

Shaoguang Li  
*Editor*

# Mouse Models of Human Blood Cancers

Basic Research and  
Pre-Clinical Applications



Springer

# Mouse Models of Human Blood Cancers

Shaoguang Li  
Editor

# Mouse Models of Human Blood Cancers

Basic Research and Pre-Clinical  
Applications



Springer

*Editor*

Shaoguang Li  
Division of Hematology/Oncology  
Department of Medicine  
University of Massachusetts  
Medical School  
Worcester, MA 01605, USA  
[Shaoguang.Li@umassmed.edu](mailto:Shaoguang.Li@umassmed.edu)

ISBN: 978-0-387-69130-5      e-ISBN: 978-0-387-69132-9  
DOI: 10.1007/978-0-387-69132-9

Library of Congress Control Number: 2008931589

© 2008 Springer Science+Business Media, LLC

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Springer Science + Business Media, LLC, 233 Spring Street, New York, NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden. The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

[springer.com](http://springer.com)

# Preface

Although it remains an open question among some people whether mice and humans are similar in disease development, the laboratory mouse has emerged as the pre-eminent animal model for human diseases. This is underscored by the recently completed mouse and human genome projects, which have revealed that mice and humans share the vast majority of their genes and thus get many of the same diseases and for the same reasons. For example, many mouse tumor models reflect at least some major characteristics of the corresponding human cancers. It is believed that continuously improved mouse models will play a critical role in understanding disease mechanisms and developing effective therapies for human cancers.

The use of mouse models for cancer research has a long history. In 1929, Dr. Clarence C. Little, a Harvard-trained geneticist, founded The Jackson Laboratory with the vision of generating and using inbred strains of mice to study the genetic basis of cancer. Since then, The Jackson Laboratory has become the world's leading and largest mouse genetics institution for the study and distribution of genetically defined mice, including those that develop cancers. In 1983, the National Institute of Health's National Cancer Institute designated the Laboratory as a National Cancer Center, a status that has been maintained since then. As a cancer researcher at The Jackson Laboratory, I took advantage of the broad range of expertise available here by inviting several Jackson Laboratory cancer researchers to participate in the writing of the book. In addition, to integrate expert opinions from other leading cancer researchers into the book, I invited several outstanding scientists in the blood cancer field outside of The Jackson Laboratory to contribute to the book. I am grateful to have had the opportunity to work with the book contributors, and I have learned a great deal by reading their chapters.

In this book, we emphasize why mouse models are valuable *in vivo* systems for understanding disease mechanisms and developing therapeutic strategies for human blood cancers. We focus on mouse models of blood cancers with the aim of presenting thorough analyses of the pathological features and the molecular bases of the diseases. However, our intent is not to cover all types of blood cancers; instead, we focus on several major types of blood cancer.

Besides the emphases on the description of variable mouse models of human blood cancers and on the study of disease mechanisms using the models, another focus area of the book is to describe translational research using mouse cancer models, including the models that would be valuable but are not yet available. Such translational research includes identification of critical signaling pathways in cancer cells and the development of novel therapeutic strategies against identified molecular targets. Other important topics are also addressed, including the influence of genome instability and dietary restriction on cancer development, and genetic and virological predisposition to lymphoid cell transformation. Furthermore, a novel method for DNA microarray data analysis is introduced as a potentially valuable method for future research using mouse cancer models. I believe that our areas of research focus will distinguish this book from others currently available that cover topics related to the study of human blood cancers in mouse models.

We acknowledge Stephen Sampson for helpful comments and Patricia Cherry for the secretarial assistance.

Bar Harbor, ME

Shaoguang Li

# Contents

<b>1</b>	<b>Mouse Models of Myeloproliferative Disease Associated with Mutant JAK2 Tyrosine Kinase: Insights into Pathophysiology and Therapy .....</b>	1
	Richard A. Van Etten	
<b>2</b>	<b>Genetic Modeling of Human Blood Cancers in Mice .....</b>	21
	Yiguo Hu and Shaoguang Li	
<b>3</b>	<b>Murine Models of Hematopoietic Disease: Pathologic Analysis and Characterization .....</b>	45
	Benjamin H. Lee and Jeffery L. Kutok	
<b>4</b>	<b>Mechanisms of DNA Double-Strand Break Repair in Hematopoietic Homeostasis and Oncogenesis .....</b>	81
	Sarah A. Maas, Lura Brianna Caddle, and Kevin D. Mills	
<b>5</b>	<b>Modeling Human Leukemia Using Immune-Compromised Mice . . . . .</b>	121
	Fumihiko Ishikawa, Yariko Saito, and Leonard D. Shultz	
<b>6</b>	<b>Dietary Restriction: A Model System Probing the Cell Fate Decision Between Cancer and Senescence .....</b>	133
	Robin P. Ertl and David E. Harrison	
<b>7</b>	<b>Modeling Human Philadelphia Chromosome-Positive Leukemia in Mice . . . . .</b>	157
	Shaoguang Li	
<b>8</b>	<b>Mouse Models of Human Mature B-Cell and Plasma Cell Neoplasms. ....</b>	179
	Siegfried Janz, Herbert C. Morse III, and Michael A. Teitell	

<b>9 Genetic and Virological Predisposition to Pre-B Lymphomagenesis in SL/Kh. . . . .</b>	<b>227</b>
Hiroshi Hiai	
<b>10 Animal Cancer Models in Anticancer Drug Discovery and Development . . . . .</b>	<b>245</b>
Francis Lee and Roberto Weinmann	
<b>11 DGL Global Strategies in DNA Microarray Gene Expression Analysis and Data Mining for Human Blood Cancers . . . . .</b>	<b>259</b>
Dongguang Li	
<b>Index . . . . .</b>	<b>283</b>

# **Contributors**

Lura Brianna Caddle

The Jackson Laboratory, Bar Harbor, ME 04609, USA,  
[brianna.caddle@jax.org](mailto:brianna.caddle@jax.org)

Robin P. Ertl

The Jackson Laboratory, Bar Harbor, ME 04609, USA,  
[robin.ertl@jax.org](mailto:robin.ertl@jax.org)

David E. Harrison

The Jackson Laboratory, Bar Harbor, ME 04609, USA,  
[david.harrison@jax.org](mailto:david.harrison@jax.org)

Hiroshi Hiai

Shiga Medical Center Research Institute, 5-4-30 Moriyama, City of Moriyama,  
Shiga 524-8524, Japan, [hiai@shigamed.jp](mailto:hiai@shigamed.jp)

Yiguo Hu

The Jackson Laboratory, Bar Harbor, ME, USA,  
[yiguo.hu@jax.org](mailto:yiguo.hu@jax.org)

Fumihiro Ishikawa

Research Unit for Human Disease Models, RIKEN Research Center for  
Allergy and Immunology, Yokohama 230-0045, Japan, [f\\_ishika@rcai.riken.jp](mailto:f_ishika@rcai.riken.jp)

Siegfried Janz

Department of Pathology, Carver College of Medicine, University of Iowa,  
500 Newton Road, 1046C ML, Iowa City, IA 52242, USA,  
[Siegfried-janz@uiowa.edu](mailto:Siegfried-janz@uiowa.edu)

Jeffery L. Kutok

Brigham and Women's Hospital, Harvard Medical School, Department of  
Pathology, Boston, MA 02115, USA, [jkutok@partners.org](mailto:jkutok@partners.org)

Benjamin H. Lee

Brigham and Women's Hospital, Harvard Medical School, Department of  
Pathology, Boston, MA 02115, USA, [bhlee@partners.org](mailto:bhlee@partners.org)

Francis Lee

Bristol-Myers Squibb Research and Development, Oncology Discovery,  
Princeton, NJ 08543, USA, francis.lee@bms.com

Dongguang Li

School of Computer and Information Science, Faculty of Computing, Health  
and Science, Edith Cowan University, Mount Lawley, WA 6050, Australia,  
d.li@ecu.edu.au

Shaoguang Li

The Jackson Laboratory, Bar Harbor, ME 04609, USA,  
shaoguang.li@jax.org

Current Address: Division of Hematology/Oncology, Department of Medicine,  
University of Massachusetts Medical School, Worcester, MA 01605, USA,  
Shaoguang.Li@umassmed.edu

Sarah A. Maas

The Jackson Laboratory, Bar Harbor, ME 04609, USA, sarah.maas@jax.org

Kevin D. Mills

The Jackson Laboratory, Bar Harbor, ME 04609, USA, kevin.mills@jax.org

Herbert C. Morse

Laboratory of Immunopathology, National Institute of Allergy  
and Infectious Diseases, National Institutes of Health, Rockville, MD 20852,  
USA, hmorse@niaid.nih.gov

Yoriko Saito

Research Unit for Human Disease Models, RIKEN Research Center for  
Allergy and Immunology, Yokohama 230-0045, Japan, ysaito@rcai.riken.jp

Leonard D. Shultz

The Jackson Laboratory, Bar Harbor, ME 04609, USA,  
lenny.shultz@jax.org

Michael A. Teitell

Department of Pathology and Laboratory Medicine, and  
Molecular Biology Institute and Jonsson Comprehensive Cancer Center,  
David Geffen School of Medicine, University of California,  
Los Angeles, CA 90095, USA,  
mteitell@ucla.edu

Richard A. Van Etten

Molecular Oncology Research Institute and Division of Hematology/  
Oncology, Tufts Medical Center, 800 Washington Street, 5609, Boston,  
MA 02111, USA, rvanetten@tuftsmedicalcenter.org

Roberto Weinmann

Bristol-Myers Squibb Oncology, Princeton, NJ 08543, USA,  
roberto.weinmann@bms.com

# **Chapter 1**

# **Mouse Models of Myeloproliferative Disease Associated with Mutant JAK2 Tyrosine Kinase: Insights into Pathophysiology and Therapy**

**Richard A. Van Etten**

## **Contents**

1.1	Dysregulated Tyrosine Kinases are the Hallmark of Chronic Myeloproliferative-Like Syndromes . . . . .	1
1.2	Mouse Models of Hematologic Malignancies Induced by Dysregulated TKs . . . . .	4
1.3	The Retroviral BM Transduction/Transplantation Model of CML . . . . .	5
1.4	Strategies to Study Mechanisms of Leukemogenesis in Mice . . . . .	7
1.5	Studies of MPD Induced by JAK2-V617F in the Mouse Retroviral Transduction/Transplantation Model . . . . .	8
1.5.1	JAK2-V617F Induces Polycythemia in Mice by Overproduction of Erythrocytes that is Independent of Epo . . . . .	8
1.5.2	JAK2-V617F Induces Strain-Dependent Leukocytosis In Mice, but not Thrombocytosis . . . . .	9
1.5.3	Histopathology of MPD Induced in Mice by JAK2-V617F . . . . .	10
1.6	Studying Signaling Mechanisms of JAK2-V617F-Induced Polycythemia in Mice . . . . .	11
1.7	Testing the Response of JAK2-V617F-Induced Polycythemia to Kinase Inhibitor Therapy . . . . .	13
1.8	Modeling MPDs Associated with Other Mutant TKs . . . . .	15
1.9	Emerging Transgenic Mouse Models of JAK2-V617F-Induced MPD . . . . .	16
1.10	Summary and Future Directions . . . . .	16

### **1.1 Dysregulated Tyrosine Kinases are the Hallmark of Chronic Myeloproliferative-Like Syndromes**

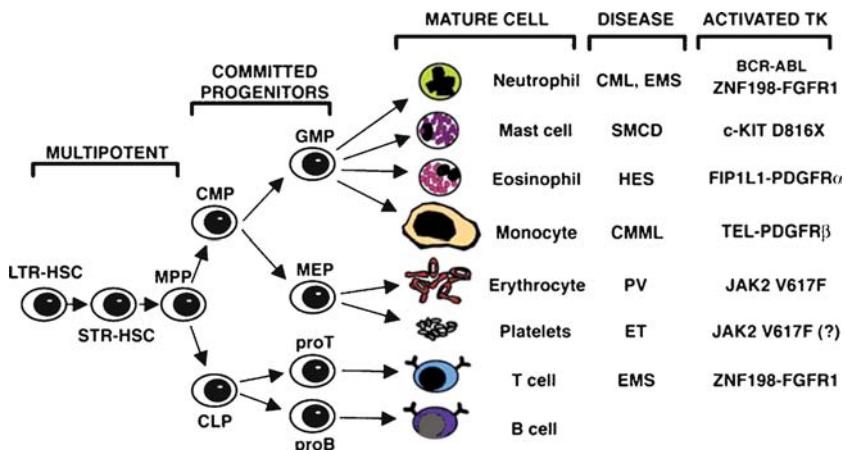
The classical myeloproliferative diseases (MPDs) include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF, also known as myelofibrosis with myeloid metaplasia or agnogenic myeloid metaplasia). These diseases were first grouped together by Dr. William Dameshek of Tufts-New England Medical Center in

