Myeloproliferative Neoplasm Animal Models

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KEYWORDS

• Myeloproliferative neoplasms • Preclinical murine models • BCR-ABL • JAK2V617F • Hematopoietic stem cells • Bone marrow microenvironment • Myelofibrosis • Oncogenes

KEY POINTS

- Retroviral transduction of *BCR-ABL* into murine bone marrow cells followed by transplantation into irradiated syngeneic mice established the field of myeloproliferative neoplasm (MPN) animal modeling.
- The effects of the *JAK2V617F* mutation in hematopoietic cells has been extensively modeled in vivo using retroviral, transgenic, knock-in, and xenograft murine models.
- The considerable phenotypic differences observed between broadly similar *JAK2V617F* murine models highlights the inherent variability in murine models that can occur as a result of multiple factors, such as promoter, oncogene expression level, murine versus human protein, and mouse strain.
- Mutant oncogenes found in human acute myelogenous leukemia (AML), such as *RAS* and *FLT3*, induce MPNs in mice, indicating that these genetic lesions are insufficient to cause AML and suggesting that additional cooperating genetic events are required for AML development.
- As the increasing genetic complexity of MPNs has become apparent, additional genetic models have been developed to investigate the functional effects and therapeutic susceptibilities of compound genetic lesions in MPNs.

INTRODUCTION

Animal models have been used extensively in the study of myeloproliferative neoplasms (MPNs) and have played a key role in advancing the biologic understanding of these diseases (**Boxes 1–3**). In general, these models have faithfully recapitulated human MPNs in mice, enabled detailed characterization of the effects of specific MPN-associated genetic abnormalities on the hematopoietic stem and progenitor

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Box 1
Retroviral bone marrow transplant murine MPN models

In the retroviral bone marrow transplantation (BMT) assay, bone marrow is harvested from donor mice that have been stimulated with 5-fluorouracil (5-FU). The 5-FU–stimulated bone marrow cells are then transduced ex vivo with a retroviral construct expressing the gene of interest. This results in stable but random integration of the transgene into the host cell genome. The transduced cells are then transplanted into irradiated syngeneic mice, where hematopoietic reconstitution is polyclonal. Transgene expression is generally high (nonphysiologic), and differences in the site of retroviral integration may result in variation in transgene expression level. Because retroviruses preferentially transduce mitotically active cells, quiescent long-term hematopoietic stem cells (LT-HSCs) are relatively resistant to retroviral integration.

cell (HSPC) compartment, and provided excellent in vivo models for testing novel MPN therapeutic agents. In this review, the authors focus primarily on murine models of the *JAK2V617F* mutation and on the insights these have provided, and also briefly outline the central role *BCR-ABL* models played in establishing and developing the field. Models of additional genetic lesions found in human MPNs are discussed, and genetic models that induce an MPN phenotype in mice are outlined. The authors describe the use of *JAK2V617F* models in the preclinical development of janus kinase 2 (JAK2) inhibitors and other MPN therapies. Studies of the bone marrow microenvironment that have been performed using MPN models are summarized, and some thoughts are provided as to how MPN animal models might be used in the future.

MPN MURINE MODELS

BCR-ABL

The faithful modeling of human MPNs in mice began in 1990 with the demonstration that retroviral transduction of *BCR-ABL* into murine bone marrow cells, followed by transplantation into irradiated syngeneic mice, recapitulated human chronic

Box 2
Genetically engineered murine MPN models

Genetically engineered murine models can be classified as transgenic or endogenous. Transgenic mice express the gene of interest under the control of ectopic promoter and enhancer elements. They are generated by pronuclear injection of the transgene into a single cell of a mouse embryo, in which it randomly integrates into the mouse genome. Knock-in mice express the gene of interest from their native promoters and thus represent endogenous genetically engineered mice. They are generated through the modification of embryonic stem (ES) cells using a DNA construct that contains sequences homologous to the target gene. The relevant mutation is thus introduced to the gene of interest under the control of its endogenous promoter via homologous recombination in ES cells. Conditional knock-in mice use site-specific recombinases, such as Cre, to control the timing and tissue-specificity of gene expression. Inducible transgenic and knock-in models use exogenous ligands (eg, doxycycline or interferon) to reversibly control the timing of target-gene expression. In general, transgenic models result in overexpression of the gene of interest through the use of exogenous promoters, whereas expression is at physiologic levels in knock-in models, in which the gene of interest is expressed from its endogenous promoter. Knock-out mice are genetically engineered mice, in which the gene of interest is inactivated by replacing it or disrupting it with an artificial piece of DNA. This is achieved in ES cells via homologous recombination. Because germline homozygous gene deletions can be embryonically lethal, conditional knock-out mice are often generated to circumvent this problem.