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Pathogenesis and treatment of Ph+ leukemia: recent insights from mouse models

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Several methods to model human Ph+ leukemia in laboratory mice are available, including propagation of BCR/ABL-expressing cells in mice, xenotransplantation of primary Ph+ leukemia cells into immunodeficient mice, BCR/ABL transgenic mice, and BCR/ABL retroviral bone marrow transduction and transplantation. Recent studies in these different model systems have yielded important advances in our knowledge of the pathogenesis and therapy of human chronic myeloid leukemia and Ph+ B-lymphoblastic leukemia, and are the subject of this review. Curr Opin Hematol 2001, 8:224–230 © 2001 Lippincott Williams & Wilkins, Inc.

Mouse models of human cancer are one of the most important areas of current molecular oncology research [1]. These models allow investigation of the molecular pathophysiology of these diseases in ways that would be difficult or impossible using primary human cancer cells and cell lines and provide a platform for the development and testing of new therapeutic approaches. Of all the models of human malignancy, murine models of the Philadelphia-positive (Ph+) leukemias are arguably the most accurate and faithful and the most advanced. In part, this is because the principal molecular abnormality of these leukemias, the BCR/ABL oncogene, was identified as the product of the t(9;22) Ph chromosome translocation more than 17 years ago, and many years of effort have been invested in expressing this gene in primary cells in mice. There are currently four main experimental approaches to modeling the 2 most common Ph+ leukemias, chronic myeloid leukemia (CML) and acute B-lymphoblastic leukemia (B-ALL), in mice: propagation of BCR/ABL-expressing cell lines in mice, xenotransplantation of primary Ph+ leukemia cells into immunodeficient mice, BCR/ABL transgenic mice, and BCR/ABL retroviral bone marrow transduction and transplantation (for more background on these models, see [2,3]). This article will focus on published advances using these four model systems during the past year.

Propagation of BCR/ABL-expressing cell lines in mice

One simple approach to modeling Ph+ leukemia in vivo is to propagate permanent cell lines expressing BCR/ABL in mice by parental inoculation. The cell lines used most often are mouse cytokine–dependent hematopoietic cell lines such as 32D or Ba/F3, which are transformed to cytokine independence by BCR/ABL. These cells can be grown in syngeneic hosts (C3H or Balb/c mice, respectively) without requiring irradiation of the recipient. Human Ph+ cell lines, derived from patients with B-ALL or blast crisis of CML, can also be grown in immunodeficient (most often SCID or nude/athymic) mice. In both cases, the BCR/ABL-expressing cells disseminate widely and cause death because of infiltration of marrow, spleen, liver, and other organs. This disease process does not accurately represent either Ph+ B-ALL or CML, so the value of this model system for studying leukemogenesis is limited. However, the system is quite useful for initial in vivo testing of new therapeutic approaches to Ph+ leukemia.

Abbreviations

AGP  acid glycoprotein
B-ALL  acute B-lymphoblastic leukemia
CML  chronic myeloid leukemia
ES  embryonic stem
GM-CSF  granulocyte-macrophage colony-stimulating factor
IL-3  interleukin 3
LTC-IC  long-term culture-initiating cells
MMTV  mouse mammary tumor virus
MT  metallothionein
Ph+  Philadelphia-positive
PI 3-K  phosphatidylinositol 3-kinase
SCID  severe combined immunodeficiency disorder
SL-IC  SCID leukemia-initiating cells
SH2  Src homology 2
SRC  SCID-repopulating cell
STAT5  signal transducer and activator of transcription-5
TRE  tetracycline-responsive promoter element

