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Retroviral Transduction Models of Ph$^+$ Leukemia: Advantages and Limitations for Modeling Human Hematological Malignancies in Mice

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ABSTRACT: There are two commonly used approaches to modeling human leukemia in mice: generation of mutant mice by traditional transgenic or knock-out/knock-in methods and retroviral bone marrow transduction and transplantation. For modeling leukemia, the retroviral model system has some distinct advantages over transgenic mice. Testing different forms and mutants of a given oncogene is much easier with the retroviral system and avoids the potential deleterious effects of expression of a transgene in nonhematopoietic tissues and during development. The retroviral provirus serves as a clonal marker of a transduced cell, facilitating analysis of clonality and transplantability of the malignancy. Finally, the retroviral system allows the assessment of the action of an oncogene in different subsets of hematopoietic precursor cells in the bone marrow, which is difficult or impossible with transgenic models. This article summarizes recent progress in modeling human Philadelphia-positive leukemia in mice with the retroviral bone marrow transduction/transplantation system and emphasizes the advantages and limitations of this approach with examples from the *BCR-ABL* leukemogenesis literature.

INTRODUCTION

Mouse models of human cancer have traditionally relied on the generation of transgenic mice or on germline gene inactivation or replacement. For hematological malignancies, which are often characterized predominantly by activation of oncogenes rather than inactivation of tumor suppressor genes, transgenic and gene knock-out/knock-in strategies pose special problems when it comes to developing accurate and faithful mouse models. An alternative approach is the introduction of leukemia oncogenes directly into murine bone marrow cells by *ex vivo* retroviral transduction, followed by transplantation of the genetically modified cells into syngeneic or immunodeficient recipient mice [for review, see (1)]. This technique was pioneered for the product of the Philadelphia chromosome, the *BCR-ABL* oncogene (2), and has subsequently been applied to several other putative leukemia oncogenes. In this review, I will summarize recent results obtained with the retroviral bone marrow transduction/transplantation model of Ph$^+$ leukemia, and point out the advantages this approach has over transgenic and knock-in models of *BCR-ABL*-induced leukemia.

RESULTS AND DISCUSSION

The BCR-ABL Retroviral Transduction/Transplantation System Yields Accurate and Quantitative Models of Chronic Myeloid Leukemia (CML) and Ph$^+$ B-Cell Acute Lymphoblastic Leukemia (B-ALL)

Recent improvements in production of high-titer, replication-defective retroviral stocks have allowed the induction of CML-like myeloproliferative disease in 100% of recipients of *BCR-ABL*-transduced bone marrow when donors are pretreated with 5-fluorouracil (5-FU) (3–5). Mice

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with the CML-like illness succumb within 4 weeks after transplantation and exhibit massive expansion of maturing myeloid cells, principally neutrophils, with involvement of peripheral blood, bone marrow, spleen, liver, and lungs. Analysis of proviral integration shows the CML-like disease to be polyclonal and involve multiple myeloid and B-lymphoid lineages, implicating a target cell that is an early multipotential progenitor or stem cell (3). The disease can be transplanted to secondary recipients, some of which develop clonally related acute lymphoid and myeloid leukemias suggestive of blast crisis (4, 6, 7). To model B-ALL with this system, donors are not pretreated with 5-FU, and marrow is transduced without prestimulation in myeloid-specific cytokines. Under these conditions, all recipients develop fatal B-lymphoid leukemia within 4–5 weeks posttransplant, characterized by lymphadenopathy, moderate splenomegaly, and a malignant pleural effusion (3, 8). The malignancy is composed of cells of late pro-B cell origin, is transplantable, and has a proviral integration pattern that is oligo- to monoclonal and involves only B-lymphoid cells (3), suggesting a lineage-restricted target cell that requires additional events in addition to *BCR-ABL* transduction for full malignant transformation.