---

R.A. Van Etten

Molecular Oncology Research Institute and Division of Hematology/Oncology,  
Tufts Medical Center, 800 Washington Street, 5609, Boston, MA 02111, USA  
rvanetten@tuftsmedicalcenter.org

a seminal paper in *Blood* in 1951 (Dameshek 1951). The MPDs are clonal disorders of hematopoiesis characterized by overproduction of mature myeloerythroid cells, abnormalities of hemostasis and thrombosis, and tendency to progress to acute leukemia (Van Etten and Shannon 2004). In the 2001 WHO classification, chronic eosinophilic leukemia (CEL) was included among the MPDs while a closely related group of diseases with mixed myelodysplastic/myeloproliferative features was also recognized (Vardiman et al. 2002). Over the last several years, there has been a revolution in our understanding of the pathogenesis of these disorders, with the recognition that somatic activating mutations in tyrosine kinases (TKs) are found in a subset of patients from each disease category (Fig. 1.1) (Krause and Van Etten 2005). Some cases of chronic myelomonocytic leukemia (CMML) are associated with activation of platelet-derived growth factor receptor beta (PDGFR $\beta$ ) through chromosome 5q translocations that fuse PDGFR $\beta$  with TEL (Golub et al. 1994) or many other partners (Jones and Cross 2004). Fusion of PDGFR $\alpha$  to FIP1L1 through interstitial deletions on chromosome 4q is found in a subset of patients with CEL/hypereosinophilic syndrome (Cools et al. 2003; Griffin et al. 2003), whereas activating point mutations in c-KIT are found in some patients with systemic mast cell disease (Furitsu et al. 1993). Patients with 8p11 myeloproliferative syndrome (EMS) (Macdonald et al. 2002) have myeloproliferation frequently accompanied by non-Hodgkin's lymphoma. Both myeloid and lymphoma cells share translocations involving chromosome 8p, which lead to fusion of the receptor TK fibroblast growth factor receptor-1 (FGFR-1) to multiple partners, including ZNF198 (Xiao et al. 1998). Together, these



**Fig. 1.1 Dysregulated tyrosine kinases (TKs) in the myeloproliferative syndromes.** Multi-potent stem cells are depicted at left, committed progenitors in the middle, and mature blood cells at right. Each mature myeloid element is overproduced in a distinct myeloproliferative-like syndrome that is associated with a specific activated tyrosine kinase (far right)

observations indicate that dysregulated TKs are found in the majority of the chronic myeloproliferative-like syndromes. This new understanding is reflected in the new 2008 WHO classification of the chronic myeloid neoplasms, which recognizes a novel category of chronic myeloid disease with eosinophilia and mutations in PDGFR $\alpha/\beta$  or FGFR-1 (Tefferi and Vardiman 2008).

Until recently, the pathogenesis of PV, ET, and PMF was less clear. PV is characterized by overproduction of mature erythrocytes, increased hematocrit and red cell mass, splenomegaly due to extramedullary hematopoiesis (Spivak 2002), and often increased circulating granulocytes and platelets. The clinical course of PV is complicated by abnormalities of hemostasis, including platelet dysfunction and bleeding, as well as arterial and venous thrombosis. The disease evolves to acute myeloid leukemia in about 20% of patients while progression to a “spent” phase, characterized by myelofibrosis and normal or low hematocrit, is more common. A hallmark of PV is the presence of endogenous erythroid colonies (EECs), erythroid progenitors that form colonies in vitro in the absence of exogenous erythropoietin (Epo) (Prchal and Axelrad 1974). Molecular studies in PV revealed no mutations in the Epo receptor, but PV granulocytes have increased transcripts for the urokinase plasminogen activator receptor PRV-1 (Temerinac et al. 2000), whereas PV platelets show decreased expression of c-Mpl, the receptor for thrombopoietin (Moliterno et al. 1998). However, whether these abnormalities are fundamental to the pathogenesis of PV was unclear.

JAK2 is a member of the Janus family of nonreceptor TKs and is required for signaling from the Epo receptor and other type I cytokine receptors (Parganas et al. 1998). In 2005, studies of erythroid progenitors from PV patients demonstrated that Epo-independent erythroid maturation was impaired by a JAK2 inhibitor (Ugo et al. 2004) and by siRNA knockdown of JAK2 (James et al. 2005). This prompted sequencing of the *JAK2* gene, which identified a G to A point mutation, resulting in substitution of phenylalanine for valine at amino acid 617 (V617F), in the JAK2 pseudokinase domain in the majority of PV patients (James et al. 2005). The JAK2-V617F mutant had constitutive kinase activity in vivo in the absence of Epo stimulation, and retroviral expression in murine bone marrow (BM) caused erythrocytosis (James et al. 2005). The JAK2-V617F mutation was independently identified through genomic sequencing of TKs in MPD patients (Baxter et al. 2005; Levine et al. 2005), and by investigation of loss of heterozygosity involving the *JAK2* gene on chromosome 9p (Kralovics et al. 2005). The JAK2-V617F mutation is found in nearly every patient with PV and is present in homozygous form through mitotic recombination in up to 30% of patients. The mutation is also found in 30–60% of ET and CMF patients but is rarely found outside the MPDs (Jones et al. 2005a; Scott et al. 2005; Steensma et al. 2005). The widespread finding of JAK2-V617F in the non-CML MPDs suggested that it may contribute to the pathogenesis of these diseases. However, it was not clear from these human studies whether JAK2-V617F could be implicated as the direct and primary cause of PV, ET, or

CIMF nor was the relationship between the different MPDs that share the JAK2 mutation understood.

## 1.2 Mouse Models of Hematologic Malignancies Induced by Dysregulated TKs

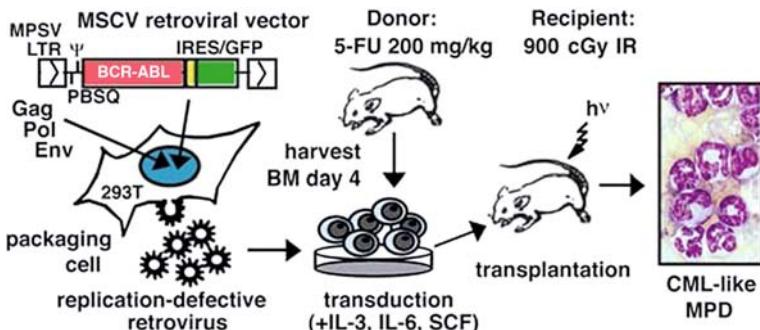
How do we know that the dysregulated TKs depicted in Fig. 1.1 play a role in the pathogenesis of these distinctly different diseases? Biochemical studies *in vitro*, in cell lines, and in primary patient cells can confirm that these TKs are dysregulated and can give insight into the signaling pathways that they activate. However, a role of a TK in disease pathogenesis can only be established by the expression of that TK in the hematopoietic system *in vivo*. Over the past decade, several laboratories have pioneered the use of laboratory mice as model systems for studying the genetics and pathophysiology of human leukemia. The strategy is conceptually straightforward and involves expression, in BM of mice, of genes that are mutated or dysregulated in human leukemia cells. A primary motivation for this effort is to determine whether and how a particular genetic abnormality that is identified in a leukemic cell contributes to the malignant phenotype. If one is successful in recapitulating some or all of the leukemia phenotype in mice, then the system can then be used for studying the molecular pathophysiology of that disease, where the goal is a description, in biochemical terms, of the specific cellular abnormalities that explain the development and clinical course of the malignancy and its response to treatment. Finally, an accurate mouse model of leukemia can serve as a platform for testing potential new therapies, particularly those directed at specific molecular targets. The power of mouse models derives from their ability to accurately recapitulate the malignant phenotype in primary cells *in vivo*. However, this accuracy comes at the price of significant complexity, and careful pathological and molecular analysis is required in order to reach correct conclusions [for review, see Van Etten (2002); see also the section on “Pathological Analysis and Characterization of Mouse Models of Hematopoietic Disease”]. If these precautions are heeded, then mouse models can be used to answer important questions about the pathophysiology of human leukemia that are difficult if not impossible to approach through studies of human primary cells and cell lines.

There are two different strategies to express dysregulated TKs in the mouse hematopoietic system: transgenic mice and retroviral gene transfer into the BM followed by transplantation. Both methods have their own advantages and limitations [reviewed in Van Etten (2001)]. Transgenic mice allow production of a uniform cohort of diseased mice by breeding, but multiple new transgenic lines must be generated for each new TK mutant to be studied, and the transgene is present in all tissues in the embryo and adult. As activated TKs can have deleterious effects during development, attempts to express BCR-ABL via a traditional transgene or knock-in have encountered problems

with toxicity and silencing (Heisterkamp et al. 1991; Jaiswal et al. 2003). With JAK2-V617F, similar difficulties have been observed. Attempts to express JAK2-V617F via a 190-kb human BAC transgene led to early lethality in three transgenic pups while a fourth viable transgenic mouse that expressed mutant JAK2 at very low levels had no erythroid phenotype (R. Skoda, personal communication). These observations suggest that more sophisticated conditional transgenic approaches [for example, see Huettner et al. (2000)] may be necessary to express dysregulated TKs in mice. By contrast, in the retroviral BM transduction/transplantation system, it is easy to test the leukemogenicity of new TKs and TK mutants by simply making new retroviral stocks. Furthermore, the effect of expression of a TK in distinct subsets of BM progenitors can be assessed. The major drawback is the labor-intensive and technically demanding nature of the experiments. This retroviral model system is now widely used by scientists across the molecular oncology field to investigate the pathogenetic role of genetic abnormalities identified in cancer cells.

### 1.3 The Retroviral BM Transduction/Transplantation Model of CML

The causative role of BCR–ABL in CML was demonstrated 17 years ago when expression of this TK by retroviral transduction in mouse BM induced MPD in recipients that closely resembled human CML (Daley et al. 1990; Kelliher et al. 1990). Subsequently, several laboratories exploited transient retroviral packaging systems to achieve efficient induction of CML-like leukemia in mice (Li et al. 1999; Pear et al. 1998; Zhang and Ren 1998), allowing the model to be used as an assay for the first time (for a detailed experimental protocol, see Gavrilescu and Van Etten (2008)). It is now possible to routinely induce CML-like leukemia in 100% of recipient mice within 4–5 weeks after transplantation when the donors are pretreated with 5-fluorouracil (5-FU) and transduced in the presence of myeloid cytokines (Fig. 1.2). Myeloid cells from these mice carry the retroviral provirus, express BCR–ABL, and exhibit increased levels of tyrosyl-phosphorylated proteins and activation of multiple cell signaling pathways (Li et al. 1999; Roumiantsev et al. 2001). Analysis of the proviral integration pattern by Southern blotting reveals that the CML-like disease is polyclonal, with the same spectrum of proviral clones present in neutrophils, macrophages, erythroid progenitors, B lymphocytes, and in some cases T lymphocytes (Li et al. 1999; Million et al. 2002). This demonstrates that the *BCR–ABL*-transduced BM cells that initiate the CML-like disease in primary recipients have multi-lineage repopulating ability, a feature of hematopoietic stem cells. The polyclonal nature and short latency of murine CML-like leukemia differ from human CML, which is monoclonal and more chronic. However, these are not important pathophysiological differences but merely reflect transplantation of multiple *BCR–ABL*-transduced progenitors into each



**Fig. 1.2** Retroviral BM transduction-transplantation model of BCR-ABL-induced chronic myeloid leukemia (CML)-like myeloproliferative disease (MPD). The BCR-ABL oncogene is cloned in the MSCV retroviral vector, which co-expresses green fluorescent protein (GFP) from an internal ribosome entry site (IRES). For induction of CML-like disease, donors are pretreated with 5-FU, bone marrow (BM) is harvested and transduced ex vivo in the presence of myeloid cytokines, followed by transplantation into irradiated syngeneic recipient mice. All recipients develop fatal MPD within 5 weeks, characterized by leukocytosis with maturing neutrophils, as illustrated by the peripheral blood smear

recipient. When lower titer virus is used or limiting numbers of transduced cells are transplanted, oligo- to monoclonal disease with a longer latency is observed (Daley et al. 1990; Jiang et al. 2003).

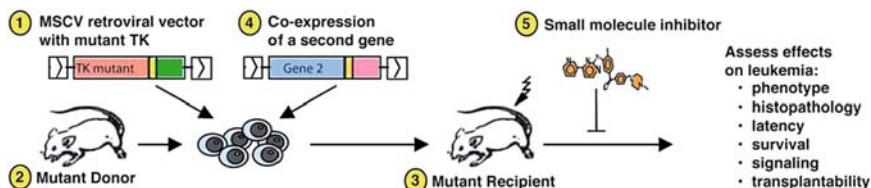
The CML-like disease induced by BCR-ABL is efficiently transplanted to irradiated secondary recipient mice with BM or spleen cells from primary diseased mice and secondary leukemic recipients also demonstrate multi-lineage involvement (Li et al. 1999). Unless treated by allogeneic hematopoietic stem cell transplantation or ABL kinase inhibitor drugs, CML patients inevitably progress to acute myeloid or lymphoid leukemia, known as blast crisis. Mice with CML-like leukemia die soon after reconstituting hematopoiesis from overwhelming granulocytosis and organ infiltration and dysfunction, and we generally do not observe evidence of progression to blast crisis in these primary recipients. However, acute leukemias of myeloid, and, more commonly, lymphoid origin are observed upon serial transplantation of CML-like disease to secondary and tertiary recipients (Daley et al. 1991; Gishizky et al. 1993; Pear et al. 1998). Analysis of proviral integration patterns demonstrates that these acute leukemias are oligo- to monoclonal and arise from clone(s) found in the primary animal with CML-like leukemia. This is evidence of clonal disease progression and demonstrates that the model recapitulates this important feature of human CML. Finally, the murine CML-like disease responds to treatment with the ABL kinase inhibitor imatinib (Hu et al. 2004; Wolff and Ilaria 2001) and to immunotherapy with allogeneic donor leukocyte infusions (Krause and Van Etten 2004).