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Several recent papers illustrate the usefulness of this model for drug testing. The Abl tyrosine kinase inhibitor STI-571 (Gleevec, formerly called CGP57148; Novartis Pharmaceuticals, East Hanover, NJ), given parenterally or orally, was able to eradicate human CML cell lines propagated in nude mice [4]. Pharmacokinetic data indicated that the effective half-life of STI-571 in mice (2–3 hours) was markedly shorter than in humans (16 hours), necessitating thrice-daily dosing. A subsequent study of mice with large burdens of Ph+ leukemia cells found that most animals relapsed after an initial response to STI-571 [5•]. Although there was evidence of in vivo resistance to the inhibitor, cells isolated from relapsing mice were fully sensitive to STI-571 in vitro. Increased plasma levels of α1 acid glycoprotein (AGP) in these mice, produced from the liver as an acute-phase reactant, bound STI-571 and blocked the inhibition of Bcr/Abl kinase activity. The in vivo resistance could be partially overcome by co-administering erythromycin, which also binds AGP. The relevance of AGP to clinical resistance to STI-571 in patients remains to be established. Another study used BCR/ABL-transformed Ba/F3 cells propagated in Balb/c mice to assess the in vivo effectiveness of an orally administered farnesyltransferase inhibitor, SCH66336 [6•]. Oral SCH66336 administered twice daily for 1 month was able to eradicate the BCR/ABL-expressing cells and led to long-term survival. Biochemical studies indicated that other farnesylated proteins in addition to Ras were targets for the action of this drug, indicating that combinations of Abl kinase inhibitors and farnesyltransferase inhibitors may be advantageous for therapy of Ph+ leukemias. The model system has also recently been used to demonstrate the potential of biologic interventions directed at BCR/ABL. Transduction of the Ph+ cell line BV173 with retrovirus expressing a novel dimeric ribozyme directed against the BCR/ABL mRNA junction sequence abolished the leukemogenicity of these cells in nonobese diabetic (NOD)/SCID recipient mice [7], suggesting this strategy might be useful for in vivo purging of marrow autografts. Conditional expression of the normal BCR gene in the Ph+ cell line K562 decreased the leukemogenicity of the cells in NOD/SCID mice [8], demonstrating that the normal Bcr protein can antagonize the actions of Bcr/Abl.

Xenotransplantation into NOD/SCID mice
Normal human bone marrow can engraft immunodeficient (SCID and NOD/SCID) mice, and this has allowed the definition of a CD34+CD38− SCID-repopulating cell (SRC) that is more primitive than most long-term culture-initiating cells (LTC-IC) [9]. Primary leukemia blasts from human Ph+ B-ALL and CML blast crisis patients are easily propagated in NOD/SCID mice [10,11], but cells from chronic-phase CML patients are more difficult to establish [10,12,13]. In 1998, three groups demonstrated efficient engraftment of immunodeficient NOD/SCID mice with large doses of primary cells from chronic-phase CML patients [14–16]. Unfortunately, the extent of engraftment of individual mice transplanted with cells from the same patient may vary by more than three orders of magnitude [15], making quantitative studies impossible. Furthermore, the engrafted mice do not develop progressive or fatal myeloproliferative disease, suggesting that the murine hematopoietic microenvironment cannot support long-term BCR/ABL-induced myelopoesis in cells of human origin.

In spite of these limitations, engraftment of NOD/SCID mice is useful because it is the only assay for the earliest malignant progenitors in patients with Ph+ leukemia. A recent study used the NOD/SCID engraftment assay to demonstrate that a primitive quiescent population of progenitors from chronic-phase CML patients contained Ph+ SRC [17]. A second paper examined the phenotype of primary cells from patients with Ph+ B-ALL and the p190 isoform of BCR/ABL that were capable of engraftment of NOD/SCID mice [18]. The SCID leukemia-initiating cells (SL-IC) were primitive CD34+CD38− cells that were more immature than most of the leukemic blasts, which were predominantly CD34+CD38−CD19−. These results support previous observations that the leukemic clone in AML patients likewise has a hierarchical organization [19]. Although the authors suggested that the SL-IC in Ph+ B-ALL might be hematopoietic stem cells, this cannot be definitively concluded from the cell surface markers used in their study. Evidence from the mouse retroviral bone marrow transduction/transplantation model system suggests that at least some non–stem cell progenitors can initiate B-ALL in mice after acquisition of the BCR/ABL oncogene [20], (see “BCR/ABL retroviral bone marrow transduction/transplantation”).

