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-like disease and down-regulate interferon consensus sequence binding protein, a transcription factor that suppresses normal and CML myelopoiesis. Other studies help resolve the controversy over the role of Stat5 activation in promoting CML pathogenesis, and link Src kinases to BCR–ABL$^+$ B lymphoid leukemia but not CML.

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12/15-LO deficiency and myeloproliferative disorder (MPD)

In a recent issue of the *Journal of Experimental Medicine*, Middleton et al. demonstrated that mice with homozygous null mutations in *Alox15*, which encodes 12/15-lipoxygenase, develop MPD (6). 12/15-LO incorporates oxygen into unsaturated lipids to generate short-lived peroxides that are ultimately converted to 12(*S*)-hydroxyecosatetraenoic acid and related products, which have pleiotropic effects on cell signaling and the inflammatory response. Hematopoietic expression of 12/15-LO was known to be restricted to myeloid progenitors. No hematologic abnormalities have been noted in the mutant mice until now, however (even though *Alox15*-null mice have been used for many years in atherosclerosis studies), possibly because of the decreased penetrance of the phenotype in mixed genetic backgrounds.

Middleton et al. reported that although young (6–8-wk old) *Alox15*$^{-/-}$ mice (in a C57BL/6 background) were normal, they uniformly developed features of an MPD by 10–12 wk of age, including increased levels of circulating myeloid cells, splenomegaly, and infiltration of spleen by what appear to be proliferating, apoptosis-resistant, immature myeloid progenitors (6). These observations in *Alox15*$^{-/-}$ mice suggest that loss or down-regulation of 12/15-LO could contribute to CML. Indeed, the authors went on to show that 12/15-LO was undetectable in a CML cell line and that ectopic expression of 12/15-LO in these cells markedly decreased their proliferation and survival, effects that were partially reversed by treatment with an inhibitor of 12/15-LO.

An important feature of human CML is the progression from the chronic phase, where differentiation of

