PERSPECTIVE

Linking the hematopoietic microenvironment to imatinib-resistant Ph⁺ B-ALL

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Chronic myeloid leukemia (CML) and a subtype of B-cell acute lymphocytic leukemia (B-ALL) result from the constitutive expression of the Philadelphia (Ph) chromosome, a reciprocal translocation between *ABL* on chromosome 9 and *BCR* located on chromosome 22 (Nowell and Hungerford 1960; Rowley 1973). The chimeric BCR-ABL fusion product encoded by this chromosomal translocation is a tyrosine kinase, and its activity accounts for >90% of CML cases and up to 15% of B-ALL cases (Deininger et al. 2000). The treatment of CML has been revolutionized by the development of Imatinib mesylate (Gleevec, STI571), a targeted inhibitor of BCR-ABL (Wong and Witte 2004). However, the response Ph⁺ B-ALL to treatment has not been as efficacious, as demonstrated by shorter remissions and relatively rapid growth of drug-resistant clones. The reason for this difference in sensitivity to imatinib has been obscure and is particularly puzzling, since expression of BCR-ABL is responsible for both diseases. A paper by Williams et al. (2007) in this issue of *Genes & Development* now provides insights into this mystery. These investigators demonstrate that the combination of BCR-ABL expression; the deletion of the *Arf* gene, which encodes a tumor suppressor protein; and the response of Ph⁺ B-ALL cells to growth-promoting stimuli from the hematopoietic microenvironment act together to create a "perfect storm," leading to imatinib-resistant lymphoproliferative disease.

When mouse B-cell progenitors are transformed with BCR-ABL, they exhibit a brief period of proliferation followed by growth arrest and high levels of death. However, following this burst of proliferation, rare, immortal populations of B-cell progenitors spontaneously emerge that are resistant to apoptosis. This occurs because of inactivation of the *INK4a/Arf* (*cdkn2a*) locus (Radfar et

al. 1998; Randle et al. 2001) that encodes the p16^{Ink4a} and ARF (p19^{ARF} in mice and p14^{ARF} in humans) tumor suppressor proteins. Both p16^{Ink4a} and Arf are potent inhibitors of cellular proliferation via their effects on regulation of retinoblastoma (Rb) and p53 activity, respectively (Sharpless 2005; Sherr 2006). Arf mediates its effects by binding to and inactivating MDM2, a negative regulator of p53. Thus, when ARF is expressed in cells, p53 is activated. Consistent with the mouse data, ~30% of Ph⁺ B-ALL patients have sustained *cdkn2a* deletions (Heerema et al. 2004; Primo et al. 2005). In spite of this knowledge, the poor response of these individuals to imatinib has been unexplained.

In order to investigate this issue, Williams et al. (2007) modeled human B-ALL by transforming B-cell progenitors from *Arf*-deficient (*Arf*^{-/-}) mice with *BCR-ABL* under conditions that promoted the growth of B-lineage cells (McLaughlin et al. 1987; Whitlock and Witte 1987). The BCR-ABL-transformed *Arf*^{-/-} cells were particularly leukemogenic. While 2 × 10⁵ of them could induce B-ALL in recipient mice by 17 d post-injection, a comparable number of *Arf*^{+/+} cells failed to do so. Most important, similar to what occurs in human B-ALL patients, recipients of BCR-ABL-transformed *Arf*^{-/-} cells did not respond to treatment with clinically efficacious doses of imatinib (Wolff and Ilaria 2001; Hu et al. 2004, 2006; Drucker et al. 2006) and mice succumbed to disease. However, the intriguing finding was that the leukemic cells recovered from the mice were imatinib sensitive in vitro (Fig. 1A). The straightforward interpretation of these results is that a cell-extrinsic effect conferred imatinib resistance in vivo, which in turn focused attention on the hematopoietic microenvironment.