To summarize, the murine CML-like disease induced by BCR-ABL upon retroviral transduction of BM is a very close pathophysiological match to the human illness in terms of the cell of origin, transplantability, disease

progression, and response to both kinase inhibitor and immunological therapies. However, the retroviral model system does have some drawbacks and limitations [reviewed in Van Etten (2001)]. The issues of disease latency and clonality were mentioned earlier. In addition, BCR–ABL is expressed from the proviral LTR at several-fold higher levels than are typically found in chronic phase CML cells (Barnes et al. 2005; Li et al. 1999), which may also affect disease latency. In addition, human CML cells are typically haploid for the *BCR* and *c-ABL* genes and can express the reciprocal ABL–BCR fusion product. However, the fact that BCR–ABL expression alone recapitulates the disease in mice argues that these differences are not critical for leukemogenesis.

## 1.4 Strategies to Study Mechanisms of Leukemogenesis in Mice

Once an accurate retroviral transplant model of human leukemia or MPD is established, there are several complementary strategies that can be used to analyze the molecular pathogenesis of the disease (Fig. 1.3). First, one can introduce mutants of the TK into BM to assess their effect on leukemogenesis. The most informative mutants are point mutations or small deletions that selectively affect the phosphorylation, protein–protein interactions, subcellular localization, or regulatory properties of the TK. Second, the investigator can utilize mice with naturally occurring or targeted mutations in signaling molecules as BM donors in the retroviral transduction/transplantation model to determine whether a given molecule plays a role in leukemogenesis. This strategy works best when the mutant mice have relatively normal baseline hematopoiesis, because it can be difficult to interpret defects in leukemogenesis when a mutation profoundly affects the production of blood cells. In a third scenario, if signaling within the BM microenvironment plays a role in leukemia development, mutant mice can be utilized as the recipients for transduced BM from either normal or mutant donors. Fourth, one can co-express other genes along with the dysregulated TK in the BM. This can be done either using a separate retroviral vector to co-transduce the BM or by co-expressing the second gene downstream of the IRES in place of GFP, in the same vector used to express the TK. The first



**Fig. 1.3 Strategies to study mechanisms of leukemogenesis by dysregulated tyrosine kinases (TKs) in the retroviral transduction model.** Five complementary approaches can be employed to study the pathogenesis of leukemias induced by dysregulated TKs. See text

approach can be useful for assessing the effect of dominantly acting genes that potentiate leukemogenesis [for example, see Dash et al. (2002)], but it is difficult to detect negative effects (such as expression of a dominant-negative mutant) because of the low probability of transducing individual HSC with more than one virus. The alternative approach, using the IRES to co-express the second gene, forces each transduced cell to express both genes and does allow negative effects to be demonstrated (Hao and Ren 2000). The latter approach can also be used in a “rescue” experiment to complement a leukemogenic defect in mutant BM cells (Ayton and Cleary 2003). Finally, the investigator can treat recipient mice with small molecule inhibitors of various signaling pathways to determine whether that pathway contributes to leukemogenesis. In order to dose the drug properly, one must know the pharmacokinetic profile of the compound in rodents, but this information is usually available for drugs that are candidates for clinical development. Using drugs to study leukemia signaling in mice is subject to the same concerns about specificity and off-target effects that are encountered in cell-culture experiments. With molecularly targeted drugs, one has the advantage of being able to assess the target directly and of using genetic approaches for target validation [for example, see Hu et al. (2004)]. With all these strategies, the endpoints that can be assessed are quantitative effects on the disease phenotype, including histopathology, latency, survival, signaling abnormalities, and transplantability.

## **1.5 Studies of MPD Induced by JAK2-V617F in the Mouse Retroviral Transduction/Transplantation Model**

To investigate the molecular pathogenesis of MPD induced by JAK2-V617F, several research groups have expressed murine JAK2 WT or JAK2-V617F in the hematopoietic system of mice using the retroviral transduction/transplantation approach (Bumm et al. 2006; Lacout et al. 2006; Wernig et al. 2006; Zaleskas et al. 2006). Two of the studies further compared the phenotype of the disease in two different inbred strains of mice, Balb/c and C57Bl/6 (B6) (Wernig et al. 2006; Zaleskas et al. 2006).

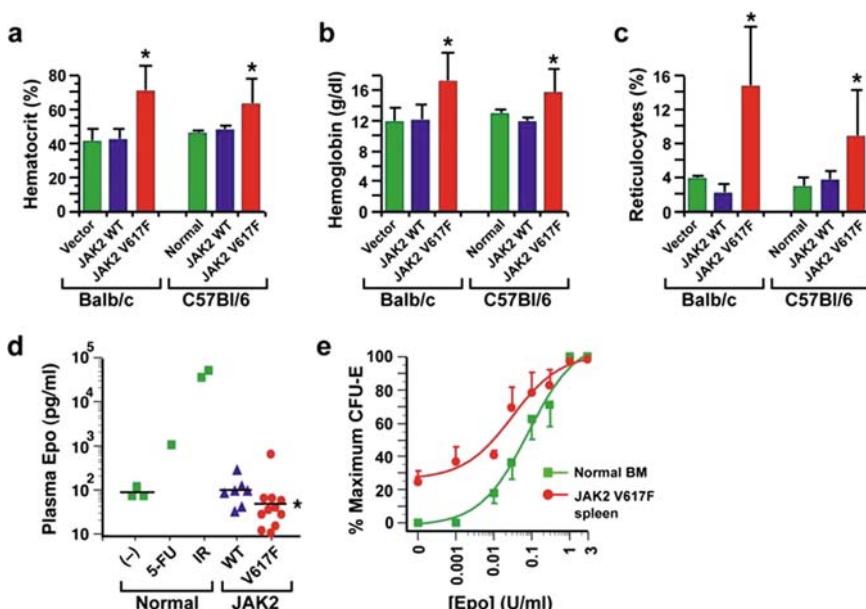
### ***1.5.1 JAK2-V617F Induces Polycythemia in Mice by Overproduction of Erythrocytes that is Independent of Epo***

In both strains of mice, recipients of JAK2-V617F-transduced BM exhibited markedly increased blood hematocrit and hemoglobin levels that were evident by three weeks after transplantation and sustained for months, whereas recipients of JAK2 WT- or vector-transduced BM were normal. Polycythemia was accompanied by a striking increase in circulating reticulocytes, representing the newest population of circulating red cells. Plasma Epo levels were suppressed in the

polycythemic mice, demonstrating that the erythropoiesis in these mice was autonomous and not driven by overproduction of Epo. CFU-E from BM or spleen of these recipients showed increased sensitivity to Epo, with around 25% of CFU-E growing in the absence of exogenous Epo. This demonstrates that EECs are found in these polycythemic mice, similar to human PV patients. Taken together, these results indicate that JAK2-V617F expression directly induces polycythemia in mice through Epo-independent overproduction of erythrocytes (Fig. 1.4). The fact that the principal erythroid features of PV are recapitulated by expression of JAK2-V617F argues that it is the primary and direct cause of human PV.

### 1.5.2 JAK2-V617F Induces Strain-Dependent Leukocytosis In Mice, but not Thrombocytosis

In contrast to the polycythemia, the effects of JAK2-V617F expression on leukocyte and platelet counts in the retroviral model were more variable.



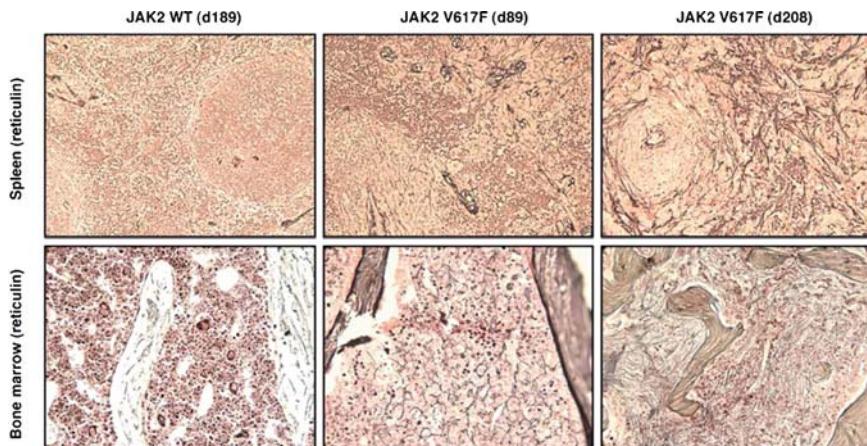
**Fig. 1.4 JAK2-V617F induces polycythemia through autonomous overproduction of erythrocytes.** (a) Hematocrit, (b) blood hemoglobin, and (c) reticulocyte counts from cohorts of Balb/c or B6 mice transplanted with syngeneic BM cells transduced with empty vector (green), or retrovirus expressing murine JAK2 WT (blue) or JAK2-V617F (red). In the case of the B6 cohorts, untransplanted mice (“normal”) were used as controls. (d) Plasma Epo levels for the three groups (B6 background). (e) Percent maximal CFU-E from normal BM (green) or spleen of JAK2-V617F recipients (red). The asterisks in the figure indicate that the differences between JAK2-V617F and JAK2 WT in each panel were statistically ( $P \leq 0.05$ , t-test) Adapted from Zaleskas et al. (2006). (See color insert)

JAK2-V617F induced significant leukocytosis in Balb/c mice, but only a modest increase in B6 mice. These results suggest that genetic differences between the two mouse strains affect the leukocytosis induced by JAK2-V617F. JAK2-V617F expression did not affect the platelet count in either strain despite evidence of proviral expression of GFP in megakaryocytes, which was associated with a significant decrease in the DNA ploidy of the megakaryocytes (Wernig et al. 2006). However, JAK2-V617F recipients had a marked defect in platelet function, with significantly prolonged tail bleeding time (Zaleskas et al. 2006). The lack of thrombocytosis suggests that additional events may be required for JAK2-V617F to cause ET, which is consistent with human studies that suggest that there may be a predisposing mutation(s) distinct from JAK2-V617F that influence the development and phenotype of the MPD (Kralovics et al. 2006). These mouse studies further suggest that qualitative platelet abnormalities induced by JAK2-V617F may contribute to the hemostatic complications of PV patients.

### ***1.5.3 Histopathology of MPD Induced in Mice by JAK2-V617F***

Despite the marked polycythemia, the MPD induced by JAK2-V617F was not fatal in either strain, with most recipients surviving for many months. This is in sharp contrast to BCR-ABL-induced CML-like MPD, which is rapidly fatal in mice because of massive infiltration of the lungs, liver, and spleen with maturing neutrophils (Roumiantsev et al. 2004). At necropsy, JAK2-V617F recipients had significant splenomegaly, particularly in Balb/c mice, but no involvement of lymph nodes or thymus and no pulmonary hemorrhages. The marrow of JAK2-V617F recipients was hypercellular, with a predominance of myeloid over erythroid cells and less than 5% blasts. Spleens exhibited massively increased erythroid precursors with partial disruption of follicular architecture and infiltration with mature myeloid cells. Livers showed moderate periportal extramedullary myeloerythropoiesis, whereas lungs had only minimal myeloid infiltration. There were increased numbers of abnormal megakaryocytes present in BM and spleen, with clustering.

The polycythemia induced by JAK2-V617F was maximal at about 4 months following transplantation but tended to decrease with time in both strains, with hematocrit and reticulocyte counts returning to nearly normal ranges by 7–8 months after transplantation and some mice developing frank anemia. This coincided with a gradual but marked increase in fibrosis in the BM and spleen of JAK2-V617F recipients that was not observed in JAK2 WT recipients (Lacout et al. 2006; Wernig et al. 2006; Zaleskas et al. 2006) (Fig. 1.5). This is reminiscent of evolution of human PV to a “spent phase” resembling PMF. In one study, polycythemia and reticulocytosis could be resurrected in secondary mice through transplantation of BM or spleen cells from primary JAK2-V617F recipients harvested either in the early



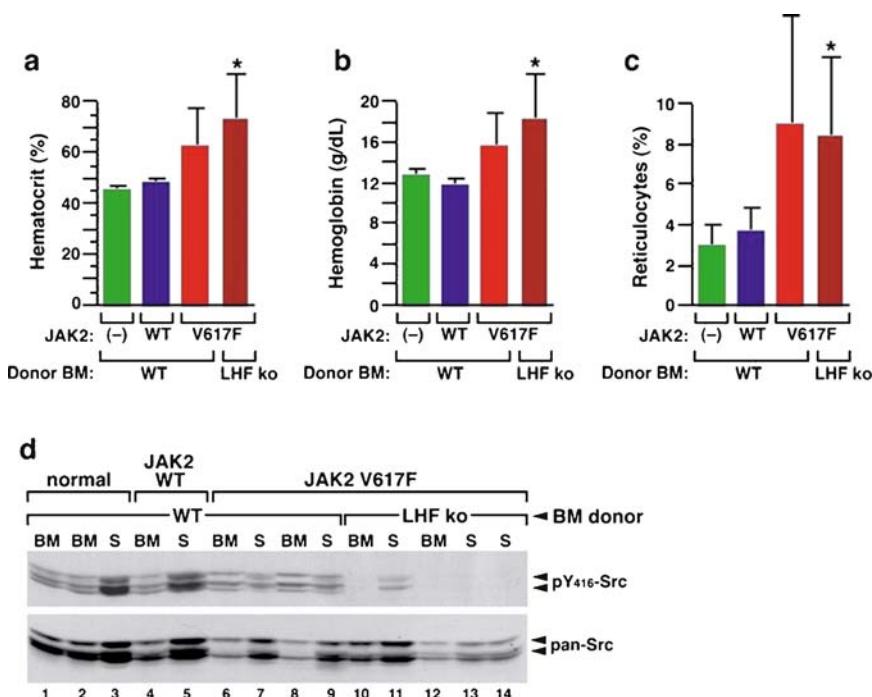
**Fig. 1.5 Development of myelofibrosis in JAK2-V617F recipient mice.** Increasing fibrosis (demonstrated by reticulin staining) in spleen (top panels) and bone marrow (BM) (bottom panels) of representative JAK2-V617F recipients at about 3 (middle panels) and 7 months (right panels) after transplantation. Note the marked increase in reticulin staining at 7 months in the JAK2-V617F recipients, but not in recipients of JAK2 wild-type (WT)-transduced BM (left panels). Adapted from Zaleskas et al. (2006). (See color insert)

polycythemic phase of the disease or in the later myelofibrotic stage (Zaleskas et al. 2006). These findings suggest that JAK2-V617F expression induces myelofibrosis, but the resulting impairment of erythropoiesis is due to a defect of the hematopoietic microenvironment rather than a deficiency of malignant hematopoietic stem cells.

