Oncogenic signaling: new insights and controversies from chronic myeloid leukemia

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Chronic myeloid leukemia (CML), which is caused by the BCR–ABL fusion tyrosine kinase, is one of the most intensively studied human cancers. ABL kinase inhibitors have been spectacularly successful in treating CML, but disease persistence and acquired drug resistance can prevent eradication and cure of the leukemia. The development of better therapies will depend on a full understanding of signaling pathways in CML, facilitated by model studies using mutant mice.

The hematologic neoplasms have been the proving ground for targeted cancer therapies, in part because the malignant cells are readily obtained and manipulated. One major lesson that has emerged is that phenotypically similar leukemias may depend on very different signaling pathways for their pathogenesis, and thus require very different therapeutic strategies. For example, CML and juvenile myelomonocytic leukemia (JMML) are both characterized by overproduction of maturing myeloid cells, but the signaling abnormalities underlying the two diseases are quite different. The direct cause of CML is the product of the Philadelphia (Ph) chromosome, the BCR–ABL fusion tyrosine kinase (1) whose leukemogenic activity depends in part on its ability to enhance survival of myeloid progenitor cells by activating antiapoptotic genes. Consistent with a critical role for BCR–ABL, the ABL kinase inhibitor imatinib has been a successful therapy, producing hematologic and cytogenetic responses in nearly all CML patients. In contrast, JMML cells lack BCR–ABL and these patients do not respond to imatinib. Instead, JMML cells frequently have activating mutations in Ras or SHP2 (a tyrosine phosphatase), or loss-of-function mutations in the neurofibromatosis-1 protein NF-1 (a negative regulator of Ras) (1), which lead to enhanced proliferation of JMML myeloid progenitors in response to granulocyte/macrophage colony-stimulating factor (2).

In CML, ABL kinase inhibitor therapy induces hematologic and cytogenetic remission in most patients, but the majority harbor residual disease detectable by PCR (3), and some (particularly those in advanced stages) will have progression of leukemia due to acquired drug resistance. Both phenomena may reflect the relative insensitivity of the leukemia-initiating or leukemic “stem” cells to drugs that inhibit BCR-ABL kinase activity (4, 5). Understanding oncogenic signaling will be of critical importance to develop strategies to cure CML and other leukemias using targeted therapies. In this commentary, several recent publications that use mutant mouse models to illuminate signaling pathways critical for the pathogenesis of CML and other leukemias will be discussed. These include the report that mice lacking 12/15-lipoxygenase (12/15-LO) develop myeloproliferative disease detectable by PCR (3), and some particularly those in advanced stages) will have progression of leukemia due to acquired drug resistance. Both phenomena may reflect the relative insensitivity of the leukemia-initiating or leukemic “stem” cells to drugs that inhibit BCR-ABL kinase activity (4, 5). Understanding oncogenic signaling will be of critical importance to develop strategies to cure CML and other leukemias using targeted therapies. In this commentary, several recent publications that use mutant mouse models to illuminate signaling pathways critical for the pathogenesis of CML and other leukemias will be discussed. These include the report that mice lacking 12/15-lipoxygenase (12/15-LO) develop myeloproliferative disease detectable by PCR (3), and some particularly those in advanced stages) will have progression of leukemia due to acquired drug resistance. 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myeloid progenitors to neutrophils is close to normal, to blast crisis (BC), a terminal phase resembling acute myeloid leukemia (AML), where there is profound impairment of hematopoietic differentiation. Interestingly, a minority (<15%) of ageing Alox15−/− mice developed a condition similar to BC, with progressive splenomegaly and increased marrow, splenic, and circulating immature myeloid cells. However, it is not clear whether these mice meet defining criteria for AML, such as >20% marrow myeloblasts (7). The cause of morbidity and death in mice with BC-like disease was thought to be severe anemia, based on a relative decrease in mature erythrocytes in the bone marrow, but it is not apparent whether this correlated with decreased blood hemoglobin or hematocrit, which are the standard criteria for anemia.

**The 12/15-LO–interferon consensus binding protein connection**

How might loss of 12/15-LO lead to a CML-like disease? Treatment of splenocytes from these mice in vitro with imatinib did not impair their proliferation or survival, nor was there any increase in tyrosine phosphorylation of the ABL substrate Crk, suggesting that dysregulated ABL activity is not involved. But the authors did establish an intriguing functional connection between 12/15-LO, the protein kinase Akt, and interferon consensus sequence binding protein (ICSBP; also known as IRF-8) (Fig. 1).

