphorylated in a significant subset of primary AML samples, consistent with constitutive activation. Thus, SYK appears to function as a critical node within an as-yet-uncharacterized AML signaling pathway that

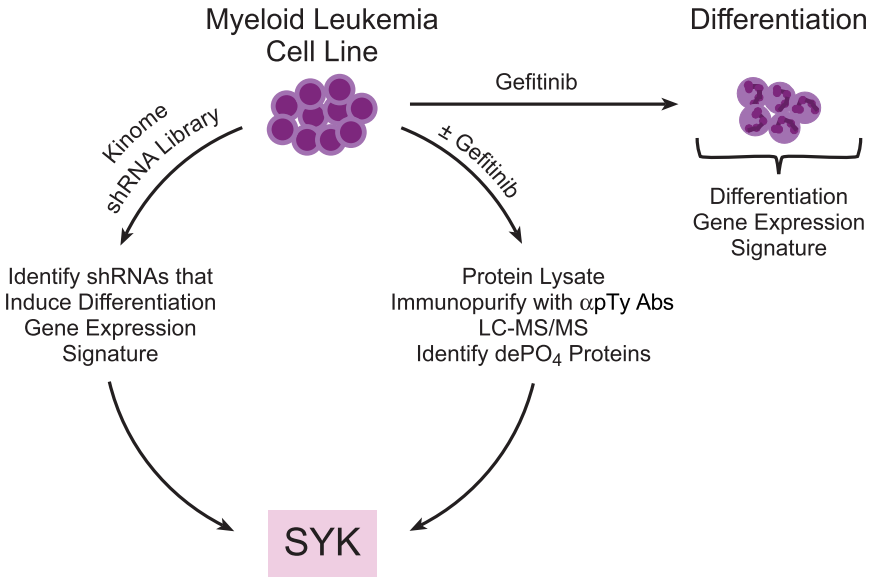

Figure 1. Orthogonal Approaches Identify SYK as a Target in AML

To define the off-target mechanism of gefitinib-induced myeloid leukemia cell differentiation, Stegmaier and colleagues used a combination of proteomics and shRNA methods. To identify the proteins most strongly dephosphorylated after gefitinib treatment, they enriched tyrosine-phosphorylated proteins from control or gefitinib-treated HL60 leukemia cells with anti-phosphotyrosine antibodies (αPTy) and analyzed them by LC-MS/MS. To complement this approach, they screened a kinome-enriched lentiviral-based shRNA library for clones capable of inducing a differentiation-associated gene expression signature. Remarkably, both approaches revealed that inhibition of SYK induced differentiation.

contributes to the block in differentiation and to enhanced cellular growth or viability.

SYK together with zeta-activated protein of 70 kDa (ZAP-70) constitute a family of nonreceptor tyrosine kinases that contain two N-terminal Src homology 2 (SH2) domains and a C-terminal kinase domain. SYK functions as a key mediator of immune receptors signaling in inflammatory cells (B and T cells, mast cells, macrophages, and neutrophils), and aberrant signaling through this kinase plays an important role in a range of autoimmune diseases including asthma, rheumatoid arthritis, and allergic rhinitis. As a result, the pharmaceutical industry has dedicated considerable resources toward developing SYK-specific inhibitors, and these drugs are quickly moving through clinical trials. Therefore, highly specific SYK inhibitors are available for direct clinical testing in AML.

As one contemplates clinical studies using SYK inhibitors to treat AML patients, there are several issues that will need to be addressed. Foremost among these is which patients will probably respond to SYK inhibition? In the present study, only a limited number of primary AML samples were analyzed, and they were heavily biased toward de novo AMLs with normal cytogenetics and/or FLT3 mutations. How will other de novo AMLs, myelodysplasia-related AMLs, or therapy-induced AMLs respond? Also, with the importance of leukemia stem cells in the pathogenesis of AML, it will be important to determine whether SYK inhibition has any effect on this population of cells—since cures will require their elimination. It will also be important to determine the mechanisms responsible for SYK activation in AML, since to date SYK has not been identified as a direct target of genetic lesions in this disease. Understanding the genetic or epigenetic alterations that lead to constitutive SYK activation will be valuable in identifying which patients have the best chance to respond to SYK-targeted therapy and whether targeting other component of the signaling pathway may be more appropriate in some patients. Also, since differentiation agents never produce durable cures on their own, the development of murine AML models with activated SYK should serve as valuable tools to help define the most effective drug combinations to move into clinical trials.

Although the identification of SYK as a therapeutic target in AML is surprising, previous studies have predicted that mutations in signal transduction pathways would be an essential step in leukemogenesis (Speck and Gilliland, 2002). The problem has been identifying the relevant targets. The approach used by Stegmaier to integrate proteomics, genome-wide expression profiling, and high-throughput RNAi-based screening should prove to be a valuable method for identifying a range of new therapeutic targets in AML and other cancers. Coupling this approach with genome-wide screens for mutation and epigenetic alterations will provide a detailed roadmap for the development of true personalized cancer therapy.

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