## 1.6 Studying Signaling Mechanisms of JAK2-V617F-Induced Polycythemia in Mice

The retroviral model of JAK2-V617F-induced polycythemia provides an excellent tool to investigate the signaling pathways critical for disease pathogenesis and to identify new potential targets for therapy. Stimulation of erythroid cells with Epo leads to activation of several Src family kinases, including Lyn (Richmond et al. 2005). Interestingly, previous studies demonstrated that a relatively nonselective Src kinase inhibitor, PP2, impaired the Epo-independent differentiation of erythroid progenitors from PV patients (Ugo et al. 2004), suggesting a role for Src kinases in the pathogenesis of PV. This has clinical relevance, because dasatinib, a dual ABL/SRC kinase inhibitor, has been approved by the FDA for the treatment of CML but could be redirected for therapy of PV. One approach to investigate the role of Src kinases in the polycythemia induced by JAK2-V617F (strategy #2 in Fig. 1.3) is to employ donor mice lacking Lyn, Hck, and Fgr, the three principal Src kinases in myeloerythroid progenitor cells (Hu et al. 2004; Meng and Lowell 1997), as

the donors for BM for transduction with JAK2-V617F. These triple Src-deficient mice have normal steady-state hematopoiesis but exhibit impaired erythropoietic responses to hemolysis as a consequence of Lyn deficiency (Ingle et al. 2005). Interestingly, recipients of *Lyn*<sup>-/-</sup>*Hck*<sup>-/-</sup>*Fgr*<sup>-/-</sup> BM transduced with JAK2-V617F developed polycythemia and reticulocytosis that tended to be greater than recipients of JAK2-V617F-transduced WT donor BM (Fig. 1.6) although this did not reach statistical significance. The results demonstrate that these particular Src kinases are not required for polycythemia induced by JAK2-V617F and might even play a negative role in JAK2-V617F signaling.



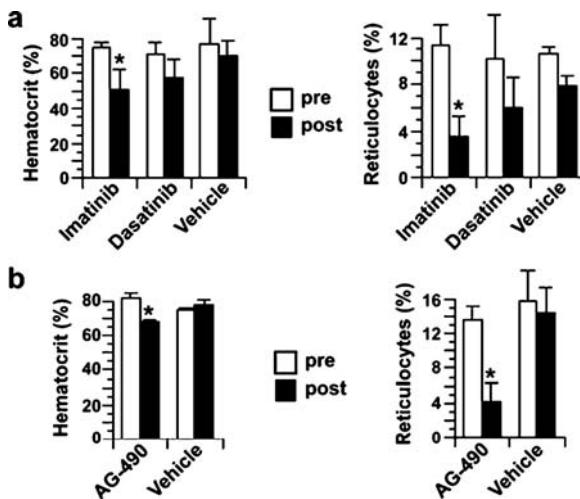
**Fig. 1.6 Polycythemia induced by JAK2-V617F is independent of Src family kinases.** (a) Hematocrit, (b) blood hemoglobin, and (c) reticulocyte counts from normal (-) B6 mice (green), B6 recipients of B6 wild-type (WT) bone marrow (BM) transduced with retrovirus expressing murine JAK2 WT (blue) or JAK2-V617F (red), and B6 *Lyn*<sup>-/-</sup>*Hck*<sup>-/-</sup>*Fgr*<sup>-/-</sup> BM transduced with retrovirus expressing JAK2-V617F (orange). (d) Western blot analysis of extracts of primary myeloerythroid cells from individual normal (lanes 1–3) B6 mice, recipients of WT BM transduced with JAK2 WT retrovirus (lanes 4–5), recipients of WT BM transduced with JAK2-V617F retrovirus (lanes 6–9), and recipients of *Lyn*<sup>-/-</sup>*Hck*<sup>-/-</sup>*Fgr*<sup>-/-</sup> BM transduced with JAK2-V617F retrovirus (lanes 10–14). The membrane was blotted with antibody recognizing the phosphorylated activation loop tyrosine (Y146 homolog) of c-Src, Lyn, Hck, Fyn, Lck, and Yes (*top panel*) and subsequently with antibody against total c-Src, Fyn, Yes, and Fgr (*bottom panel*). The asterisks in the figure indicate that the differences between JAK2-V617F and JAK2 WT in each panel were statistically ( $P \leq 0.05$ , t-test) Adapted from Zaleskas et al. (2006). (See color insert)

There is a formal possibility that one or more of the other six vertebrate Src family kinases might compensate for the lack of Lyn/Hck/Fgr, particularly Fyn, Yes, and c-Src, which are expressed in myeloid cells. However, there was no overexpression of Fyn, Yes, or c-Src in myeloerythroid cells from polycythemic recipients of JAK2-V617F-transduced *Lyn<sup>-/-</sup>Hck<sup>-/-</sup>Fgr<sup>-/-</sup>* BM and little or no detectable activation of these Src kinases, as assessed by an antibody against the phosphorylated activation loop tyrosine in Src (Fig. 1.6). These results suggest that compensation by other Src family members is unlikely to play a role in polycythemia induced by JAK2-V617F in the mutant cells.

## 1.7 Testing the Response of JAK2-V617F-Induced Polycythemia to Kinase Inhibitor Therapy

The retroviral model of JAK2-V617F-induced MPD is also an excellent platform for preclinical testing of new therapies, particularly molecularly targeted drugs. By analogy to BCR-ABL in CML, JAK2 is a rational therapeutic target in PV. Several published studies have already begun to address this topic (Pardanani 2008). In one study, cohorts of polycythemic JAK2-V617F recipient mice were treated for a 2-week period with small molecule TK inhibitors, including imatinib and the dual ABL/Src inhibitor dasatinib (Zaleskas et al. 2006). Imatinib therapy can reduce the hematocrit in some human PV patients but has minimal effects on the level of JAK2-V617F (Jones et al. 2005b). Relative to vehicle-treated controls, imatinib-treated mice demonstrated significant decreases in hematocrit and reticulocyte counts, while the corresponding responses to dasatinib were less robust and did not reach statistical significance (Fig. 1.7a, b). These results suggest that imatinib impairs JAK2-V617F-induced erythropoiesis through inhibition of a target other than ABL or c-Kit and confirm the genetic data that Src kinases may not be good targets for therapy in PV. AG-490 is a parenteral kinase inhibitor of the tyrphostin family that inhibits JAK2 but is relatively nonspecific. The compound is not orally bioavailable, and parenteral administration is complicated by its short half-life and low solubility, but it has been previously shown to be efficacious against acute lymphoid leukemia cells in a mouse xenotransplant model (Meydan et al. 1996). Continuous parenteral administration of AG-490 to mice with JAK2-V617F-induced polycythemia over a 2-week period caused a modest but significant decrease in hematocrit with a more pronounced drop in reticulocytes (Fig. 1.7b), suggesting that chronic treatment with a JAK2 inhibitor would have therapeutic benefit in PV (Zaleskas et al. 2006).

A second study employed an orally bioavailable small molecule JAK2 inhibitor, TG101209, in mice transplanted with cell lines expressing JAK2-V617F (Pardanani et al. 2007). Pharmacodynamic studies documented a marked inhibition of Stat5 phosphorylation in the leukemic cells following a single oral 100 mg/kg dose, and sustained daily therapy was associated with



**Fig. 1.7 Polycythemia and reticulocytosis induced by JAK2-V617F responds to kinase inhibitor therapy.** (a) Hematocrit (left panel) and reticulocyte counts (right panel) of cohorts of mice treated with twice daily oral gavage with 100 mg/kg imatinib, 10 mg/kg dasatinib, or vehicle, determined before initiation of therapy (white bars, “pre”) or after 2 weeks of treatment (black bars, “post”). The hematocrit and reticulocyte count were significantly decreased in response to imatinib therapy, whereas imatinib had no effect on these parameters in normal mice (data not shown). (b) Hematocrit (left panel) and reticulocyte counts (right panel) of mice treated with continuous parenteral ad ministration of 300 µg/day AG-490 or vehicle, determined before initiation of therapy (white bars) or after 2 weeks of treatment (black bars). The hematocrit and reticulocyte count were significantly decreased in response to AG-490 therapy. The asterisks in the figure indicate that the difference between pre- and post-treatment values in each panel were statistically significant ( $P < 0.05$ , t-test) Adapted from Zaleskas et al. (2006)

a significant decrease in leukemic cell burden and with prolonged survival. Together, these studies demonstrate that JAK2-V617F is a very promising target for rational therapy of PV. However, several critical questions have not yet been addressed in the model. Initially, there is great interest in determining whether erythropoiesis driven by JAK2-V617F may be particularly sensitive to pharmacological inhibition of JAK2, relative to normal Epo-dependent erythropoiesis. In CML, depending on the cytokine conditions used, Ph<sup>+</sup> myeloid progenitors are selectively killed in vitro by ABL kinase inhibitors such as imatinib (Deininger et al. 1997; Druker et al. 1996), a phenomenon referred to as “oncogene addiction” (Weinstein 2002). If JAK2-V617F<sup>+</sup> erythroid progenitors also exhibit heightened sensitivity to a JAK2 inhibitor, this may create a therapeutic “window” that allows elimination of the malignant clone in PV without causing generalized myelosuppression. Another important question is whether inhibition of JAK2-V617F will reverse the nonerythroid phenotypic abnormalities found in PV, such as myelofibrosis, platelet dysfunction, and predisposition to thrombosis. This is a critical question for clinical trial design, because it is very likely that JAK2 inhibitors will enter human trials in patients

with PMF, not classical PV, because of the dismal prognosis of the former patients. We may expect these important preclinical questions to be addressed in the retroviral model system in the near future.

## 1.8 Modeling MPDs Associated with Other Mutant TKs

Although the great majority of patients with the clinical diagnosis of PV have the JAK2-V617F mutation if the analysis is done with sensitive allele-specific PCR, a small subset of patients are negative for this mutation, which prompted a search for mutations elsewhere in the JAK2 molecule. This led to the identification of a cluster of somatic mutations in exon 12 of the JAK2 gene, including a point mutation (K539L) and several small amino acid deletions and insertions (Scott et al. 2007). These exon 12 mutant JAK2 kinases, when co-expressed in cytokine-dependent hematopoietic cell lines with Epo-R, transformed the cells to cytokine-independent growth. When expressed in murine BM via the retroviral transduction/transplantation model, the JAK2 exon 12 mutants induced MPD that was indistinguishable from that induced by JAK2-V617F, with erythrocytosis and polycythemia, leukocytosis, and splenomegaly. Interestingly, although a subset of patients with JAK2 exon 12 mutations had a clinical phenotype of isolated erythrocytosis (i.e., without leukocytosis or thrombocytosis), this was not completely reproduced in mice, as the leukocyte counts were increased in mice that received either JAK2-V167F or K539L although the latter cohort did have significantly lower leukocyte levels (Scott et al. 2007). These studies suggest that mutant JAK2 kinases are the fundamental cause of virtually all cases of clinical PV that present with an increased red cell mass.

By contrast, about half of patients with ET or PMF lack JAK2 mutations, and genomic sequencing of the other JAK family kinases or of the Stat5 genes has failed to reveal any mutations in these signaling molecules. Inherited mutations in EpoR and MPL, the receptor for thrombopoietin, are found in patients with familial erythrocytosis and thrombocytosis, respectively (Kralovics et al. 1997), suggesting that these cytokine receptors might also be targets for somatic mutation in the MPDs. Indeed, systematic exonic sequencing of the MPL gene revealed a somatic juxtamembrane mutation (W515K or L) in about 6–8% of patients with JAK2-V617F-negative PMF and a smaller proportion of JAK2-negative ET patients (Pardanani et al. 2006; Pikman et al. 2006). Once again, the mutant MPL receptor transformed cytokine-dependent hematopoietic cells to cytokine independence. In the retroviral BM transduction/transplantation model, MPL W515K/L induced marked megakaryocytic hyperplasia with thrombocytosis and myelofibrosis. The recipients also developed leukocytosis with very high white blood cell counts reminiscent of BCR-ABL-induced CML-like disease but no polycythemia (Pikman et al. 2006). Interestingly, the mice were found to have frequent infarcts in the spleen at necropsy, but

because similar infarcts are observed in mice with BCR–ABL-induced disease (where the platelet count is normal), it is not clear whether this can be attributed to the increased leukocytes or platelets.