Analysis of Different Forms and Mutants of BCR-ABL Is Facilitated with the Retroviral Model System

In transgenic models of *BCR-ABL* leukemogenesis, comparing the leukemogenic activity of different forms of *BCR-ABL* requires the generation of many new transgenic founder mice, an expensive and time-consuming task. Similarly, p190 *BCR-ABL* knock-in mice have been generated by homologous recombination in embryonic stem (ES) cells (9), but testing different forms and mutants in this system requires performing new rounds of gene targeting. A comparison of the lymphoid leukemogenic activity of the p190 and p210 forms of *BCR-ABL* in transgenic mice has demonstrated that p210 transgenic mice develop lymphoid leukemia less efficiently than p190 transgenic mice, and in addition develop T-lymphoid tumors in addition to B-lymphoid neoplasms (10). In contrast, testing the leukemogenic activity of different forms of *BCR-ABL* in the retroviral model system requires only making new virus stocks, which can then be used quickly to assess induction of CML-like disease and B-ALL. In this manner, it has recently been demonstrated that the p190, p210, and p230 forms of BCR-ABL are equivalent at inducing CML-like leukemia in mice, but p190 has more potent B-lymphoid leukemogenic activity (3). Similarly, the analysis of mutants of *BCR-ABL* is much easier with the retroviral model system. Several recent studies have tested the role of the *BCR-ABL* SH3 domain (11), Grb2 binding site (12), and SH2 domain (8) in the retroviral transduction/transplantation model of CML. The C-terminal F-actin-binding domain of Abl was recently shown to be necessary for efficient induction of B-ALL in p190 transgenic mice (13), but this study required generation of multiple founder mice and illustrates the effort required to analyze a single mutant in this system. Both model systems can be used to test leukemogenesis in different genetic backgrounds. Crossing the *BCR-ABL* transgenic mice with $bcr^{-/-}$ mice demonstrated that lymphoid leukemogenesis by *BCR-ABL* does not require the normal Bcr protein (14), while use of mice with inactivation of the *Il3* gene as donors, recipients, or both demonstrated that IL-3 is not required for induction of CML-like leukemia by *BCR-ABL* (15).

The Retroviral Model System Avoids the Potential Toxicity of a BCR-ABL Transgene

An additional problem with traditional *BCR-ABL* transgenic mice is that all cells in the mouse contain the *BCR-ABL* gene. Because activated Abl kinases have cytostatic and cytotoxic effects (16), the inappropriate expression of a *BCR-ABL* transgene outside the hematopoietic system or high level expression during embryonic development can cause transgene toxicity (manifest as decreased yield of transgenic offspring) or transgene silencing (decreased or absent transgene expression in the expected target tissues). For example, transgenic expression of *BCR-ABL* under

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the murine *bcr* promoter causes embryonic lethality (17), while expression from the immunoglobulin heavy chain enhancer/promoter causes both toxicity and silencing (18). In part, this problem might be avoided by the use of more stringent promoters to express *BCR-ABL*, or by conditional transgenic approaches such as the tetracycline-regulated binary transgene system (19) or a conditional knock-in. However, these strategies are currently limited by lack of suitable promoters and "transactivator" transgenic mice. Transgene toxicity and silencing may account for the lack of an accurate transgenic mouse model of CML. In contrast, the retroviral model system completely avoids the problem of inappropriate expression of *BCR-ABL* outside the bone marrow and during development.

The Retroviral Model System Facilitates the Analysis of Clonality and Transplantation of the Leukemias

Because the retroviral stocks employed are replication-defective, each proviral integration event serves as a unique clonal marker of the transduced cell and its progeny. This allows easy determination of the clonality of a leukemia, and permits the fate of the clone to be followed in secondary transplantation experiments. For example, the B-lymphoid leukemias induced by retroviral transduction of *BCR-ABL* are oligo- to monoclonal (3), suggesting that multiple events in addition to *BCR-ABL* transduction are required for full malignant transformation. This must be definitively established by defining the bone marrow target cell for induction of B-ALL (see below), and repopulating recipient mice with defined numbers of transduced cells. In contrast, determining whether the leukemias arising in transgenic mice are clonal or polyclonal is extremely difficult, because the *BCR-ABL* gene cannot serve as a useful clonal marker. Even immunoglobulin gene rearrangement or karyotypic studies cannot provide definitive evidence of clonality in these mice (20). As a consequence, the clonal status of the leukemias that arise in p190 transgenic mice is unknown.

