Persistent LYN Signaling in Imatinib-Resistant, BCR-ABL–Independent Chronic Myelogenous Leukemia

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Resistance to imatinib is commonly associated with reactivation of BCR-ABL signaling. Many such patients harbor a mutation in BCR-ABL that confers resistance to imatinib. In other patients, the leukemia cells appear to express increased amounts of BCR-ABL kinase. Identification of a BCR-ABL mutation provides an explanation for onset of imatinib resistance and indicates a clear treatment strategy: second-line therapy with an ABL kinase inhibitor that is active against the particular BCR-ABL variant kinase. In the clinical setting, nilotinib and dasatinib are used for this purpose; additional inhibitors are under development.

However, not all cases of resistance can be attributed to a BCR-ABL mutation or enhanced expression. The phenomenon of BCR-ABL–independent resistance is probably more frequent than generally appreciated and of considerable clinical importance. At a mechanistic level, BCR-ABL–independent resistance is poorly understood. Although these patients may progress via clonal evolution, thus far relatively few key molecular players have been convincingly implicated.

SRC kinases are generally regarded to be important but not crucial downstream targets of BCR-ABL kinase in chronic-phase chronic myelogenous leukemia (CML). However, the SRC family member LYN kinase may take on added importance in some situations. Specifically, Donato et al. (1) and others (2) have previously shown that overexpression and activation of LYN are hallmarks of cells from some patients with imatinib resistance and progressing disease. In this issue of the Journal, an article from the Donato group (3) addresses this question in primary cells with well-designed ex vivo LYN silencing (short interfering RNA [siRNA]) and LYN add-in (transfection) experiments.

The authors consistently observed that cells from patients with imatinib-resistant, BCR-ABL mutation-negative CML exhibit attenuated BCR-ABL tyrosine kinase activity in response to imatinib and yet resist apoptosis. They also observed increased expression of LYN and persistent LYN activity, leading them to propose that this phenomenon may be a case of “transferred addiction,” in which LYN regulation is no longer primarily downstream of and dependent upon BCR-ABL signaling (Figure 1A and B).

This finding raises the question of whether the regulation of LYN activity is fundamentally different in CML cells from patients with BCR-ABL–dependent resistance and from those with BCR-ABL–independent resistance. In support of this possibility, the authors found a distinct LYN phosphorylation profile in cells from imatinib-resistant CML patients and also demonstrated coimmunoprecipitation of phosphorylated LYN, c-Cbl, and an 80-kDa protein that may be Gab2 (3). The use of primary cells is a particular strength of these studies, and several clues as to how persistent LYN signaling is facilitated were obtained, but a more complete mechanistic explanation will require further investigation (4).

The authors note that the imatinib-resistant patients in this cohort responded to dasatinib (Figure 1C), an established inhibitor of BCR-ABL and LYN kinases, as well as all other SRC family members. Although both the siRNA and inhibitor studies are consistent with LYN kinase involvement, one potentially informative experiment to further gauge the extent of dependence on LYN signaling would be to examine whether ex vivo treatment with the ABL and LYN dual tyrosine kinase inhibitor INNO-406 reproduces the cellular and biochemical responses observed after dasatinib treatment. Testing with nilotinib, which does not block kinase activity of the SRC family but can be effective in imatinib-resistant CML, would address whether more complete inhibition of BCR-ABL kinase activity relative to imatinib can shut down the putative BCR-ABL–independent LYN axis. Additionally, although the authors find no evidence of activating point mutations in LYN in imatinib-resistant patients, it would be interesting to see whether expression of a “gatekeeper-mutated” variant of LYN (the equivalent of T315I in ABL) in imatinib–resistant cells that overexpress LYN kinase would render these cells dasatinib resistant. Clearly, detecting such a mutation in a patient who does not respond to dasatinib treatment and also lacks a BCR-ABL mutation would be the ideal “experiment of nature” to support the importance of LYN for resistance.

An intriguing question arising from this work centers on the mechanism of activation of LYN kinase in the absence of upstream BCR-ABL signaling. One possibility is that cells from at least some patients with imatinib-resistant, BCR-ABL mutation–negative CML have instigated a “rewiring” of the LYN signaling cascade to establish or increase the reliance on an auxiliary BCR-ABL–independent mechanism (Figure 1B). Of note, overexpression of LYN kinase in imatinib-sensitive CML cells resulted in moderately reduced imatinib sensitivity resembling the level observed for weakly imatinib-resistant BCR-ABL mutations that can be overridden by dose escalation. This observation may imply that most or all of the components necessary for BCR-ABL–independent LYN activation are present but that events leading to rewiring...
have not yet occurred. Introduction of a constitutively activated LYN construct into imatinib-sensitive cells could potentially shed light on this issue. If phosphorylation of LYN is a crucial step toward BCR-ABL–independent disease, these cells might be expected to exhibit more profound resistance to imatinib. It would also be of interest to carry out LYN coimmunoprecipitation experiments with lysates from these cells to see if increased association with c-Cbl and/or the 80-kDa protein is observed. Also, it should be noted that, although an accurate estimate of incidence will require surveying a much larger patient population, persistent LYN signaling probably does not account for all patients with mutation-negative BCR-ABL who fail to respond to treatment (eg, dasatinib nonresponders).

We know that BCR-ABL participates in the activation of SRC family kinases, although likely not although direct phosphorylation. Although studies (5) in mice that are null for the three SRC kinases that are principally expressed in the myeloid lineage (LYN, HCK, and FGR) have revealed that BCR-ABL does not require SRC kinases to induce a myeloproliferative disease, this result does not preclude the possibility that a SRC kinase could replace BCR-ABL kinase in a cell that has managed to rewire. In this scenario, however, what is driving LYN activation? Although the authors detected no mutations in LYN, they found an abnormal phosphorylation pattern, implicating an as yet unidentified tyrosine kinase upstream of LYN or an abnormal adaptor protein that facilitates abnormal substrate utilization. We can only speculate whether this putative mechanism of transferred addiction in the face of sustained inhibition of BCR-ABL is driven by somatic mutations or is the result of plasticity that allows some cells to adapt to a situation in which BCR-ABL kinase is inhibited. Dissecting the underlying mechanism will be challenging. The work of Wu et al. in this issue (3) takes us outside of the realm of thoroughly studied kinase domain mutation-based resistance and toward an improved understanding of BCR-ABL–independent disease. In addition to opening new questions for exploration, these results suggest that therapies targeting both BCR-ABL and LYN kinases may prove beneficial in certain circumstances of imatinib-resistant CML.

References