## 1.9 Emerging Transgenic Mouse Models of JAK2-V617F-Induced MPD

Although the retroviral models of JAK2-induced MPD have already proven very valuable, transgenic mice offer an alternative that is attractive because of the ease of generation of diseased mice, and the more uniform phenotype of disease within a cohort. As mentioned in Section 1.2, successful generation of transgenic mice expressing JAK2-V617F within the hematopoietic system may require a system to conditionally express the transgene in order to avoid deleterious effects of the dysregulated TK on embryonic development. One method to accomplish this is to use tissue-specific or conditional expression of a transgene expressing Cre recombinase to modify the genomic structure of the TK transgene allowing expression. Most commonly, this is done by inserting a transcriptional “stop” cassette flanked by loxP sites upstream of the TK gene. In a recently published study, the authors took a slightly different approach. They generated *JAK2*-V617F transgenic mice where the sequences encoding the JAK2 kinase domain were in the inverse orientation, flanked by antiparallel loxP sites. These JAK2 transgenic mice were then crossed with a second transgenic strain expressing Cre recombinase under the control of the hematopoiesis-specific *Vav* promoter. In these double transgenic offspring, the level of expression of *JAK2*-V617F was lower than the endogenous wild-type (WT) JAK2, and the mice developed a phenotype resembling ET with strongly elevated platelet counts and moderate neutrophilia (Tiedt et al. 2008). By contrast, when the same JAK2-V617F transgenic mice were crossed to Mx–Cre transgenic mice and Cre expression induced by poly-I : C injection, this resulted in expression of *JAK2*-V617F that was approximately equal to WT JAK2 and resulted in a PV-like phenotype, with increased hemoglobin, thrombocytosis, and neutrophilia (Tiedt et al. 2008). These studies suggest that the ratio of mutant to WT JAK2 influences the MPD phenotype that results *in vivo*.

## 1.10 Summary and Future Directions

Mouse models of the MPDs have already proven their worth by allowing novel insights into the molecular pathogenesis of these diseases, an understanding of disease pathophysiology and identification of new potential targets for therapy. This knowledge would be difficult if not impossible to obtain if one was limited to studying primary human cells or cell lines. Going forward, we should expect to see continued use of these model systems for validation of new molecular

targets and for the preclinical testing of targeted therapeutic agents, particularly combinations of agents. The diversity of inbred mouse strains and the ongoing Mouse Phenome Project at The Jackson Laboratory also offer a unique opportunity to identify genetic modifiers of the MPD phenotype in mice, which may have clinical relevance in human MPD patients.

## References

- Ayton PM, Cleary ML (2003) Transformation of myeloid progenitors by MLL oncproteins is dependent on Hoxa7 and Hoxa9. *Genes Dev* 17:2298–2307
- Barnes DJ, Schultheis B, Adedeji S, Melo JV (2005) Dose-dependent effects of Bcr-Abl in cell line models of different stages of chronic myeloid leukemia. *Oncogene* 24:6432–6440
- Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, Vassiliou GS, Bench AJ, Boyd EM, Curtin N et al. (2005) Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 365:1054–1061
- Bumm TG, Elsea C, Corbin AS, Loriaux M, Sherbenou D, Wood L, Deininger J, Silver RT, Druker BJ, Deininger MW (2006) Characterization of murine JAK2V617F-positive myeloproliferative disease. *Cancer Res* 66:11156–11165
- Cools J, DeAngelo DJ, Gotlib J, Stover EH, Lagare RD, Cottet J, Kutok J, Clark J, Galinsky I, Griffin JD et al. (2003) A tyrosine kinase created by fusion of the PDGFA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N Engl J Med* 348:1201–1214
- Daley GQ, Van Etten RA, Baltimore D (1990) Induction of chronic myelogenous leukemia in mice by the P210<sup>bcr/abl</sup> gene of the Philadelphia chromosome. *Science* 247:824–830
- Daley GQ, Van Etten RA, Baltimore D (1991) Blast crisis in a murine model of chronic myelogenous leukemia. *Proc Natl Acad Sci USA* 88:11335–11338
- Dameshek W (1951) Some speculations on the myeloproliferative disorders. *Blood* 6:372–375
- Dash AB, Williams IR, Kutok JL, Tomasson MH, Anastasiadou E, Lindahl K, Li S, Van Etten RA, Borrow J, Housman D et al. (2002) A murine model of CML blast crisis induced by cooperation between *BCR/ABL* and *NUP98/HOX49*. *Proc Natl Acad Sci USA* 99:7622–7627
- Deininger MW, Goldman JM, Lydon N, Melo JV (1997) The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells. *Blood* 90:3691–3698
- Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 2:561–566
- Furitsu T, Tsujimura T, Tono T, Ikeda H, Kitayama H, Koshimizu U, Sugahara H, Butterfield JH, Ashman LK, Kanayama Y (1993) Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. *J Clin Invest* 92:1736–1744
- Gavrilescu LC, Van Etten RA (2008). Applications of murine retroviral bone marrow transplantation models for the study of human myeloproliferative disorders. In: Enna SJ, Williams M, Ferkany JW, Kenakin T, Moser P, Ruggeri B (eds) *Current protocols in pharmacology*, John Wiley & Sons, Inc., Pagosa Springs, CO
- Gishizky MI, Johnson-White J, Witte ON (1993) Efficient transplantation of *BCR-ABL*-induced chronic myelogenous leukemia-like syndrome in mice. *Proc Natl Acad Sci USA* 90:3755–3759
- Golub TR, Barker GF, Lovett M, Gilliland DG (1994) Fusion of the PDGF receptor  $\beta$  to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell* 77:307–316

- Griffin JH, Leung J, Bruner RJ, Caligiuri MA, Briesewitz R (2003) Discovery of a fusion kinase in EOL-1 cells and idiopathic hypereosinophilic syndrome. *Proc Natl Acad Sci USA* 100:7830–7835
- Hao SX, Ren R (2000) Expression of interferon consensus sequence binding protein (ICSBP) is downregulated in Bcr-Abl-induced murine chronic myelogenous leukemia-like disease, and enforced coexpression of ICSBP inhibits Bcr-Abl induced myeloproliferative disorder. *Mol Cell Biol* 20:1149–1161
- Heisterkamp N, Jenster G, Kioussis D, Pattengale PK, Groffen J (1991) Human *bcr-abl* gene has a lethal effect on embryogenesis. *Transgenic Res* 1:45–53
- Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, Hallek M, Van Etten RA, Li S (2004) Requirement of Src kinases Lyn, Hck and Fgr for *BCR-ABL1*-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. *Nat Genet* 36:453–461
- Huettnner CS, Zhang P, Van Etten RA, Tenen DG (2000) Reversibility of acute B-cell leukaemia induced by *BCR-ABL1*. *Nat Genet* 24:57–60
- Ingle E, McCarthy DJ, Pore JR, Sarna MK, Adenan AS, Wright MJ, Erber W, Tilbrook PA, Klinken SP (2005) Lyn deficiency reduces GATA-1, EKLF and STAT5, and induces extramedullary stress erythropoiesis. *Oncogene* 24:336–343
- Jaiswal S, Traver D, Miyamoto T, Akashi K, Lagasse E, Weissman IL (2003) Expression of BCR/ABL and BCL2 in myeloid progenitors leads to myeloid leukemias. *Proc Natl Acad Sci USA* 100:10002–10007
- James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, Garcon L, Raslova H, Berger R, Bennaceur-Griscelli A et al. (2005) A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 434:1144–1148
- Jiang X, Stuible M, Chalandon Y, Li A, Chan WY, Eisterer W, Krystal G, Eaves A, Eaves C (2003) Evidence for a positive role of SHIP in BCR-ABL-mediated transformation of primitive murine hematopoietic cells and in human chronic myeloid leukemia. *Blood* 102:2976–2984
- Jones AV, Cross NC (2004) Oncogenic derivatives of platelet-derived growth factor receptors. *Cell Mol Life Sci* 61:2912–2923
- Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, Score J, Seear R, Chase AJ, Grand FH et al. (2005a) Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood* 106:2162–2168
- Jones AV, Silver RT, Waghorn K, Curtis C, Kreil S, Zoi K, Hochhaus A, Oscier D, Metzgeroth G, Lengfelder E et al. (2005b) Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. *Blood* 107:3339–3341
- Kelliher MA, McLaughlin J, Witte ON, Rosenberg N (1990) Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and bcrabl. *Proc Natl Acad Sci USA* 87:6649–6653
- Kralovics R, Indrak K, Stopka T, Berman BW, Prchal JF, Prchal JT (1997) Two new EPO receptor mutations: truncated EPO receptors are most frequently associated with primary familial and congenital polycythemias. *Blood* 90:2057–2061
- Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, Skoda RC (2005) A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 352:1779–1790
- Kralovics R, Teo SS, Li S, Theocharides A, Buser AS, Tichelli A, Skoda RC (2006) Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. *Blood* 108:1377–1380
- Krause DS, Van Etten RA (2004) Adoptive immunotherapy of *BCR-ABL*-induced chronic myeloid leukemia-like myeloproliferative disease in a murine model. *Blood* 104:4236–4244
- Krause DS, Van Etten RA (2005) Tyrosine kinases as targets for cancer therapy. *N Engl J Med* 353:172–187
- Lacout C, Pisani DF, Tulliez M, Gachelin FM, Vainchenker W, Villevie JL (2006) JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. *Blood* 108:1652–1660

- Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, Boggon TJ, Wlodarska I, Clark JJ, Moore S et al. (2005) Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 7:387–397
- Li S, Ilaria RL, Million RP, Daley GQ, Van Etten RA (1999) The P190, P210, and P230 forms of the *BCR/ABL* oncogene induce a similar chronic myeloid leukemia-like syndrome in mice but have different lymphoid leukemogenic activity. *J Exp Med* 189:1399–1412
- Macdonald D, Reiter A, Cross NCP (2002) The 8p11 myeloproliferative syndrome: a distinct clinical entity caused by constitutive activation of FGFR1. *Acta Haematol* 107:101–107
- Meng F, Lowell CA (1997) Lipopolysaccharide (LPS)-induced macrophage activation and signal transduction in the absence of Src-family kinases Hck, Fgr, and Lyn. *J Exp Med* 185:1661–1670
- Meydan N, Grunberger T, Dadi H, Shahar M, Arpaia E, Lapidot Z, Leeder JS, Freedman M, Cohen A, Gazit A et al. (1996) Inhibition of acute lymphoblastic leukaemia by a Jak-2 inhibitor. *Nature* 379:645–648
- Million RP, Aster J, Gilliland DG, Van Etten RA (2002) The Tel-Abl (ETV6-Abl) tyrosine kinase, product of complex (9;12) translocations in human leukemia, induces distinct myeloproliferative disease in mice. *Blood* 99:4568–4577
- Moliterno AR, Hanks WD, Spivak JL (1998) Impaired expression of the thrombopoietin receptor by platelets from patients with polycythemia vera. *N Engl J Med* 338:572–580
- Pardanani A (2008) JAK2 inhibitor therapy in myeloproliferative disorders: rationale, pre-clinical studies and ongoing clinical trials. *Leukemia* 22:23–30
- Pardanani A, Hood J, Lasho T, Levine RL, Martin MB, Noronha G, Finke C, Mak CC, Mesa R, Zhu H et al. (2007) TG101209, a small molecule JAK2-selective kinase inhibitor potently inhibits myeloproliferative disorder-associated JAK2V617F and MPLW515L/K mutations. *Leukemia* 21:1658–1668
- Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, Steensma DP, Elliott MA, Wolanskyj AP, Hogan WJ et al. (2006) MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood* 108:3472–3476
- Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, Vanin EF, Bodner S, Colamonti OR, van Deursen JM et al. (1998) Jak2 is essential for signaling through a variety of cytokine receptors. *Cell* 93:385–395
- Pear WS, Miller JP, Xu L, Pui JC, Soffer B, Quackenbush RC, Pendergast AM, Bronson R, Aster JC, Scott ML, Baltimore D (1998) Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. *Blood* 92:3780–3792
- Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, Cuker A, Wernig G, Moore S, Galinsky I et al. (2006) MPLW515L Is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med* 3:e270
- Prchal JF, Axelrad AA (1974) Letter: bone-marrow responses in polycythemia vera. *N Engl J Med* 290:1382
- Richmond TD, Chohan M, Barber DL (2005) Turning cells red: signal transduction mediated by erythropoietin. *Trends Biochem Sci* 15:146–155
- Roumiantsev S, de Aos I, Varticovski L, Ilaria RL, Van Etten RA (2001) The Src homology 2 domain of Bcr/Abl is required for efficient induction of chronic myeloid leukemia-like disease in mice but not for lymphoid leukemogenesis or activation of phosphatidylinositol 3-kinase. *Blood* 97:4–13
- Roumiantsev S, Krause DS, Neumann CA, Dimitri CA, Asiedu F, Cross NC, Van Etten RA (2004) Distinct stem cell myeloproliferative/T lymphoma syndromes induced by ZNF198-FGFR1 and BCR-FGFR1 fusion genes from 8p11 translocations. *Cancer Cell* 5:287–298
- Scott LM, Campbell PJ, Baxter EJ, Todd T, Stephens P, Edkins S, Wooster R, Stratton MR, Futreal PA, Green AR (2005) The V617F JAK2 mutation is uncommon in cancers and in

