Imatinib spells BAD news for Bcr/abl-positive leukemias

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One of the medical success stories of the past decade has been the development of new agents to treat chronic myelogenous leukemia (CML). Building on earlier studies that identified the t(9;22) chromosomal translocation in CML, cloned the *BCR/ABL* fusion gene, and demonstrated the ability of the resulting kinase to transform cells, investigators identified imatinib mesylate as an inhibitor of that kinase, demonstrated that imatinib inhibits proliferation and survival of Bcr/abl-transformed hematopoietic cells *in vitro* and *in vivo*, and performed clinical trials showing unprecedented activity in chronic-phase CML (1). When the earliest trials of imatinib in aggressive-phase CML and Bcr/abl-driven acute lymphocytic leukemia revealed emergence of resistance as a problem, the contribution of point mutations in the *BCR/ABL* gene was demonstrated (2), and kinase inhibitors that are unaffected by some of these mutations, including the recently approved dasatinib and nilotinib, entered clinical testing (3). It is important to emphasize, however, that *BCR/ABL* mutations are found in only a subset of imatinib-resistant Bcr/abl-driven leukemias (4). In this issue of PNAS, a report by Kuroda *et al.* (5) not only provides new insight into the cytotoxic action of imatinib but also potentially identifies another mechanism of resistance and a means for overcoming it.

The study of Kuroda *et al.* (5) builds on 20 years of research into the mechanism of action of Bcl-2 and its homologs. Based on structural and functional criteria, these polypeptides are divided into three subfamilies: an antiapoptotic group that includes Bcl-2, Bcl-x_L, Bcl-w, Mcl-1, A1, and possibly others; a proapoptotic multidomain group consisting of Bax, Bak, and Bok; and a "BH3-only" group that includes Bid, Bad, Bim, Noxa, Puma, and a handful of additional polypeptides that exhibit sequence homology with other Bcl-2 family members only in their 15-aa BH3 (Bcl-2 homology 3) domains (6). According to current models, proapoptotic stimuli cause Bax and Bak to breach the outer mitochondrial membrane (6, 7), leading to the appearance of cytochrome *c* and other mitochondrial intermembrane proteins in the cytoplasm (8), where they contribute to biochemical changes that result in cellular demise. Antiapoptotic family members such as Bcl-2, Bcl-x_L, and Mcl-1, which reside on the cytoplasmic surfaces of mitochondria and other cellular organelles, bind and neutralize Bax

Fig. 1. Schematic representation of Bcr/abl-mediated survival signaling. Bcr/abl activates several signal transduction pathways that are known to inhibit apoptosis. Bcl-2 family members shown by Kuroda *et al.* (5) to affect imatinib sensitivity are enclosed in yellow boxes. Activating protein–protein interactions or phosphorylations are indicated by black arrows, activating protein–protein interactions are indicated by black bars, inactivating phosphorylation events are indicated by red bars, and transcriptional activation events are indicated by green arrows.

and Bak before membrane disruption occurs. Conversely, BH3-only polypeptides facilitate the action of Bax and Bak, at least in part, by binding and neutralizing various antiapoptotic Bcl-2 family members so that they cannot intercept Bax and Bak (6). It is important to emphasize, however, that the assorted BH3-only polypeptides involved in activating apoptosis are not equivalent. Different BH3 polypeptides not only exhibit a wide range of affinities for various antiapoptotic Bcl-2 family members (9) but also are activated by different cellular stresses (10). For example, Bad is dephosphorylated and released from cytoplasmic 14-3-3 proteins upon withdrawal of cytokines or after other changes that diminish Akt signaling; Bmf is released from actin filaments when cell–substrate attachment is disrupted; and Bim is activated by detachment from microtubules or JNK-mediated phosphorylation. In addition, the genes encoding Bim, Noxa, and Puma are up-regulated by various stimuli (10, 11).

Earlier studies demonstrated that imatinib induces apoptosis in Bcr/abl-transformed cells (12, 13). In experiments that evaluated the role of Bcl-2 family members in this process, Kuroda *et al.* (5) demonstrated that imatinib causes Bad dephosphorylation as well as up-regulation of Bim and Bmf. Further experiments examining Bcr/abl-transformed hematopoietic cell lines from *Bim*^{−/−}, *Bad*^{−/−}, and double-knockout mice demonstrated a predominant role for Bim and a less

dominant but nonetheless important role for Bad in imatinib-induced cell death. In light of earlier studies showing that inhibition of Akt signaling can lead to Bad activation (14) as well as *BIM* induction (11, 15), the results of Kuroda *et al.* (5) can be largely explained by inhibition of a single signal transduction pathway downstream of Bcr/abl (Fig. 1), although other processes such as interruption of ERK-induced proteasome-mediated Bim degradation might also contribute. These observations fit nicely with recent suggestions that the Akt pathway, one of several prosurvival pathways activated by Bcr/abl (Fig. 1), plays a unique and critical role in Bcr/abl-mediated transformation (reviewed in ref. 16). Collectively, these results invite the speculation that Akt inhibitors, alone or in combination with MEK inhibitors, might also be active against Bcr/abl-driven leukemias, including those that are imatinib-resistant as a consequence of *BCR/ABL* mutations.