Several lines of evidence have previously implicated ICSBP, an interferon-stimulated transcriptional repressor, as a suppressor of normal and CML myelopoiesis. ICSBP transcripts are low to absent in chronic phase CML (8), and ICSBP-deficient mice develop an MPD-like syndrome (9), in which the myeloid progenitors are hypersensitive to myeloid growth factors including granulocyte/macrophage colony-stimulating factor and interleukin-3 (10). In mouse bone marrow, ICSBP activation is decreased by BCR–ABL, whereas enforced coexpression of ICSBP attenuates both normal and BCR–ABL

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**Figure 1. Schematic representation of signaling in myeloid progenitors.** (A) Interferon-α (IFN-α) induces ICSBP transcription through Stat1. Increased ICSBP mediates an antileukemic effect through an unknown mechanism. (B) The BCR–ABL kinase represses ICSBP transcription through an unknown mechanism, but also activates multiple signaling pathways, including Ras–MAPK (leading to induction of Bcl-2 gene transcription), Stat5 (leading to Bcl-X gene transcription), PI3K (through a Grb2–Gab2 interaction) leading to Akt activation, and Src family kinases (Lyn and Hck). The net effect of BCR–ABL activity is to promote Bcl-2 and Bcl-X expression and to inhibit ICSBP transcription. (C) In contrast, 12/15-LO may either activate PTEN or inhibit PDK1, both regulators of Akt, leading to increased phosphorylation and cytoplasmic localization of ICSBP, an effect mediated in part through an unknown tyrosine kinase. This may increase survival in myeloid progenitors through relief of ICSBP-mediated inhibition of Bcl-2 and Bcl-X.
granulopoiesis (11). Direct repression targets of ICSBP in myeloid cells, which may account for this activity, include the antiapoptotic genes Bcl-X (12) and Bcl-2 (13).

Middleton et al. found that nuclear ICSBP protein levels were reduced in splenocytes from Alox15−/− mice with MPD (6). This decrease in nuclear ICSBP correlated with increased activation of Akt, enhanced tyrosyl phosphorylation of ICSBP, and elevated expression of Bcl-2. Prevention of Akt activation by treatment with a phosphatidylinositol 3-kinase (PI3K) inhibitor reduced ICSBP tyrosyl phosphorylation and increased nuclear ICSBP levels, coincident with reduced Bcl-2 levels and increased apoptosis. The mechanism through which 12/15-LO deficiency activates PI3K was not defined, but it is possible that some 12/15-LO products affect PI3K regulators such as PTEN or PDK1 (Fig. 1). The findings further suggest that a tyrosine kinase is involved in regulating ICSBP, but aside from excluding ABL, the authors did not pursue the identity of this kinase.

**Nice model, but is it CML?**

Alox15−/−-deficient mice clearly have overproduction of maturing myeloid cells and splenomegaly. But is this model relevant to CML? One problem in diagnosing MPD is that the malignant cells are virtually identical to normal maturing myeloerythroid cells, so that distinguishing MPD from reactive conditions is difficult (1). An undiagnosed generalized inflammatory state in older Alox15−/− mice could explain the myeloproliferation, but 12/15-LO and its products are generally considered to be proinflammatory, arguing against this possibility. Two cardinal features that distinguish leukemia from reactive conditions are clonality and transplantability. Because Alox15−/− mice are initially normal, 12/15-LO deficiency alone may be insufficient for development of MPD; additional events might be required for progression to MPD and subsequently to the blastic phase. In the Middleton et al. paper, the authors could not determine whether the MPD or blast phase are clonal processes (6), but future retroviral marking studies may resolve this. They were unable to adaptively transfer disease from Alox15−/− MPD mice by transplantation of bone marrow and/or splenocytes to syngeneic, unirradiated recipients, which probably reflects the very poor engraftment of donor hematopoietic stem cells under these conditions (14). However, they were able to efficiently transfer hematopoietic disease (defined as modest splenic enlargement with disruption of architecture) when donors in the “blast” phase were used, although the extent of donor engraftment and whether recipients developed fatal AML were not documented. These results suggest that the Alox15−/− donor cells capable of transferring disease (i.e., the leukemia-initiating or leukemic “stem” cells) differ between the MPD and blast phases of the disease, which is reminiscent of human CML (15). It would also be informative to test whether Alox15−/−-deficient mice are certain to provide important new insights into normal and malignant myelopoiesis.

**Stat5 and CML: superfluous or significant?**

ICSBP intersects with another well-studied hematopoietic signaling system that has been implicated in CML oncogenesis, the JAK-Stat pathway. Activation of the latent transcription factor, signal transducer and activation of transcription 5 (Stat5), in BCR–ABL-expressing cell lines and primary leukemia cells was recognized a decade ago (18), but the role of Stat5 in the pathogenesis of CML has been controversial. BCR–ABL may activate Stat5 through direct phosphorylation, or the activation could be indirect, via phosphorylation by JAK2 (19) or by Src family kinases (20), both of which are activated in BCR–ABL-expressing cells (Fig. 1). In a mouse retroviral bone marrow transplantation model of CML, initial studies using donor mice with targeted mutations in Stat5a and Stat5b suggested that Stat5 was not absolutely required for induction of CML-like leukemia by BCR–ABL (21). However, it is now widely recognized that the Stat5a/b mutations used in these studies were hypomorphic rather than true null alleles (22).