BCR/ABL transgenic mice
Reproducible expression of BCR/ABL in the hematopoietic system of transgenic mice has been hampered by the toxicity of this oncogene during mouse embryonic development, leading to down-regulation and silencing of the transgene [21] and even embryonic lethality [22]. The most successful BCR/ABL transgenic model system has used the metallothionein (MT) promoter to express either the p190 or p210 isoforms of BCR/ABL [23,24]. These mice express BCR/ABL in many tissues at very low levels [25], reproducibly develop B-ALL after several months’ latency, and have proven valuable for investigating the pathophysiology of Ph+ B-ALL and for testing treatments.

Expression of BCR/ABL in hematopoietic cell lines is associated with chromosomal instability [26] and increased frequency of point mutations [27], but it has been difficult to prove that these abnormalities are directly induced by Bcr/Abl rather than a secondary consequence of selection for increased growth in culture. A recent study in preleukemic MT-p190 BCR/ABL trans-
genic mice demonstrated an increased frequency of point mutations in a chromosomally integrated lacI reporter gene from both spleen and kidney, offering the first direct evidence that BCR/ABL expression causes a mutator phenotype in primary tissues [28•]. The precise mechanism of the increased mutation frequency induced by BCR/ABL is unknown. Previous studies in Ba/F3 cells demonstrated that BCR/ABL expression conferred resistance to apoptosis after ionizing radiation, accompanied by a prolonged G2/M arrest that was hypothesized to account for this genetic instability [29]. Surprisingly, primary cells from preleukemic p190 BCR/ABL transgenic mice showed no evidence of decreased apoptosis or abnormal checkpoint arrest after ionizing radiation [30]. Although the level of expression of p190 Bcr/Abl protein is low in the preleukemic mice, similar results were obtained with malignant lymphoblasts from leukemic mice, which express much higher levels of p190. These results suggest that the effects of BCR/ABL on cell cycle checkpoints in cultured cells may be irrelevant to the initiation and progression of leukemia in vivo, and further studies will be necessary to determine the mechanism of the increased mutation frequency induced by BCR/ABL.

The MT-BCR/ABL transgenic mice have also been used to study the signaling pathways important for leukemogenesis by BCR/ABL. One approach is to study leukemogenesis by different forms and mutants of BCR/ABL in this model. This is laborious, because testing each BCR/ABL mutant requires the generation, breeding, and characterization of multiple additional transgenic founder animals. In spite of this limitation, the approach has been used in the past year to demonstrate that both the coiled-coil oligomerization domain of Bcr [31] and the F-actin binding domain of Abl [32] are required for efficient development of B-ALL in BCR/ABL transgenic mice. A complementary strategy is breeding the MT-BCR/ABL transgene into different knockout and transgenic backgrounds to examine the effect on leukemogenesis. In this way, it has been shown that induction of B-ALL by BCR/ABL is independent of the normal BCR gene product [33], whereas overexpression of the CrkL adapter protein accelerates B-lymphoid leukemogenesis by BCR/ABL [34]. Finally, the MT-BCR/ABL mice have also been used to test the effectiveness of the farnesyltransferase inhibitor SCH66336. Administration of this compound to early leukemic mice caused suppression of the leukemic phenotype and allowed disease-free survival in 80% of treated mice for the duration of the study [35]. These results suggest that inhibition of farnesyltransferase may be of therapeutic benefit in Ph+ B-ALL, a disease where STI-571 is of only temporary benefit [36].

For unknown reasons, the MT-BCR/ABL transgenic mice do not develop myeloid leukemia or myeloproliferative disease. The only BCR/ABL transgenic model of myeloproliferative disease is a line of mice expressing p210 BCR/ABL from the hematopoietic-specific tec promoter, which develop gradual increases in circulating neutrophils, thrombocytosis, anemia, and morbidity by 8 to 12 months of age [37]. Crossing the tec-p210 transgene into mice with heterozygous mutation of the p53 tumor suppressor gene resulted in rapidly developing BCR/ABL-expressing T-lymphomas, most of which showed evidence of loss of the second p53 allele [38]. This study provides convincing evidence of cooperation between BCR/ABL and loss of p53, but the lack of clear myeloproliferative disease in these mice prior to T-lymphoma development casts doubt on whether this represents blast crisis.