- myeloid malignancies other than the classic myeloproliferative disorders. *Blood* 106:2920–2921
- Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, Futreal PA, Erber WN, McMullin MF, Harrison CN et al. (2007) JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med* 356:459–468
- Spivak JL (2002) Polycythemia vera: myths, mechanisms, and management. *Blood* 100:4272–4290
- Steensma DP, Dewald GW, Lasho TL, Powell HL, McClure RF, Levine RL, Gilliland DG, Tefferi A (2005) The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both “atypical” myeloproliferative disorders and myelodysplastic syndromes. *Blood* 106:1207–1209
- Tefferi A, Vardiman JW (2008) Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia* 22:14–22
- Temerina S, Klippel S, Strunck E, Roder S, Lubbert M, Lange W, Azemar M, Meinhardt G, Schaefer HE, Pahl HL (2000) Cloning of PRV-1, a novel member of the uPAR receptor superfamily, which is overexpressed in polycythemia rubra vera. *Blood* 95:2569–2576
- Tiedt R, Hao-Shen H, Looser R, Dirnhofer S, Schwaller J, Skoda RC (2008) Ratio of mutant JAK2-V617F to wild type Jak2 determines the MPD phenotypes in transgenic mice. *Blood* 111:3931–3940
- Ugo V, Marzac C, Teyssandier I, Larbret F, Lecluse Y, Debili N, Vainchenker W, Casadevall N (2004) Multiple signaling pathways are involved in erythropoietin-independent differentiation of erythroid progenitors in polycythemia vera. *Exp Hematol* 32:179–187
- Van Etten RA (2001) Models of chronic myeloid leukemia. *Curr Oncol Rep* 3:228–237
- Van Etten RA (2002) Studying the pathogenesis of *BCR-ABL*<sup>+</sup> leukemia in mice. *Oncogene* 21:8643–8651
- Van Etten RA, Shannon KM (2004) Focus on myeloproliferative diseases and myelodysplastic syndromes. *Cancer Cell* 6:547–552
- Vardiman JW, Harris NL, Brunning RD (2002) The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood* 100:2292–2302
- Weinstein IB (2002) Addiction to oncogenes—the Achilles heel of cancer. *Science* 297:63–64
- Wernig G, Mercher T, Okabe R, Levine RL, Lee BH, Gilliland DG (2006) Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. *Blood* 107:4274–4281
- Wolff NC, Ilaria RL Jr (2001) Establishment of a murine model for therapy-treated chronic myelogenous leukemia using the tyrosine kinase inhibitor ST1571. *Blood* 98:2808–2816
- Xiao S, Nalabolu SR, Aster JC, Ma J, Abruzzo L, Jaffe ES, Stone R, Weissman SM, Hudson TJ, Fletcher JA (1998) *FGFR1* is fused with a novel zinc-finger gene, *ZNF198*, in the t(8;13) leukaemia/lymphoma syndrome. *Nature Genet* 18:84–87
- Zaleskas VM, Krause DS, Lazarides K, Patel N, Hu Y, Li S, Van Etten RA (2006) Molecular Pathogenesis and Therapy of Polycythemia Induced in Mice by JAK2 V617F. *PLoS ONE* 1:e18
- Zhang X, Ren R (1998) Bcr-Abl efficiently induces a myeloproliferative disease and production of excess interleukin-3 and granulocyte-macrophage colony-stimulating factor in mice: a novel model for chronic myelogenous leukemia. *Blood* 92:3829–3840

# **Chapter 2**

## **Genetic Modeling of Human Blood Cancers in Mice**

**Yiguo Hu and Shaoguang Li**

### **Contents**

2.1	Introduction . . . . .	21
2.2	Mouse Leukemia Models . . . . .	22
2.2.1	Modeling Acute Myeloid Leukemia . . . . .	22
2.2.2	Modeling Chronic Myeloid Leukemia-Like Diseases . . . . .	27
2.2.3	Modeling Acute Lymphoblastic Leukemia . . . . .	28
2.2.4	Modeling Chronic Lymphocytic Leukemia . . . . .	29
2.3	Conclusion . . . . .	34

### **2.1 Introduction**

Leukemia is a broad term describing a spectrum of diseases involving white blood cells and is divided into four categories: acute or chronic myelogenous and acute or chronic lymphocytic leukemia (CLL). Acute leukemia is characterized by the rapid proliferation of immature blood cells that cannot carry out their normal functions. Acute leukemia generally occurs in children and young adults and needs immediate treatment because of the rapid progression and accumulation of the malignant cells in the body. Chronic leukemia is distinguished by the excessive and slow build-up of relatively mature white blood cells, which can still carry out some of their normal functions. Chronic leukemia mostly occurs in older people but can theoretically occur in any age group. Whereas acute leukemia must be treated immediately, chronic forms are sometimes monitored for some time before treatment to ensure maximum effectiveness of therapy. Classification of leukemia into myeloid or lymphoid form is based on the type of abnormal white blood cells found most in the blood or bone marrow. Acute lymphocytic leukemia (also known as acute lymphoblastic leukemia, or ALL) is the most common type of leukemia in young children and

---

S. Li

Division of Hematology/Oncology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA 010605, USA  
Shaoguang.Li@umassmed.edu

also affects adults, especially those age 65 and older. CLL most often affects adults over the age of 55. CLL sometimes occurs in younger adults, but it almost never affects children.

There is no single known cause for all different types of leukemia. Four possible causes are (1) natural or artificial ionizing radiation, (2) certain kinds of chemicals, (3) some viruses, and (4) genetic predispositions. Leukemia, like other cancers, can result from somatic mutations in the DNA, which leads to disruption of the regulation of cell death, proliferation, and differentiation. These mutations may occur spontaneously or as a result of exposure to radiation or carcinogenic substances (such as benzene, hair dyes, etc.), and sensitivity of humans to these cancer-causing agents are likely to be influenced by genetic factors. Viruses have also been linked to some forms of leukemia. For example, certain cases of ALL are associated with viral infections by either the human immunodeficiency virus (HIV, responsible for AIDS) (Murray et al., 1999) or human T-lymphotropic virus [HTLV-1 and HTLV-2, causing adult T-cell leukemia/lymphoma (TCL)] (Poiesz et al., 2001). Fanconi anemia is also a risk factor for developing acute myelogenous leukemia (Bhatia et al., 2007). All these risk factors end up causing aberrant activation or inactivation of cellular genes that control normal cell proliferation and differentiation. In human blood cancers, formation of a fusion gene from two normal cellular genes, which is caused by chromosomal translocation, is a frequent way to abnormally activate a cellular gene that becomes oncogenic after forming a chimeric gene with another cellular gene. Large numbers of mouse models of human blood cancers are generated by expressing these chimeric or active oncogenes in mice. Mouse leukemia models provide powerful tools to investigate the disease mechanisms and help to develop new therapies.

## 2.2 Mouse Leukemia Models

In principle, leukemia mouse models are generated based on three major mechanisms: (1) expressing human oncogene(s) in hematopoietic progenitor cells, (2) inactivating tumor suppressor gene(s) (including DNA repair genes) in hematopoietic cells, and (3) combining these two methods. Described below are examples of established mouse models for different forms of human blood cancers.

### 2.2.1 Modeling Acute Myeloid Leukemia

*Expression of a human acute myeloid leukemia-inducing gene in mouse bone marrow cells using retrovirus.* Fusion genes involving transcriptional coactivators and generated through chromosomal translocations are frequently found in human acute myeloid leukemia (AML). Examples of these fusion genes are MLL/CBP (Satake et al., 1997; Sobulo et al., 1997; Taki et al., 1997), MLL/

p300 (Ida et al., 1997), MOZ (monocytic leukemia zinc finger)/CBP (Borrow et al., 1996), MOZ/p300 (Chaffanet et al., 2000; Kitabayashi et al., 2001b), MORF/CBP (Panagopoulos et al., 2001), and MOZ-TIF2 (Carapeti et al., 1998; Liang et al., 1998). Each of these fusion proteins contains one or more histone acetyltransferase (HAT) domain(s) that function to modify chromatin by acetylation of the N-terminal histone tail. Because MOZ-TIF2 is a common and well-understood fusion oncogene causing human AML, here we use this fusion gene as an example to describe the retroviral bone marrow transduction/transplantation mouse model of AML induced by MOZ-TIF2.

MOZ belongs to the MYST family of HATs and was first cloned as a fusion partner of CBP as a consequence of t(8;16)(p11;p13) chromosomal translocation associated with the French-American-British M4/M5 subtype of AML (Borrow et al., 1996). MOZ regulates transcriptional activation mediated by the hematopoietic transcription factor, Runx1 (AML1) (Kitabayashi et al., 2001a), and a related osteogenic transcriptional factor, Runx2 (Kitabayashi et al., 2001a). TIF2 belongs to p160 nuclear receptor transcriptional coactivator family (NRCoAs) (Glass et al., 1997; Horwitz et al., 1996), which includes SRC-1, TIF2/GRIP1, and ACTR/RAC3/pCIP/AIB-1. p160 family coactivators have a conserved N-terminal bHLH-PAS domain, a centrally located receptor interaction domain (RID), and a C-terminal transcriptional activation domain (AD). The RID contains three conserved motifs, LXXLL (where L is leucine and X is any amino acid), that are required to mediate interactions between coactivators and liganded nuclear receptors (Ding et al., 1998; Heery et al., 1997; Torchia et al., 1997). TIF2 can directly interact with CBP via its three conserved LXXLL motifs (Demarest et al., 2002; Torchia et al., 1997). P160 family members interact with nuclear receptors and enhance transcriptional activation by the receptor via histone acetylation/methylation (Leo and Chen, 2000).

In the MOZ-TIF2 fusion protein, MOZ retains the C4HC3-type PHD zinc finger domain and the HAT (MYST) domain and TIF2 retains the CBP interaction domain (CID) and CBP-independent activation domain (called AD2) of TIF2. MOZ-TIF2 lacks the C-terminus of MOZ and the PAS-bHLH DNA-binding/protein heterodimerization domain, and nuclear RID of TIF2 (Deguchi et al., 2003). To assess the transforming properties of MOZ-TIF2 *in vivo*, the *MOZ-TIF2* gene was cloned into the MSCV retroviral vector (see Fig. 1.2 in Chapter 1 for the viral vector structure); mouse bone marrow cells transduced with the *MOZ-TIF2* containing retrovirus were transplanted into irradiated syngeneic mice (Deguchi et al., 2003). Recipients receiving bone marrow transduced with either the *MOZ-TIF2(I)* or the *MOZ-TIF2(II)* variant fusion genes developed fatal hematopoietic malignant disease, with high white blood cell (WBC) counts and splenomegaly. In addition, the mice demonstrated the presence of peripheral blood and bone marrow blasts and extensive tissue infiltration of organs including the liver, spleen, and lungs by leukemic blasts (Deguchi et al., 2003). This study provides sufficient evidence

showing that MOZ–TIF2, which is associated with human AML, induces similar disease in mice.

*Transgenic AML mouse model.* A good example of transgenic AML model is to express the *CBFβ–SMMHC* gene in mice. *CBFβ–SMMHC* resulted from the inversion of chromosome 16 inv(16)(p13.1;1q22), which breaks and joins the *CBFβ* gene with the myosin gene *MYH11* (Liu et al., 1993, 1996) and causes about 12% of human AML. To avoid embryonic lethality caused by expression of the *CBFβ–SMMHC* gene<sup>6–10</sup>, a conditional *CBFβ–SMMHC* knock-in mouse was generated to analyze the preleukemic effects of *CBFβ–SMMHC* in hematopoiesis and AML development in adult mice. The *CBFβ–SMMHC* gene caused appearance of abnormal progenitor cells that are leukemic precursors. Mice expressing *CBFβ–SMMHC* developed AML with a median latency of approximately 5 months. Interestingly, the number of *CBFβ–SMMHC*-expressing hematopoietic stem cells (HSCs) was maintained at a normal level, but their ability to differentiate into multiple lineages of blood cells was severely impaired. This AML model is key for the study of early target genes in progenitor cells and provides an *in vivo* validation system for studying cooperative oncogenes and for testing candidate drugs for improved treatment of AML.

*Collaborative induction of AML with multiple oncogenes.* It is generally believed that multiple genetic alterations are required for the initiation and progression of malignant diseases. There are many examples that show the failure of a single AML-inducing oncogene to efficiently induce AML, as evident by no induction of leukemia or induction of leukemia with low penetrance and long latency. Additional genetic events (secondary “hits”) are needed to promote the pathogenesis of leukemia. In this case, coexpression of more than one oncogene in the same hematopoietic progenitor cells helps to successfully induce human AML in mice.

The PML–RAR $\alpha$  fusion oncogene is found in acute promyelocytic leukemia (APL). APL comprises about 5–10% of cases of AML, and approximately 90% of APL patients are associated with a balanced t(15;17)(q22;q21) reciprocal chromosomal translocation. This translocation results in the fusion of the *PML* gene on chromosome 15 to the retinoic acid receptor alpha (*RAR $\alpha$* ) gene on chromosome 17, forming two new oncogenes, PML–RAR $\alpha$  and RAR $\alpha$ –PML. The *RAR $\alpha$*  gene encodes a hormone-inducible nuclear receptor that has been shown to be involved in myeloid development (Collins et al., 1990; Dawson et al., 1994; Onodera et al., 1995; Tsai and Collins, 1993). Both PML–RAR $\alpha$  and RAR $\alpha$ –PML play roles in APL pathogenesis.