The role of Stat5 in CML has been readdressed in two recent papers. In the first paper, induction of murine CML-like MPD was attenuated in donor hematopoietic cells with a single null mutation in Stat5a (23), indicating that Stat5a isoform has a nonredundant function in BCR–ABL leukemogenesis. The second paper used novel mice that have the entire Stat5b locus deleted (24). Stat5b−/− mice die perinatally, but fetal liver hematopoietic progenitors from these mice were incapable of generating leukemia in recipient mice after retroviral transduction
with BCR–ABL (25). Together with a recent report that siRNA against Stat5 in human CML patient samples impairs Ph+ myeloid colony formation (26), these studies suggest that Stat5 signaling contributes to BCR–ABL leukemogenesis. However, several important issues must be clarified. The Stat5ab−/− experiments addressed principally B lymphoid transformation and leukemogenesis rather than CML-like MPD, and the extent that nonmalignant Stat5ab−/− hematopoietic stem cells can contribute to stable myeloid transformation and leukemogenesis after transplantation has not been defined. Lastly, the important transcriptional targets of Stat5 in CML must be determined. In this regard, there is considerable evidence that Bcl-X, a target for repression by ICSBP (12), is transcriptionally activated by Stat5a in CML cells (27) and may contribute to increased survival (Fig. 1).

**Src kinases in BCR–ABL leukemogenesis: a lineage-specific role**

As mentioned in the previous section, BCR–ABL activates multiple Src family kinases through a mechanism that does not involve direct phosphorylation, and Src kinase inhibitors and dominant-negative mutants impair BCR–ABL transformation in cultured cells (28). However, BCR–ABL can efficiently induce CML-like MPD in marrow from mice lacking the three Src kinases principally expressed in myeloid progenitor and stem cells (Lyn, Hck, and Fgr), suggesting that these Src kinases have no role in the pathogenesis of chronic phase CML (29). In contrast, induction of B cell acute lymphoblastic leukemia (B-ALL), which is also BCR–ABL dependent, was dramatically impaired in the absence of any two of these three Src kinases, suggesting a partially redundant requirement for Src kinases in the pathogenesis of Ph+ B-ALL and the B lymphoid BC stage of CML (29). Subsequently, these findings were supported by the demonstration that siRNA knockdown of Lyn in primary CML cells impaired leukemic cell viability and colony formation in cells from patients with Ph+ lymphoid BC, but had less effect on myeloid BC cells (30).

Several pharmaceutical companies have developed drugs that inhibit the kinase activity of both BCR–ABL and Src kinases, some of which are active against many but not all of the BCR–ABL imatinib-resistant mutants (31, 32). The studies with Lyn/Hck/Fgr-deficient mice showed that those particular Src kinases were not required for induction of CML-like disease by BCR–ABL (29), but involvement of the other six Src family members could not be excluded. When administered to mice with BCR–ABL-induced CML-like MPD or B-ALL, the ABL kinase inhibitor imatinib (Gleevec) prolonged the survival of mice with either disease, whereas CGP76030, a small molecule inhibitor of Src kinases that also inhibits BCR–ABL at higher concentrations, was effective alone and synergized with imatinib in mice with B-ALL, but had no effect in mice with CML-like leukemia (29). Biochemical analysis of primary leukemia cells showed that, at the doses used, CGP76030 inhibited Src family kinases but not BCR–ABL. A novel genetic strategy has been developed to verify the in vivo therapeutic target of dual ABL/Src kinase inhibitors in the Ph+ leukemia. The BCR–ABL T315I mutant is resistant to both imatinib (33) and the dual ABL/Src inhibitor dasatinib (BMS-354825) (31). B lymphoid blasts transformed by BCR–ABL T315I are relatively resistant to imatinib but susceptible to dasatinib in vitro and in vivo (5), implying that inhibition of BCR–ABL alone is insufficient for therapeutic responses in Ph+ B-ALL. The results further suggest that pure Src kinase inhibitors will have little therapeutic activity in chronic phase CML patients, but could be useful in the treatment of Ph+ B-ALL and CML lymphoid blast crisis. This prediction has been born out clinically, as patients with chronic and accelerated phase CML and the T315I mutation do not respond to dasatinib (34).

**REFERENCES**


