IN THE SPOTLIGHT

Discovering What Makes STAT Signaling TYK in T-ALL

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Summary: RNA interference screening establishes TYK2 dependence in T-cell acute lymphoblastic leukemia (T-ALL), leading to identification of TYK2-activating mutations and increased IL-10 receptor signaling in T-ALL cell lines. Cancer Discov; 3(5): 494–6. © 2013 AACR.

See related article by Sanda et al., p. 564 (2).

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy derived from thymic T-cell progenitor cells that undergo malignant transformation. T-ALL represents 10% to 15% of pediatric and 25% of adult ALL cases (1). From the molecular standpoint, T-ALL is a complex disease featuring many different recurrent somatic genetic alterations, affecting HOX11, TAL1, LYL1, LMO1, and LMO2, among others, which have been used to define molecular genetic subtypes in T-ALL (1). Among recurrent genetic lesions, Notch1 pathway activation (>60% of cases) and CDKN2A/2B inactivation (70% of cases) are the most frequent alterations (1). Although cure rates have improved with intensive chemotherapy—up to 75% in children and 50% in adults—the outcome of patients with refractory or relapsed disease remains poor (1). Accordingly, research efforts are focused on finding new treatments that can improve the outcome of these patients and reduce the toxicity of current treatments.

In this issue of Cancer Discovery, Sanda and colleagues (2) use RNA interference screening specifically directed for tyrosine kinases to look for the so-called “Achilles heel” of T-ALL. siRNA screening in primary cells from a pediatric T-ALL patient revealed dependence of these cells on TYK2, a member of the Janus-activated kinase (JAK) family of tyrosine kinases. TYK2 is known to associate with cytokine receptors and mediates signal transduction in combination with other JAK proteins to phosphorylate and activate STAT proteins (3). Tyk2−/− mice manifest mild immunodeficiency and increased susceptibility to viral or bacterial infections because of attenuated response to IFN-α/β and defective IFN-γ production, linked to defective interleukin (IL)-12 signaling (3). TYK2 also plays a role in development of T helper (Th)17 cells, responsible for recruitment of phagocytic cells at the sites of infection (3). Tyk2−/− mice are more susceptible to Abelson murine leukemia virus-induced B-cell leukemia/lymphoma, TEL-JAK2–induced T-cell lymphoid leukemia, and breast cancer due, at least in part, to a decrease in immune surveillance (4).

The current report by Sanda and colleagues (2) is the first to directly implicate somatic mutation of TYK2 in oncogenesis. To validate the relevance of TYK2 in T-ALL, Sanda and colleagues (2) knocked down 1,740 genes including the whole tyrosine kinome in 3 T-ALL cell lines. Consistent with their previous observation, a TYK2 shRNA was depleted from the T-ALL cell lines tested but had little effect on diffuse large B-cell lymphoma cell lines. Subsequently, short hairpin RNA (shRNA) knockdown effect was assessed in a larger panel of cells, among which TYK2 silencing significantly inhibited the growth of 14 of 16 T-ALL cell lines and 5 of 8 “primagraft” samples, which are primary cell lines derived from patient samples through expansion in immunocompromised mice. Decreased expression of TYK2 diminished cell growth rate by inducing apoptosis in sensitive cell lines (2).

TYK2, along with JAK1, has been reported to phosphorylate and activate STAT1 in response to IFNs type I and type II signaling (3). In an effort to characterize the TYK2 pathway in T-ALL cell lines, Sanda and colleagues (2) determined that STAT1 is phosphorylated downstream of TYK2. Use of gene expression profiling after the silencing of either TYK2 or STAT1 in the Jurkat T-cell line pointed toward BCL2 as a downstream target of this pathway, whereas other antiapoptotic and proapoptotic genes were not affected. Elegant rescue experiments overexpressing wild-type or kinase-dead TYK2 were carried out, showing that BCL2 expression is dependent on TYK2 kinase activity in this system. Similarly, expression of a knockdown-resistant STAT1 rescued the phenotype, whereas a nonphosphorylatable STAT1 did not, indicating that phosphorylation of STAT1 is required for BCL2 expression (Fig. 1).

