Advances in the Development of Animal Models of Myeloid Leukemias

Olga A. Guryanova[a] *and Ross L. Levine*[a,b]

The ideal in vivo models for studies of leukemia pathogenesis and treatment would have several important features. First, these animals would have phenotypes that are robust and reproducible. Second, these models would reliably develop phenotypes that accurately recapitulate disease observed in the clinic with short latency and high penetrance sufficient for informative preclinical therapeutic studies. Leukemia models based on retroviral transduction of oncogenes followed by transplantation into irradiated syngeneic hosts offer a rapid and cost-efficient way to evaluate the effects of a gene or mutation of interest but in some cases result in inconsistent phenotypes due to non-physiological levels of expression and random genomic integration. Xenotransplantation models enable the propagation and analysis of primary human samples but may be difficult to interpret due to variability of genetic background, the effects of immune recognition, a limited knowledge of genetic alterations in samples before and after xenotransplantation, and incomplete interaction with the microenvironment. Genetically engineered mice represent tractable models of human myeloid malignancies, and in many cases empower the study of the molecular pathogenesis of leukemia and evaluation of novel chemotherapeutics. Our understanding of the molecular pathogenesis of myeloid malignancies relies largely on the knowledge of cytogenetic abnormalities and recurrent chromosomal translocations, many of which have been successfully modeled in laboratory animals. In addition, as our knowledge of the genetics of myeloid leukemia improves with additional integrated profiling efforts, it will lead to more accurate animal models and facilitate translational efforts in the leukemia field. Here we review the most recent advances in the generation of mouse models of myeloid leukemias.

Semin Hematol 50:145–155. Published by Elsevier Inc.

The most widely used strategies for modeling leukemia in mice are xenotransplantation, retroviral transduction followed by transplantation, and generation of genetically modified mice, including transgenesis, gene ablation (''knock-out''), and gene targeting with a mutated copy of the gene of interest in a specific locus (''knock-in''). Retroviral transduction method currently represents a widely used technique to perform rapid and cost-effective assessment of the effect of expression of a specific gene of interest in vivo. The main caveats of this method are insufficient control of the level of expression (usually overly high), silencing of viral promoter-driven expression, inability to manipulate the niche, and random genomic integration that may lead to insertional mutagenesis.

The observation that human cells can be successfully engrafted in immunodeficient mice has opened a new era in leukemia research and preclinical therapeutic studies. Using this approach, neoplastic cells derived from leukemia patients could be propagated and studied in the in vivo setting. After years of refinement, a series of immunodeficient strains of mice have been developed, including non-obese diabetic/severe combined immunodeficient/*IL2Rγ*^{null} (NOD/SCID/*IL2Rγ*^{null} or NSG) mice that lack functional natural killer (NK) cells and support the highest level of engraftment of primary unmanipulated human leukemic cells reported to date.[1–4] Xenotransplantation has been most useful in the characterization of the immunophenotype and frequency of leukemic stem cells (LSCs),[5–8] poses no requirement

[a]Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY.
[b]Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.
Conflicts of interest: the authors declare no competing financial interests.
Address correspondence to Ross L. Levine, MD, Human Oncology and Pathogenesis Program, and Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, Box 20, New York, NY 10065. E-mail: leviner@mskcc.org
0037-1963/$ - see front matter
Published by Elsevier Inc.
http://dx.doi.org/10.1053/j.seminhematol.2013.03.027

for prior knowledge of genetic or epigenetic changes in these samples, and represents an attractive platform for evaluation of novel pharmacologic agents. However, variability in engraftment and partial reconstitution of the clinical phenotype in the majority of cases have necessitated continued efforts to improve xenotransplant systems, and limited their use in detailed preclinical therapeutic studies.