An exciting development in transgenic BCR/ABL models during the past year is the conditional expression of BCR/ABL in mice using the tetracycline-regulated system. In this system, two transgenic strains of mice are generated, one (transactivator) expressing the tetracycline-responsive transcriptional activator protein tTA, the other (transresponder) expressing BCR/ABL under control of a DNA response element with multiple tTA binding sites (Fig. 1A). The two strains are mated to generate double-transgenic mice, but the expression of the BCR/ABL transgene is suppressed during embryonic and postnatal development by dosing the drinking water of pregnant females and their offspring with tetracycline. Double-transgenic mice derived from four different p210 BCR/ABL transresponder lines remained healthy as long as tetracycline administration was continued, but developed fatal B-lymphoid leukemia with 100% incidence on withdrawal of the antibiotic [39•] (Fig. 1B). Surprisingly, retreatment of diseased mice with tetracycline led to the rapid death of the leukemic cells by apoptosis, demonstrating that BCR/ABL-induced B-ALL is absolutely dependent on continued expression of the oncogene, even at advanced stages of the leukemia (Fig. 1C). This model system should be useful for studying the earliest events in B-lymphoid leukemogenesis by BCR/ABL both in vivo and in vitro, and for analyzing the anti-apoptotic action of BCR/ABL in primary cells. The mice do not develop myeloid leukemia or CML because the tTA protein is not highly expressed in myeloid and stem cells [39•], but generation of different transactivator mice with myeloid-specific promoters might circumvent this problem. In related studies, another group expressed p210 BCR/ABL in totipotent embryonic stem (ES) cells using the tetracycline-regulated system, and demonstrated that BCR/ABL expression stimulated multipotential progenitor expansion and myeloid lineage commitment in vitro [40]. Generation of chimeric mice with these conditional BCR/ABL ES cells is the obvious next step, but has not been reported to date.
The most accurate and quantitative mouse model of CML involves retroviral transduction of the BCR/ABL gene into primary murine bone marrow ex vivo, followed by transplantation of the cells into irradiated syngeneic recipient mice [41]. Two years ago, three groups reported efficient induction of CML-like myeloproliferative disease in recipients of BCR/ABL-transduced marrow, allowing the system to be used as an assay for the first time [42–44]. The retroviral marrow transduction/transplantation model has several distinct advantages over transgenic and knock-in strategies for modeling Ph⁺ leukemia in mice [45], including ease of testing different mutants of BCR/ABL and the ability to assess leukemogenicity in different bone marrow target cells.

This model system has been used recently to determine the role of particular signaling molecules in the pathogenesis of BCR/ABL-induced CML-like disease by using mice with naturally occurring or targeted mutations as donors, recipients, or both. BCR/ABL induces constitutive activation of the transcription factor signal transducer and activator of transcription-5 (STAT5) in hematopoietic cells, but some recipients of BCR/ABL-transduced marrow from mice with homozygous mutations in both Stat5a and Stat5b genes still developed myeloproliferative disease [46], demonstrating that STAT5 is not absolutely required for the pathogenesis of BCR/ABL-induced CML-like disease. In contrast, a similar myeloproliferative illness induced by the TEL/JAK2 fusion oncogene was absolutely dependent on STAT5 [47]. These studies emphasize that important pathophysiologic differences exist between phenotypically similar leukemias and suggest that molecularly targeted therapy of these diseases must be individualized. In another recent study, the role of cytokines in the pathogenesis of BCR/ABL-induced CML-like disease was investigated. Aberrant transcripts for interleukin 3 (IL-3) and granulocyte colony-stimulating factor (G-CSF) have been reported in primitive Ph⁺ progenitors from chronic-phase CML patients, raising the possibility of an autocrine growth mechanism in CML [48]. Although increased circulating IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are detected in mice with BCR/ABL-induced CML-like leukemia [43], neither cytokine is required for induction of myeloproliferative disease in mice by BCR/ABL [49•]. These results argue that IL-3 does not play an essential role in human chronic-phase CML but do not exclude a role for cytokines in the long latent phase of CML, which is not modeled accurately in mice.