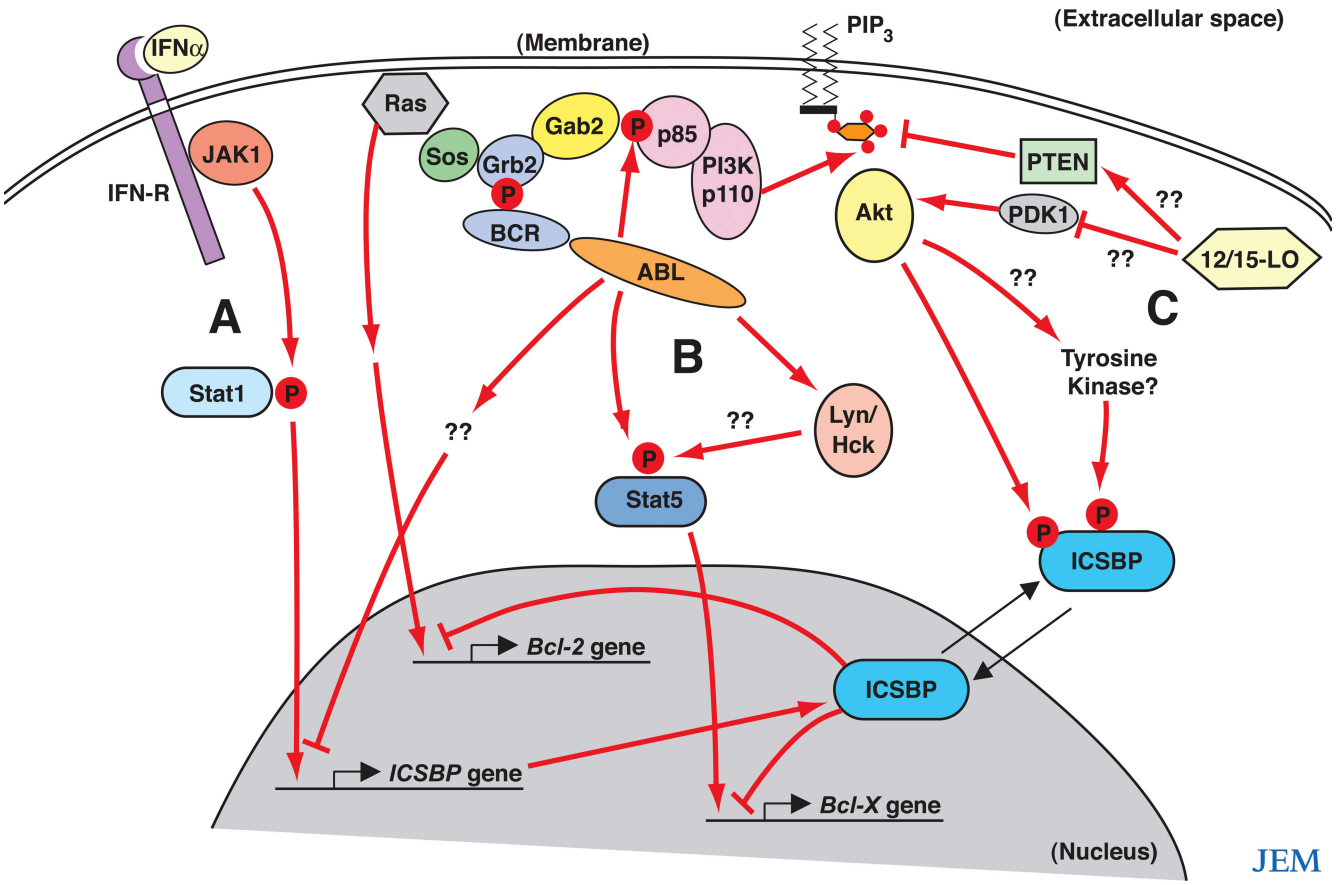

Figure 1. Schematic representation of signaling in myeloid progenitors. (A) Interferon-α (IFN-α) induces ICSBP transcription through Stat1. Increased ICSBP mediates an antileukemic effect through an unknown mechanism. (B) The BCR–ABL kinase represses ICSBP transcription through an unknown mechanism, 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 ISCBP-mediated inhibition of Bcl-2 and Bcl-X. PIP_3, phosphatidyl inositol-3,4,5-triphosphate.

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 tyrosyl 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 ISCBP-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^{+}

granulopoiesis (11). Direct repression targets of ICSBP in myeloid cells, which may account for this activity, include the antiapototic 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 adoptively 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*$^{-/-}$ myeloid progenitors were hypersensitive to cytokines, as in ICSBP deficiency (10). Cytokine hypersensitivity would connect decreased ICSBP function directly with the enhanced myelopoiesis in *Alox15*$^{-/-}$ mice, but it would also suggest that the disease is more like JMML (2) than CML, where the cytokine response is normal (16).

Ultimately, whether *Alox15*$^{-/-}$ mice represent an informative model of CML will require further study. There are some tantalizing clinical correlates, including decreased 12-LO activity in CML marrow cells (17) and frequent loss at chromosome 17p, where *ALOX15* is located, in CML disease progression. However, some functional connection between BCR–ABL and 12/15-LO (for example, does BCR–ABL alter 12/15-LO activity or expression?) is needed to support the proposed role of 12/15 LO in CML (Fig. 1). Although both BCR–ABL activity and 12/15-LO deficiency activate Akt in myeloid progenitors, the effects on ICSBP in the two myeloproliferative syndromes are distinct, with BCR–ABL decreasing ICSBP transcripts and protein expression, whereas 12/15-LO deficiency impairs nuclear localization of ICSBP but not its overall expression. Abnormalities of the 12/15-LO pathway should be sought in Ph-negative (atypical) CML and in chronic neutrophilic leukemia, two CML-like MPDs that lack BCR–ABL (1), and in myeloid blast crisis of CML. Whether or not the analogy to CML holds up, the *Alox15*$^{-/-}$ 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 transduction/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 this Stat5 isoform has a nonredundant function in BCR–ABL leukemogenesis. The second paper used novel mice that have the entire *Stat5ab* locus deleted (24). *Stat5ab*$^{-/-}$ 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 myeloerythropoiesis 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 Stat5 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 (28), 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^+ leukemias. 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).

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