The development of genetically modified mice brought about a revolution in how cancer genes in general could be studied in an in vivo context. The present review will focus on the most recent advances in generation and characterization of genetically engineered mice harboring acute myeloid leukemia (AML)-associated genomic alterations, many of which have been developed and reported in the last few years. Table 1

MODELS OF CLASSIC GENOMIC ALTERATIONS IDENTIFIED IN MYELOID LEUKEMIAS—HEMATOPOIETIC TRANSCRIPTION FACTORS

RUNX1/CBFβ is a heterodimeric transcription regulator essential in hematopoiesis. Both genes are subject to leukemogenic chromosomal translocations/inversions with *RUNX1* (also known as *AML1* or *CBF*α) being most often fused to *ETO* as a result of t(8;21), and *CBF*β most commonly fused to the smooth muscle myosin heavy chain gene (*SMMHC*, or *MYH11*) as a result of inv(16). The function and in vivo models of *RUNX1/CBF*β and their derivatives have been reviewed elsewhere.[9,10] The CCAAT-enhancer binding protein alpha (*C/EBP*α) leucine zipper transcription factor is a critical regulator of proliferation and differentiation in multiple cell types including the myeloid lineage. Conditional ablation of *Cebpa* in the hematopoietic system led to a block in granulocyte differentiation. *Cebpa*-deficient hematopoietic stem cells (HSCs) acquire competitive advantage in transplantation assays, likely due to upregulation of the self-renewal gene *Bmi-1*, but fail to initiate leukemia.[11] *C/EBP*α is a single-exon gene expressed as two isoforms (p42 and p30), which are expressed from alternative translation initiation sites. Two distinct classes of *C/EBP*α alterations have been described in human AML: C-terminal mutations, which disrupt DNA binding and dimerization; and N-terminal mutations, which abrogate the expression of the longer p42 isoform, with biallelic mutations being the most common. Knock-in of the C-terminal *Cebpa* mutation promotes loss of quiescence and expansion of HSCs and impairs myeloid lineage commitment without conferring self-renewal.[12] The N-terminal *Cebpa* mutation knock-in, leading to p42-specific loss, maintains granulomonocytic differentiation potential but leads to acquisition of self-renewal by the committed myeloid progenitor population (c-Kit$^+$Mac1$^+$). A double knock-in combines both phenotypes, bestows LSC properties onto committed myeloid progenitor population, and accelerates the development of frank AML.[13]

Acute promyelocytic leukemia (APL) is characterized by the presence of a balanced t(15;17) chromosomal translocation resulting in the PML-RARα fusion protein, or other translocations involving retinoic acid receptor α (*RARA*) gene. The majority of APL patients can be cured by administration of regimens including all-*trans* retinoic acid (ATRA). Transgenic expression of the APL-associated RARα-fusions under control of the *hCG* or *hMRP8* promoters, or targeted into endogenous cathepsin G locus, results in the development of a myeloproliferative disorder (MPN) that progresses to leukemia in 15%–20% of cases.[14–16] More recently, Welch and colleagues developed an inducible *PML-RAR*α knocked-in into the endogenous *PML* locus, which results in expansion of the HSCs with concurrent enhancement of self-renewal phenotype and competitive repopulation capability without myeloproliferation.[17] To uncover secondary cooperating mutations, Ley and colleagues[18] sequenced a diploid mouse APL genome and identified a number of somatic events that occurred at progression to frank APL, including recurrent *Jak1* V657F mutations that they showed could cooperate with expression of *PML-RARA*, to induce transplantable leukemia.

The transcription factor Wilms tumor (*WT1*) is recurrently mutated in human AML. However, the adequate mouse models of *Wt1*-mutant leukemia are still lacking. Mice with inducible *Wt1* knock-out show an almost complete absence of erythrocytes and a reduction in the megakaryocyte-erythrocyte progenitors, suggesting a defect in myeloid differentiation. A more detailed analysis of the hematopoietic compartment was precluded by lethality due to multiple organ failure.[19]

MOUSE MODELS OF NPM1 ALTERATIONS

Nucleophosmin (*NPM1*) alterations are present in almost a third of all examined cases, making *NPM1* the second most frequently mutated gene in human AML.[20] These mutations result in aberrant cytoplasmic, instead of nuclear, localization of the protein. *Npm1*$^{-/-}$ mutants were embryonic lethal, mainly due to severe anemia; *Npm1*$^{+/-}$ heterozygous mice develop a hematologic syndrome with salient features of human myelodysplastic syndrome (MDS),[21] and suffer an increased rate of both myeloid (about 2/3) and lymphoid malignancies with features of genomic instability, especially in older animals.[22] Analysis of leukemic cells from *Npm1*$^{+/-}$ mice showed normal expression of the wild-type allele, indicating that *Npm1* haploinsufficiency, rather than biallelic loss, is sufficient to promote