Another series of recent studies examined leukemogenesis by BCR/ABL mutants with alterations in well-defined structural domains of the polypeptide. Two critical domains in the Bcr portion of the fusion protein were found to be necessary for efficient induction of myeloproliferative disease by Bcr/Abl: the N-terminal coiled-coil oligomerization domain [50] and the direct binding site for the Grb2 adapter protein at Bcr tyrosine 177 [50,51]. Although the requirement for the oligomeriza-
tion domain was anticipated from in vitro studies [52], the necessity of direct Grb2 binding was unexpected [53] and validates this pathway as a target for rational drug design. The importance of direct binding of Grb2 is further emphasized by the fact that p160 v-Abl, the product of the transforming gene of Abelson murine leukemia virus, lacks Grb2 binding and is completely defective for induction of myeloproliferative disease in mice [51,54].

The effect of mutations in the Src homology 2 (SH2) domain of Bcr/Abl are worth emphasizing because they illustrate the complexity of this model system. The SH2 domain of Bcr/Abl was previously reported to be absolutely and specifically required for activation of phosphatidylinositol 3-kinase (PI 3-K) [55]. Recent studies demonstrated that the SH2 domain is required for efficient induction of myeloproliferative disease in mice [51,54]. The effect of mutations in the Src homology 2 (SH2) domain of Bcr/Abl are worth emphasizing because they illustrate the complexity of this model system. The SH2 domain of Bcr/Abl was previously reported to be absolutely and specifically required for activation of phosphatidylinositol 3-kinase (PI 3-K) [55]. Recent studies demonstrated that the SH2 domain is required for efficient induction of CML-like disease by Bcr/Abl [20•,56] but not for B-lymphoid leukemogenesis [20•]. SH2 mutation decreased the intrinsic tyrosine kinase activity of Bcr/Abl, yet malignant lymphoblasts and myeloid cells from the diseased mice showed no impairment of PI 3-K activation [20•]. These results argue that the decreased induction of myeloproliferative disease by SH2-mutated Bcr/Abl is not the effect of an isolated defect in one specific signaling pathway, but is rather a consequence of slightly dampened signaling in many pathways (Fig. 2).

As a corollary, maximal Bcr/Abl tyrosine kinase activity is required for induction of myeloproliferative disease but not for B-lymphoid leukemogenesis, which may in part explain the resistance of Ph+ B-ALL to kinase inhibitor therapy [36].

Conclusions

Human Ph+ leukemia is arguably the best understood of all human malignant disorders, in part because of what we have learned from mouse models of these diseases. These models are valuable because they accurately recapitulate the complex pathophysiology of the leukemias in vivo. We can expect the existing model systems to be improved, and they will continue to provide important new knowledge about the pathophysiology and therapy of the Ph+ leukemias that would impossible to derive from clinical studies. However, continued correlative studies between human and murine BCR/ABL-induced leukemias are essential, and conclusions reached in mouse model systems require validation, if possible, in human patients and cells.

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This article presents a comprehensive assessment of the role of SH2 domain mutations on leukemogenesis and signaling by Bcr/Ab1.


40 This is the first report of conditional expression of p210 BCR/ABL in mice using a binary tetracycline-regulated transgene system.


45 Li S, Iaria RL, Million RP, et al.: The P190, P210, and P230 forms of the BCR/ABL oncogene induce a similar chronic myeloid leukemia-like syn-


This article provides an example of using mutant mice in the retroviral bone marrow transduction/transplantation system to determine the role of cytokines in the pathogenesis of BCR ABL-induced CML-like leukemia.


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