To induce APL in mice, a transgene containing a human PML–RAR $\alpha$  cDNA under the control of sequence that regulates the promyelocyte-specific expression of the human CG gene allows expression of PML–RAR $\alpha$  in the early myeloid cells of the transgenic mice (Grisolano et al., 1997). At the early stage, these transgenic mice were found to have altered myeloid development with an expansion of myeloid cells in their bone marrows and spleens. After a long latent period, approximately 30% of the transgenic mice developed

leukemia, with massive splenomegaly, high percentage of immature myeloid cells in peripheral blood and bone marrow of the mice (Grisolano et al., 1997). In addition, approximately 40% of human APL patients are found to contain an activating mutation in the *FLT3* gene, containing internal tandem duplication (ITD) in the juxtamembrane domain. ITDs in *FLT3* (*FLT3*-ITD) are found in 27% of all AML cases (Stirewalt et al., 2001; Yamamoto et al., 2001; Yokota et al., 1997) and 37% of APL patients (Kottaridis et al., 2001). *FLT3*-ITDs induce a myeloproliferative disease in a murine bone marrow transplantation model but are insufficient to induce AML (Kelly et al., 2002b). This low frequency and long latency of APL pathogenesis induced by PML-RAR $\alpha$  or *FLT3*-ITDs can be overcome by coexpression of both genes in the same animal. In this model, bone marrow cells derived from hCG-PML-RAR $\alpha$  transgenic mice (Grisolano et al., 1997) were transduced with the *FLT3*-ITD retrovirus, followed by transplantation of transduced cells into lethally irradiated syngeneic recipient mice. These recipients developed APL-like disease with complete penetrance and a short latency. The pathogenesis of this disease resembles the APL-like disease that occurs with a long latency in the PML/RAR $\alpha$  transgenic mice, suggesting that activating mutations in *FLT3*-ITD services as the additional mutations in APL progression in the hCG-PML-RAR $\alpha$  transgenic mice.

Another example is the Ras oncogene. Ras mutations are commonly found in AML. N-ras and K-ras mutations are found in 4 (Callens et al., 2005) and 10% of APL patients (Bowen et al., 2005), respectively. Overexpressing oncogenic K-ras under the control of its endogenous promoter in the mouse hematopoietic system, K-ras induces a myeloproliferative disease, but it is not sufficient to induce AML (Braun et al., 2004; Chan et al., 2004). To test whether K-ras serves as a cooperative secondary genetic event in induction of AML, LSL-K-ras G12D mice (Jackson et al., 2001), in which K-ras expression is controlled by the conditional knock-in Lox-stop-Lox, were crossed with cathepsin G-PML-RAR $\alpha$  mice (Grisolano et al., 1997) to generate LSL-K-ras G12D $^{+/-}$ /cathepsin G-PML-RAR $\alpha^{+/-}$  mice (KP mice). Subsequently, these mice were crossed with Mx-1-Cre mice (Kuhn et al., 1995) to generate triple-transgenic LSL-K-ras G12D $^{+/-}$ /cathepsin G-PML-RAR $\alpha^{+/-}$ /Mx-1-Cre $^{+/-}$  mice (KPM mice). K-ras expression was induced by deletion of the Lox-stop-Lox with Cre, whose expression was induced with polyinosinic–polycytidylc acid (pI–pC) (Chan et al., 2006). Mice expressing oncogenic K-ras and PML-RAR $\alpha$  developed an APL-like disease with a high penetrance and short latency compared to cathepsin G-PML-RAR $\alpha$  transgene mice (Chan et al., 2006).

*Acceleration of AML development with a chemical mutagen.* As pointed out above, some oncogenes are, by themselves, insufficient to transform cells and induce leukemia. However, genetic modifications or changes of the model-making procedures, or the oncogene itself, or even mouse background would dramatically increase the penetrance of leukemogenesis. Mouse model of *AML1*–ETO-induced AML is such an example.

*AML1-ETO* (also known as RUNX1-ETO) is a fusion gene resulted from translocation between chromosomes 8 and 21. The translocation is highly associated with human AML and is present in up to 40% of leukemias of the French-American-British M2 subtype (Hess and Hug, 2004). *AML1* is a key regulator of normal blood formation and is frequently altered in leukemias. However, it has been difficult to clarify the role of *AML1-ETO* in leukemogenesis, because *AML1-ETO* alone is not sufficient to cause AML, and *AML1-ETO* transgene causes embryonic lethality (Okuda et al., 1998, 2000). To bypass the embryonic lethality caused by *AML1-ETO*, conditional and inducible transgenic models, and bone marrow transplantation system were used to express *AML1-ETO* in mice; all these strategies were unable to reliably induce AML even after 24 months (de Guzman et al., 2002; Fenske et al., 2004; Higuchi et al., 2002; Rhoades et al., 2000), suggesting that induction of AML by *AML1-ETO* requires additional genetic events. However, when stem cells were transduced with *AML1-ETO* and transplanted into lethally irradiated recipient animals, the stem cell compartment expanded dramatically (de Guzman et al., 2002). Similarly, direct targeting of *AML1-ETO* expression to stem cells by using the SCA-1 promoter enhanced myeloid progenitor expansion (Fenske et al., 2004). These results imply that retroviral insertion sites or large numbers of leukemia-initiating progenitors provide the additional "hits" for *AML1-ETO*-induced leukemia. To assess the ability of *AML1-ETO* to induce leukemia in the context of cooperating mutations, animals expressing *AML1-ETO* were mutagenized with the alkylating agent *N*-ethyl-*N*-nitrosourea (ENU). In two independent systems, mutagenized *AML1-ETO*-expressing mice developed myeloid leukemia or granulocytic sarcoma at frequencies greater than ENU-treated wild-type animals (Higuchi et al., 2002; Yuan et al., 2001). These results confirm that *AML1-ETO* predisposes a myeloid precursor population to cellular transformation (Hess et al., 2004).

The *AML1-ETO* mouse model provides an excellent assay system to investigate *AML1-ETO* downstream signaling pathways. *AML1-ETO* was found to suppress cell proliferation by inhibiting its targeting genes, including cyclin D3 and CDK4 (Bernardin-Fried et al., 2004; Burel et al., 2001; Lou et al., 2000), and impair cell cycle in the transition of G1 to S phase (Burel et al., 2001). In addition, an *AML1-ETO* truncated protein (loss of C-terminal Nervy homology regions 3 and 4 domain), which binds the corepressor complexes associated with N-CoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptor) (Lutterbach et al., 1998), can induce high penetrance of leukemia with a short disease latency (mean survival of 20 weeks) in the retroviral transduction/transplantation model (Yan et al., 2004). In this study, the results also showed that expression of cyclin A and D3 was increased in truncated *AML1-ETO*-transformed cells compared with full-length *AML1-ETO*-transformed cells. Taken together, these studies demonstrate that *AML1-ETO* alone is not sufficient to cause leukemia, and additional genetic changes that cooperate with *AML1-ETO* are required for the

development of AML. Obviously, AML1–ETO mouse models of AML will be helpful in study of genetic pathways involved in AML development.

*Deletion of a tumor suppressor gene causes AML.* Tumor suppressor genes play critical roles in regulating biological properties of cells, including cell cycle control, apoptosis, proliferation and differentiation, detecting and repairing DNA damage, and protein ubiquitination and degradation (Sherr, 2004). Deletions of tumor suppressor genes are associated with many types of tumors, and examples of tumor suppressor genes are *P53*, *RB*, *INK4a*, *ARF*, *APC*, *PTCH*, *SAMAD4/DPC4*, *PTEN*, *TSC1/2*, *NF1*, *WT1*, *MSH2*, *MLH1*, *ATM*, *NBS1*, *CHK2*, *BRCA1/2*, *FA*, and *VHL* (Sherr, 2004). The best AML model established by the deletion of a tumor suppressor gene is the removal of the *PTEN* gene in mice.

The *PTEN* gene was initially identified based on the observation that a loss of heterozygosity (LOH) at 10q23 was frequently detected in a variety of human tumors, and *PTEN* was later identified as the corresponding gene (Li et al., 1997). Further studies indicate that *PTEN* suppresses tumor cell growth by modulating G1 cell cycle progression through negatively regulating the PI3 K/Akt signaling pathway, and a critical target gene of this pathway is the cyclin-dependent kinase inhibitor p27 (KIP1) (Li and Sun, 1998). *PTEN* has been found to be associated with a series of primary acute leukemias and non-Hodgkin lymphomas (NHLs) as well as many tumor cell lines, and 40% of these cell lines carried *PTEN* mutations or hemizygous *PTEN* deletions. On the other hand, one-third of these cell lines had low *PTEN* transcript levels, and 60% of them had low or absent *PTEN* protein. Furthermore, a smaller number of primary hematologic malignancies, in particular NHLs, carried *PTEN* mutations (Dahia et al., 1999). To model AML induced by the deletion of the *PTEN* gene,

*Ptenfl/fl* mice (Lessard and Sauvageau, 2003) were crossed with *Ptenfl/+* mice carrying an *Mx-1-Cre* (Park et al., 2003) transgene to generate litters containing *Mx-1-Cre<sup>+</sup>* and *Ptenfl/fl*. *PTEN* deletion was induced by injection of pI-pC to mice at weaning. After the induction of *PTEN* deletion, mice had an increased representation of myeloid and T-lymphoid lineages in bone marrow and developed myeloproliferative disorder. Notably, the cell populations that expanded in *PTEN*-deficient mice matched those that became dominant in the acute myeloid/lymphoid leukemia that developed in later stages of myeloproliferative disorder. This study demonstrates that *PTEN* has essential roles in restricting the activation of HSCs, in lineage fate determination, and in the prevention of leukemogenesis (Zhang et al., 2006).

### **2.2.2 Modeling Chronic Myeloid Leukemia-Like Diseases**

Chronic myeloid leukemia (CML) is represented by myeloproliferative disease induced by the BCR–ABL oncogene that results from the t(9;22)(q34;q22)

chromosomal translocation. Other CML-like diseases are induced by the fusion genes *TEL/PDGF $\beta$ R* (Golub et al., 1994), *TEL/ABL* (Golub et al., 1995), *TEL/JAK2* (Lacronique et al., 1997), and *H4/PDGF $\beta$ R* (Kulkarni et al., 2000; Schwaller et al., 2001), which are associated with t(5;12)(q33;p13), t(9;12)(q34;p13), t(9;12)(p24;p13), and t(5;10)(q33;q11.2) translocations, respectively. These fusion genes encode constitutively activated tyrosine kinases and are sufficient to induce myeloproliferative diseases in mice (Daley et al., 1990; Schwaller et al., 1998; Tomasson et al., 2000). Because BCR–ABL oncogene is associated with over 95% of human CML, we describe BCR–ABL-induced mouse CML models in detail in Chapter 7.

### 2.2.3 Modeling Acute Lymphoblastic Leukemia

ETV6/RUNX1 (TEL/AML1) results from a t(12;21)(p13;q22) chromosomal translocation and is the most common known gene rearrangement in childhood cancer. Twenty-seven percent of childhood ALL samples contain an ETV6/RUNX1 fusion transcript detected by the PCR screening. RUNX1 is a member of the heterodimeric core-binding factor (CBF) family of transcription factors and has been shown to regulate a number of genes relevant to myeloid and lymphoid development (Tenen et al., 1997). RUNX1 contains conserved Runt homology domain (RHD) in the N-terminal half, which can bind to DNA, and this DNA-binding activity is enhanced by interaction with the C-terminal portion of the CBF beta subunit (Fenrick et al., 1999; Kitabayashi et al., 1998; Levanon et al., 1998; Meyers et al., 1993). Recruitment of the AML1 complex to the enhancers of its target genes can be direct or cooperatively with other proteins (Pabst et al., 2001). ETV6 protein contains a helix-loop-helix (HLH) motif and an ETS DNA-binding domain. 12p13 translocations and deletions are highly associated with childhood ALL, suggesting that there is a tumor suppressor gene that is disturbed by these chromosomal changes. Detailed examination shows that the critically deleted region includes two candidate suppressor genes: *ETV6* and *KIP* (Stegmaier et al., 1995). ETV6/RUNX1 forms homodimers and forms heterodimers with the normal ETV6 protein when the two proteins were expressed together (Hess and Hug, 2004). Besides ETV6/RUNX1, ETV6 variably forms fusion genes with other genes, including *ETV6/MN1* (Raynaud et al., 1996), *ETV6/AML1* (Ford et al., 1998), *ETV6/JAK2* (Schwaller et al., 2000), *ETV6/ARNT* (Salomon-Nguyen et al., 2000b), *ETV6/MDS2* (Odero et al., 2002), *ETV6/PER1*, and *ETV6/ABL* (Papadopoulos et al., 1995).

To elucidate the mechanism of lymphoid transformation by ETV6/RUNX1, the ETV6/AML1 coding region was inserted into retroviral vector to allow expression of ETV6/AML1 in lineage-negative donor bone marrow cells in mice (Fischer et al., 2005). Although mice receiving ETV6/RUNX1-transduced bone marrow cells did not develop B cell ALL, ETV6/RUNX1 perturbed B-cell

differentiation by increasing the proportion of pro-B cells with low level of mature lymphoid cells in the blood and spleen, which is consistent with human precursor B cell ALL at an early stage. This mouse ALL model can be used for studying the mechanism of early stage of ETV6/RUNX1-induced ALL. Apparently, better disease models need to be developed with *ETV6/RUNX1* or other *ETV6*-related fusion genes to study the molecular basis of ALL.