Blood cell development takes place within the medullary cavity of bones in association with a three-dimensional network of supporting stromal cells (Kincade et al. 1989; Dorshkind 1990; Nagasawa 2006). Stromal cells mediate their effects on developing blood cell progenitors through direct cell-to-cell interactions and via the secretion of a variety of soluble mediators that regulate progenitor cell growth, survival, and differentiation. Mu-

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Figure 1. p185 BCR-ABL-transformed *Arf*^{-/-} B-cell progenitors are resistant to imatinib in vivo. (A) Imatinib cannot control the growth of BCR-ABL-transformed *Arf*^{-/-} B-ALL cells in vivo. In addition to the absence of the Arf tumor suppressor protein, the B-ALL cells receive a proliferative stimulus from stromal cell-derived IL-7 that binds to the heterodimeric IL-7 receptor, formed by the IL-7Rα and γ_c. The leukemic cells replicate and mice ultimately succumb to disease. However, the leukemic cells recovered from the mice are imatinib sensitive in vitro in the absence of the IL-7 stimulus. Together, these data indicate that both cell-intrinsic and -extrinsic influences combine to promote B-ALL, and that imatinib cannot block these growth-promoting effects. (B) When the response to IL-7 is attenuated by preventing the assembly of its receptor in *Arf*^{-/-} × γ_c^{-/-} mice, B-ALL cells are now sensitive to imatinib treatment in vivo and a proportion of mice survive long term.

rine B lymphopoiesis is particularly dependent on stromal cell-derived interleukin-7 (IL-7), as B-cell development is effectively blocked in animals that cannot produce it or express its receptor (Peschon et al. 1994; von Freeden-Jeffry et al. 1995). The heterodimeric IL-7 receptor (CD127) is formed by the IL-7 receptor α (IL-7Rα) chain and the common γ chain (γ_c) (Giliani et al. 2005). CD127 is widely expressed on B-lineage-specified progenitors that include the common lymphoid progenitor (CLP), pre-pro-B cells, and pro-B cells, and following binding to IL-7, signaling through the JAK-STAT pathway results in cell growth and/or survival.

That signals from the hematopoietic microenvironment were responsible for the resistance of BCR-ABL-transformed *Arf*^{-/-} cells to imatinib was tested by generating *Arf*^{-/-} × γ_c^{-/-} mice. As noted above, γ_c forms part of the IL-7 receptor, so these double-knockout mice cannot respond to IL-7. BCR-ABL-transformed B-lineage cells from *Arf*^{-/-} × γ_c^{-/-}-deficient donors generated B-ALL when transferred to recipient mice and, in contrast to *Arf*^{-/-} mice, a significant proportion of the animals were now sensitive to imatinib and survived long term following treatment (Fig. 1B). This is a striking result that for the first time makes it necessary to consider cell-extrinsic effects, in addition to cell-intrinsic ones, in B-ALL pathogenesis.

A presumption in these studies (Williams et al. 2007) is that IL-7 was the cytokine that conferred imatinib resistance on the BCR-ABL-transformed *Arf*^{-/-} cells in vivo. However, in addition to forming part of the IL-7 receptor, γ_c also forms part of the receptor for additional cytokines that include IL-2, IL-4, IL-9, IL-15, and IL-21. Since these factors have not been implicated in B lymphopoiesis, the focus on IL-7 seems appropriate. Another consideration is that the BCR-ABL targets in *Arf*^{-/-} × γ_c^{-/-} mice, which were imatinib sensitive, and *Arf*^{-/-} mice, which were not, are overlapping but not identical populations. In this regard, all stages of B-cell development including CLP, pre-pro-B cells, pro-B cells, and pre-B cells are presumably present in the bone marrow of *Arf*^{-/-}-deficient mice. However, while CLP are present in normal numbers in γ_c^{-/-} mice, all downstream stages of B-cell development are absent (Miller et al. 2002). While it is important to acknowledge these points, the simplest interpretation of these results is that the IL-7-mediated signal received by BCR-ABL-transformed *Arf*^{-/-} cells provides an important growth stimulus that contributed to the imatinib resistance.