The Retroviral Model System Allows Leukemogenesis to Be Studied in Distinct Bone Marrow Target Cells

One of the most powerful advantages of the retroviral technology is the ability to transduce different types of hematopoietic progenitor cells in the bone marrow. This is of particular importance with *BCR-ABL*, which can induce distinct forms of leukemia upon transduction of different target cells. For example, the cell that initiates the CML-like disease has multilineage repopulating ability (3) but is heterogenous for self-renewal, as assessed by secondary transplantation (3, 5) and generation of secondary day 12 spleen colonies (3). In contrast, the cell that initiates B-ALL after *BCR-ABL* transduction has very different properties: it is abundant in normal bone marrow, highly transducible without 5-FU treatment, and lacks the ability to contribute to myeloid lineages or generate day 12 spleen colonies in secondary transplants (3). These issues are of clinical relevance. For example, the Bcr/Abl SH2 domain is required for efficient induction of CML-like disease in mice, but not for induction of B-ALL (8). These results demonstrate that the critical signaling pathways for *BCR-ABL* leukemogenesis in these distinct target cells are different, and suggest that small molecules that block SH2 function might be effective for therapy of CML but not B-ALL.

Although xenotransplantation studies have suggested that the cell initiating human Ph^+ B-ALL is a primitive hematopoietic progenitor (21), these studies do not distinguish between a committed lymphoid progenitor and an earlier cell. Similarly, establishing the nature of the leukemogenic cell in transgenic models of *BCR-ABL* leukemia is extremely difficult because of the presence of the transgene in all marrow cells. In theory, this type of analysis should be possible with the use of promoters that tightly restrict *BCR-ABL* expression to distinct subsets of hematopoietic progenitors. However, suitable hematopoietic-restricted promoters are not currently available (with the possible exception of the MRP8 promoter), and their use would be compli-

cated by problems of transgene toxicity and silencing, as described above.

Limitations of the Retroviral Model System

The retroviral model system does have several distinct disadvantages. The assay is labor intensive and involves several steps, including generation of viral stocks, ensuring the different stocks have equivalent titer, priming donors, harvesting marrow, prestimulation and transduction, and transplantation. Variability in any of these steps can lead to discrepancies in the leukemogenesis assay, necessitating that the utmost care is taken at all times during the procedure. This effort must be compared with the simple breeding required to generate more *BCR-ABL* transgenic mice. In the current system, the *BCR-ABL* gene is expressed from the retroviral long terminal repeat promoter/enhancer, which is obviously different from (and probably stronger than) the human *BCR* promoter. For this reason and the fact that cells must be cycling in order to be productively transduced by type C retroviruses, the current retroviral transduction/transplantation system does not model the long latent period characteristic of human CML. Also, recipients are irradiated, which is immunosuppressive and results in two competing processes (reconstitution and leukemogenesis) occurring in recipients simultaneously. It is plausible that modifications to the vectors and transduction/transplantation conditions may alleviate these problems in the future. Finally, the fact that many subtypes of hematopoietic progenitors are transduced is an advantage of the system (as described above) but it can also be a liability, as induction of multiple leukemias from different target cells can greatly complicate the analysis of leukemogenesis. Extreme care in the characterization and analysis of the leukemias that arise in this system is required to avoid erroneous conclusions.

CONCLUSIONS

The retroviral bone marrow transduction/transplantation model system affords accurate and quantitative models of human CML and Philadelphia-positive B-lymphoid leukemia, and has several unique advantages over transgenic and knock-in models of *BCR-ABL* leukemogenesis. Careful and creative application of this model system should continue to yield important new knowledge about the molecular pathogenesis of these leukemias that would be difficult or impossible to derive from studies of human patients or primary leukemia cells.

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