The authors were able to find TYK2 mutations in 4 of the 16 T-ALL cell lines with TYK2 dependence. Mutations were present in the different domains of the protein and not restricted to the kinase domain. Five of 6 mutations found rescued Ba/F3 cells from IL-3 withdrawal, and protein extracts from these cells showed increased STAT1 phosphorylation and total levels. A kinase-dead TYK2 protein containing one of the newly identified activating mutations was unable to transform Ba/F3 cells; therefore, kinase activity is apparently necessary for TYK2-dependent cell transformation. However, many of TYK2-dependent T-ALL cell lines had no mutations in TYK2, so other mechanisms must be responsible for this dependence. To address this, cytokine receptor signaling through TYK2 was evaluated using shRNA knockdown, which identified IL-10 signaling as responsible for the increased cell

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growth, STAT1 phosphorylation, and increased expression of BCL2. In fact, IL-10 had already been reported as a survival factor for primary T-cells through upregulation of BCL2 (5).

This new report (2) suggests an important role for TYK2 in transducing survival signals in T-ALL. The phenomenon of TYK2 dependency is compelling, as this was shown in a primary T-ALL case, with functional validation targeting TYK2 addiction conducted both in cell lines and in primary cell–derived xenografts. It is notable in these studies that TYK2 dependence may extend beyond the situation where T-ALL cells harbor TYK2 somatic mutation. Hence, there may be other mechanisms causing biologic dependence on TYK2 in T-ALLs. It will be important to identify whether genetic background or perhaps other features, such as the differentiation stage of the cell of origin, are linked to this phenotype. It will also be important to confirm whether activating TYK2 somatic mutations can be identified from primary T-ALL specimens as well as the spectrum of T-ALLs that are TYK2 dependent. In spite of T-ALL cell lines and primary specimens being TYK2 dependent, none of the 45 patient samples studied in this report presented somatic mutations in TYK2. With the noted caveat that these are rare tumors and much remains to be explored in the genetic landscape of T-ALL, other mutation profiling studies have also not detected TYK2 somatic mutations (e.g., ref. 6). Mutations in TYK2 in T-ALL cell lines were proposed to be a consequence of time in culture by others (7). TYK2 mutation may thus be a rare event in patients with T-ALL. Nonetheless, several TYK2 single-nucleotide polymorphisms have been associated with increased susceptibility to various pathologic conditions, including multiple sclerosis, experimental allergic encephalomyelitis, and acute myeloid leukemia (8), although little is known about the effect of these variants on protein activity. In addition, Tyk2-deficient mice are predisposed to develop tumors because of their impaired IFN type I signaling causing an inefficient cytotoxic CD8+ antitumoral response (4). Yet, because TYK2 works commonly downstream of cytokine receptors hyperactivated in cancer, inhibiting TYK2 may have beneficial effects for cancer treatment.

Selective TYK2 inhibition in patients is not feasible with the JAK inhibitors that are currently in clinical trials, because they have been developed to differentially target JAK1 and/or JAK2. New inhibitors more specific to TYK2 are needed, and, indeed, some have already been identified (9) and need to be translated for use in humans. Along with TYK2 inhibition, addiction to IL-10 signaling and BCL2 expression can also be exploited therapeutically in T-ALL treatment. In fact, elevated IL-10 levels have been reported in more than 70% of adult T-cell leukemia cases (Fig. 1; ref. 10). Along these lines, rituximab treatment induced downregulation of IL-10 expression and may be responsible for the downregulation of BCL2 expression in T-ALL, sensitization of B-cell non-Hodgkin lymphoma cells by therapeutic drugs (11). Indeed, current clinical trials are in place to test the BCL2 inhibitors Obatoclax (NCT00933985: phase I in combination with vincristine/doxorubicin/dexrazoxane) and Oblimersen (NCT00060112: phase I in combination with gemcitabine) in T-ALL. In summary, TYK2 could represent a viable therapeutic target in T-ALL, potentially benefiting patients with IL-10- and/or BCL2-addicted tumors. A larger number of genetically characterized patients should be surveyed for TYK2 dependence to determine which patients will benefit from such intervention.
Disclosure of Potential Conflicts of Interest

A. Melnick has received honoraria from the Speakers’ Bureau of Genentech and is a consultant/advisory board member of Celgene. No potential conflicts of interest were disclosed by the other author.

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