The key question is how the various events described above converge to mediate imatinib resistance in B-ALL. The development of progenitors with mutations in the *cdkn2a* locus seems to be central in this regard. As noted, this commonly occurs in clinical Ph⁺ B-ALL but apparently not in myeloid progenitors (Heerema et al. 2004; Primo et al. 2005). It is not clear why B-cell progenitors are prone to delete this locus, but doing so clearly places them at a disadvantage, because one of their main tools with which dysregulated growth is controlled is disabled. Part of the efficacy of imatinib is thought to be its ability to suppress multiple kinases in addition to BCR-ABL (Wong et al. 2004). However, in the context of BCR-ABL transformation, the absence of a potent tumor suppressor like Arf may literally be the straw that breaks the camel's back, because in the face of this, imatinib alone cannot control multiple growth signals received by B-lineage progenitors in vivo.

In fact, imatinib treatment of Ph⁺ B-ALL may be a double-edged sword that, rather than mitigating disease, actually selects for cells with mutations in the BCR-ABL kinase domain. For example, imatinib treatment of *Arf*-deficient, BCR-ABL-transformed mouse B-cell progenitors results in the emergence of cells that express relatively high levels of BCR-ABL (Williams et al. 2006). Based on the results of a recent human study of B-ALL patients, it is likely that these cells harbored BCR-ABL mutations. Interestingly, that report demonstrated that these were not necessarily imatinib induced, and instead existed in mutant clones that had already expanded prior to the initiation of therapy (Pfeifer et al. 2007). Thus, while imatinib might deplete clones in which mutations have not occurred, the most resistant populations remain and are mediators of aggressive disease.

In view of these points, new approaches to the treatment of B-ALL are necessary. One obvious way forward is to test new inhibitors that effectively target BCR-ABL mutations. However, in view of the effects of the micro-

environment on the growth of B-ALL cells in which *Arf* has been deleted, it may be necessary to combine them with additional targeted inhibitors. These could include JAK kinase inhibitors, since both BCR-ABL and IL-7 signal through these intermediates (Weisberg et al. 2007). Another possibility is to use molecules that interfere with the binding of ligand to the IL-7 receptor. Ideally, they would be engineered to interfere with binding to the IL-7R α and not γ_c chain so as to leave other cytokine signaling pathways in normal cells intact. Although this strategy would interfere with IL-7-dependent thymopoiesis, this should not be an issue in adults where this process has waned. B lymphopoiesis would not be affected, regardless of the age of the patient; while human B-cell progenitors are IL-7 responsive, in contrast to the mouse, their development is not IL-7 dependent (Giliani et al. 2005). Thymic stromal lymphopoietin (TSLP), whose heterodimeric receptor is composed of IL-7R α and the TSLP receptor, is the only other known cytokine whose binding would be blocked by such an approach (Giliani et al. 2005).

Another interesting observation by Williams et al. (2007) was that virtually every BCR-ABL-transformed *Arf*^{-/-} B-cell progenitor had leukemic potential. This result is similar to what was recently reported for pre-B/B-cell lymphomas from E μ -myc transgenic mice, E μ -N-RAS thymic lymphomas, and PU.1-deficient AMLs (Kelly et al. 2007). These reports raise questions regarding the cancer stem cell hypothesis, which states that only a few self-renewing cells within a tumor have the capacity to regenerate it (Lobo et al. 2007). At the very least, they suggest a modification of any strict definition, indicating that cancer stem cells are defined by frequency. Thus, some tumors, like selected leukemias and lymphomas, may include a high proportion of cancer stem cells, while in other malignancies, like chronic-phase CML, the tumor primarily consists of differentiated cells and only rare cancer stem cells.

Taken together, the Williams et al. (2007) study once again points out the importance of Arf as a key tumor suppressor that regulates the growth of malignant cells and indicates that its lack of expression could serve as an important biomarker indicative of poor prognosis in B-ALL. This conclusion is consistent with a recent report that expression of the polycomb family member Bmi1, which inhibits p16^{INK4a} and Arf expression, is a biomarker of poor prognosis in CML (Mohty et al. 2007). However, the new finding that extends our understanding of B-ALL is the demonstration that signals from the hematopoietic microenvironment also play a critical role in promoting the growth of leukemic cells. The challenge is to now exploit this observation to develop additional therapies.

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