There are several additional take-home lessons from the current study. First, Kuroda *et al.* (5) failed to observe the imatinib-induced down-

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regulation of Bcl-x_L and Mcl-1 that has been reported in several Bcr/abl-transformed cell lines (17, 18). These results serve as a reminder that critical pathways regulating the apoptotic machinery might differ in important details between various cell lines and even within subclones of the same line. Second, Kuroda *et al.* (5) observed imatinib-induced Bmf up-regulation but could not demonstrate a critical role for this change in imatinib-induced death. These results emphasize the importance of analyzing the functional consequences of observed changes in Bcl-2 family members.

In the course of their studies, Kuroda *et al.* (5) demonstrated that Bcr/abl-transformed hematopoietic cells lacking Bim and, to a lesser extent, Bad, are resistant to imatinib-induced killing. Similar resistance was observed in cells engineered to overexpress Bcl-2 or Bcl-x_L. The potential implications of these observations for clinical imatinib resistance require further study. Much recent attention has been focused on the role of *BCR/ABL* mutations in recurrence of disease that initially responds to imatinib (so-called secondary resistance). Depending on the assay used (2), however, 10–50% of patients with secondary resistance lack detectable *BCR/ABL* mutations (4). Moreover, a number of the observed mutations scarcely alter imatinib sensitivity of the Bcr/abl kinase or transfected cells (19, 20), raising the possibility that additional mechanisms might play a role in secondary resistance. Furthermore, imatinib induces fewer remissions in patients with aggressive-phase CML and

other Bcr/abl-driven leukemias than in chronic-phase CML (4). Mechanisms of this so-called primary resistance remain incompletely defined (4, 21).

On the other hand, a role for elevated Bcl-2 or Bcl-x_L, diminished Bim or Bad, or even altered activation of the Akt and ERK pathways in primary or secondary resistance is not a foregone conclusion. A previous study analyzing samples from patients with CML in blast crisis failed to demonstrate a significant correlation between pretreatment elevation of Bcl-2 or Bcl-x_L transcripts and

Earlier studies demonstrated that imatinib induces apoptosis in Bcr/abl-transformed cells.

imatinib response (22). Moreover, when samples harvested from individual chronic-phase CML patients before imatinib treatment and at the time of relapse were compared, no statistically significant recurring alterations in levels of Bcl-2 family member mRNA were observed (23). Because these studies examined mRNA rather than the individual polypeptides, their posttranslational modifications, and their functional status, further investigation is required to assess the importance of Bcl-2 family members in clinical resistance to Bcr/abl inhibitors. If these components of

the apoptotic machinery are ultimately found to be altered in drug-resistant disease, the results of Kuroda *et al.* (5) indicate that ABT-737 might resensitize the resistant cells.

The results under discussion also have interesting implications for one of the unsolved problems in CML management. Recent analysis indicates that BCR/ABL-positive cells can be detected indefinitely in imatinib-treated chronic-phase CML patients (4), providing a reservoir that can contribute to relapse when imatinib is discontinued (24). Additional investigation has identified the persistent *BCR/ABL*-positive cells as CD34⁺ immature myeloid progenitors (25). The previous observation that early myeloid progenitors express high levels of Bcl-x_L (26), which would make them particularly resistant to induction of apoptosis by a variety of agents, not only provides a potential explanation for the persistence of the CD34⁺ *BCR/ABL*-positive cells in imatinib-treated chronic-phase patients, but also raises the possibility that imatinib might be able to eradicate this reservoir if combined with a Bcl-x_L antagonist such as ABT-737. It is hoped that this extrapolation from the results of Kuroda *et al.* (5) will be tested in the near future.

In summary, the provocative results of Kuroda *et al.* (5) provide new insight into the mechanism of imatinib-induced cytotoxicity, identify a potential mechanism of imatinib resistance that requires further investigation, and suggest a means for improving the efficacy of existing CML therapy. Studies that further explore the ramifications of these findings are awaited with interest.

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