#### 2.2.4 Modeling Chronic Lymphocytic Leukemia

CLL is a common type of leukemia. There are about 10,000 new CLL cases in United States every year (Bichi et al., 2002; Landis et al., 1998), and CLL accounts for almost 30% of all adult leukemia cases. Most cases of CLL are of B-cell origin, and a few are of T-cell origin. B-CLL is believed to be derived from CD5<sup>+</sup> B lymphocyte through clonal expansion. Several common genomic abnormalities in CLL have been identified, and *TCL1* is involved in the pathogenesis of CLL. The *TCL1* gene locates at chromosome 14q32.1 (Virgilio et al., 1994) and is commonly activated by inversions or translocations that juxtapose it to a T-cell receptor locus at 14q11 or 7q35. *TCL1* has been found to be overexpressed in sporadic and ataxia telangiectasia-associated T-prolymphocytic leukemia (T-PLL) (Narducci et al., 1997; Thick et al., 1996). *TCL1* is also highly expressed in a broad variety of human tumor-derived B-cell lines and in many cases of B-cell neoplasias (Narducci et al., 2000; Takizawa et al., 1998). To elucidate the role of *TCL1* in B-cell development and in B-cell leukemia pathogenesis, *TCL1* transgenic mouse has been generated by cloning human *TCL1* coding region into the pBSVE6BK (pEμ) plasmid containing a mouse VH promoter (V186.2) and the IgH-μ enhancer along with the 3'-untranslated region and the poly(A) site of the human beta-globin gene, followed by injecting the *TCL1*-containing construct free from vector sequences into fertilized oocytes from B6C3 mice. In this model, *TCL1* was under the control of a promoter and enhancer whose activity specifically targets expression of the *TCL1* transgene to the B-cell compartment. Eμ-*TCL1* transgenic mice developed a disease similar to human CLL. The mice first developed a preleukemic phenotype and later developed a frank leukemia with all characteristics of CLL (Bichi et al., 2002).

TNF receptor-associated factors (TRAFs) are a family of adapter proteins that link TNF-family receptors (TNFRs) to intracellular signaling pathways. It has been demonstrated that TRAF-family members participate in signaling cascades involved in gene expression, cell proliferation, and control of apoptosis. Elevated expression of some TRAF-family proteins, in particular TRAF1, is found in hematopoietic malignancies such as CLL and NHL (Munzert et al., 2002; Zapata et al., 2000). A study shows that TRAF1 and TRAF2 mediated apoptosis protection (Arron et al., 2002; Lin et al., 2003; Wang et al., 1998), suggesting that these TRAF family members could participate in the

**Table 2.1** Examples of mouse models of human blood cancers

Chromosomal translocation	Fusion gene	Leukemia type	Disease frequency	Mouse models (approaches)	Citations
t(15;17)(q22;q11.2-12)	<i>PML-RAR<math>\alpha</math></i>	AML	10% (98%)	Transgene, retroviral transduction of bone marrow cells	(Chan et al., 2006; Grisolano et al., 1997; Kelly et al., 2000a; Westervelt et al., 2003)
t(8;21)(q22;q22)	<i>AML1-ETO</i>	AML	18% (30%)	Transgene, retroviral transduction of bone marrow cells	(de Guzman et al., 2002; Feniske et al., 2004; Yan et al., 2004)
t(11;17)(q23;q21) inv(16) or t(16;16)	<i>PLZF-RAR<math>\alpha</math></i>	AML	Rare	Transgene	(Rego et al., 2006)
t(9;11)(p22;q23)	<i>CBF<math>\beta</math>-MYH11</i>	AML	8% (~100%)	Conditional transgene	(Kuo et al., 2006)
t(6;11)(q27;q23)	<i>MLL-AF9</i>	AML	11% (30%)	Knock-in	(Dobson et al., 1999)
t(10;11)	<i>MLL-AF10, CALM-AF10</i>	AML	11q23 abnormalities are detected in ~35% of all AML		(Joh et al., 1997; Poirel et al., 1996)
t(11;17)(q23;q21)	<i>MLL-AF17</i>				(Borkhardt et al., 1993a)
t(11;19)(q23;p3.3)	<i>MLL-ENL/ENL/EEN</i>			Retroviral transduction of bone marrow cell Knock-in	(Suzukawa et al., 2005) (DiMartino et al., 2000; Rubnitz et al., 1999)
t(4;11)(q21;q23)	<i>MLL-AF4</i>				(Chen et al., 2006; Domer et al., 1993)
t(6;9)(p23;q34)	<i>DEK-CAN</i>	AML	1%		(Soekarnan et al., 1992; von Lindern et al., 1992)

**Table 2.1** (continued)

Chromosomal translocation	Fusion gene	Leukemia type	Disease frequency	Mouse models (approaches)	Citations
t(16;21)(p11;q22)	<i>TLS (FUS)-ERG</i>	AML	<1%		(Ichikawa et al., 1994; Kong et al., 1997; Panagopoulos et al., 1994)
t(16;21)(q24;q22)	<i>AML1-MTG16</i>	t-AML, MDS	<1%		(La Starza et al., 2001; Salomon-Nguyen et al., 2000a)
t(3;21)	<i>AML1-EVII</i> <i>AML1-EAP</i> <i>AML1-MDSL</i> <i>NUP98-HOXA9</i> <i>NUP98-PMX1</i>	AML	<1%	Knock-in	(Maki et al., 2005)
t(7;11)(p15;p15) t(1;11)(q23;p15)		AML	<1%	Retroviral transduction of bone marrow cells	(Kroon et al., 2001; Nakamura et al., 1996)
inv(8)(p11q13)	<i>MOZ-CBP</i> <i>MOZ-TIF2</i>	AML	<1%		(Borrow et al., 1996)
t(8;22)(p11;p13)	<i>MOZ-p300</i>	AML	<1%	Retroviral transduction of bone marrow cells	(Deguchi et al., 2003; Imamura et al., 2003; Kitabayashi et al., 2001b)
t(12;22)(p12;q23)	<i>TEL-MNI</i>	AML, CML	<1%		(Buijs et al., 1995; Nakazato et al., 2001)

Table 2.1 (continued)

Chromosomal translocation	Fusion gene	Leukemia type	Disease frequency	Mouse models (approaches)	Citations
t(1;22)(p13;q13)	<i>OTT-MAL</i>	AML	<1%		(Ma et al., 2001;
t(5;12)(q33;p12)	<i>TEL-PDGFR<math>\beta</math></i>	CMMoL	2–5%	Transgene; retroviral transduction of bone marrow cells	Mercher et al., 2001; (Ritchie et al., 1999;
					Sawyers and Denny, 1994; Tomasson et al., 2000; Wlodarska et al., 1995)
t(9;22)(q34;q11.2)	<i>BCR-ABL</i>	CML	~98%	Retroviral transduction of bone marrow cells; tetracycline inducible system	(Koschmieder et al., 2005; Li et al., 1999)
t(1;19)(q23;p13.3)	<i>E2A-PBX1</i>	Pre-B ALL	6% (30%)		(Kamps et al., 1991)
t(17;19)(q22;p13.3)	<i>E2A-HLF</i>	Pro-B ALL	1%	Transgene	(Honda et al., 1999;
t(12;21)(p12;q22)	<i>TEL-AML1</i>	Pre-B ALL	25%	Retroviral transduction of bone marrow cells	Hunger et al., 1992) (Bernardin et al., 2002;
t(9;22)(q34;q11.2)	<i>BCR-ABL</i>	ALL	~5% of childhood ALL; 30% of adult B-ALL	retroviral transduction of bone marrow cells; knock-in	Tsuzuki et al., 2004) (Castellanos et al., 1997; Li et al., 1999)
t(4;11)(q21;q23)	<i>MLL-AF4</i>	Pre-B ALL	5%	Knock-in	(Chen et al., 2006;
					Domer et al., 1993)

Table 2.1 (continued)

Chromosomal translocation	Fusion gene	Leukemia type	Disease frequency	Mouse models (approaches)	Citations
t(9;11)(p22;q23)	<i>MLL-AF9</i>	Pre-B ALL	<1%	Knock-in	(Dobson et al., 1999)
t(11;19)(q23;p13.3)	<i>MLL-ENL</i>	Pre-B ALL, T-ALL	1% <1%	Retroviral transduction of bone marrow cell	(DiMartino et al., 2000; Rubnitz et al., 1999)
t(X;11)(q13;q23)	<i>MLL-AFX1</i>	T-ALL	<1%		(Corral et al., 1993)
t(1;11)(p32;q23)	<i>MLL-AFP1</i>	ALL	<1%		(Borkhardt et al., 1995b)
t(6;11)(q27;q23)	<i>MLL-AF6</i>	ALL	<1%		(Joh et al., 1997; Poirier et al., 1996)
t(2;5)(p23;q35)	<i>NPM-ALK</i>	Lymphoma ALCL, NHL	75%	Retroviral transduction of bone marrow cell; transgene	(Chiari et al., 2003; Jager et al., 2005; Kuefer et al., 1997; Miethling et al., 2003)

The percentage refers to the frequency of the translocations within the disease overall. The values within parentheses refer to the frequency within the morphologic or immunologic subtype of the disease. ALCL, anaplastic large cell lymphoma; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma. (Referred and modified from [http://enmice.nci.nih.gov/enmice/mouse\\_models/hema\\_models/organ\\_models/](http://enmice.nci.nih.gov/enmice/mouse_models/hema_models/organ_models/) [hema\_models/organ\_models/hema\_ appendix\_two].)

apoptosis-resistant phenotype of CLL and NHL. To model TRAF-mediated CLL, transgenic mice, which expressed in lymphocytes a TRAF2 mutant lacking the RING and zinc finger domains located at the N-terminus of TRAF2 (TRAF2DN), developed splenomegaly and lymphadenopathy, as a result of a polyclonal expansion of B lymphocytes (Lee et al., 1997). In addition, transgenic mouse expressing Bcl-2 in B lymphocytes developed age-dependent lymphadenopathy and splenomegaly (Katsumata et al., 1992), associated with lymphoid cell expansions resembling certain human low-grade B-cell malignancies (Katsumata et al., 1992; Strasser et al., 1993). When both TRAF2DN and Bcl-2 transgenic mice were crossed to generate double transgenic mice, the double homozygous mice develop an age-dependent B-cell leukemia and lymphoma, with striking similarities to human CLL. These findings also provide direct evidence that TRAFs contribute to CLL development and that the high coexpression levels of TRAF1 and Bcl-2 commonly found in human CLL contribute to the pathogenesis of this leukemia (Zapata et al., 2000).

## 2.3 Conclusion

Although many mouse models of human blood cancers (Table 2.1) are available for the study of disease mechanisms and the development of new therapeutic strategies, improvements are needed to more accurately mimic human blood cancers. On the other hand, mouse models of many types of human leukemia induced or accelerated by fusion genes and other mutated genes are not yet available (Table 2.1), and generation of these disease models will be of important value.

## References

- Arron, J.R., Pewzner-Jung, Y., Walsh, M.C., Kobayashi, T., and Choi, Y. (2002). Regulation of the subcellular localization of tumor necrosis factor receptor-associated factor (TRAF)2 by TRAF1 reveals mechanisms of TRAF2 signaling. *J Exp Med* 196, 923–934.
- Bernardin, F., Yang, Y., Cleaves, R., Zahurak, M., Cheng, L., Civin, C.I., and Friedman, A.D. (2002). TEL-AML1, expressed from t(12;21) in human acute lymphocytic leukemia, induces acute leukemia in mice. *Cancer Res* 62, 3904–3908.
- Bernardin-Fried, F., Kummalue, T., Leijen, S., Collector, M.I., Ravid, K., and Friedman, A.D. (2004). AML1/RUNX1 increases during G1 to S cell cycle progression independent of cytokine-dependent phosphorylation and induces cyclin D3 gene expression. *J Biol Chem* 279, 15678–15687.
- Bhatia, A., Dash, S., Varma, N., and Marwaha, R.K. (2007). Fanconi anemia presenting as acute myeloid leukemia: a case report. *Indian J Pathol Microbiol* 50, 441–443.
- Bichi, R., Shinton, S.A., Martin, E.S., Koval, A., Calin, G.A., Cesari, R., Russo, G., Hardy, R.R., and Croce, C.M. (2002). Human chronic lymphocytic leukemia modeled in mouse by targeted TCL1 expression. *Proc Natl Acad Sci USA* 99, 6955–6960.

- Borkhardt, A., Haas, O.A., Strobl, W., Repp, R., Mann, G., Gadner, H., and Lampert, F. (1995a). A novel type of MLL/AF10 fusion transcript in a child with acute megakaryocytic leukemia (AML-M7). *Leukemia* 9, 1796–1797.
- Borkhardt, A., Mitteis, M., Brettreich, S., Schlieben, S., Hammermann, J., Repp, R., Kreuder, J., Buchen, U., and Lampert, F. (1995b). Rapid synthesis of hybrid RNA molecules associated with leukemia-specific chromosomal translocations. *Leukemia* 9, 719–722.
- Borrow, J., Stanton, V.P., Jr., Andresen, J.M., Becher, R., Behm, F.G., Chaganti, R.S., Civin, C.I., Distechi, C., Dube, I., Frischaufer, A.M., et al. (1996). The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. *Nat Genet* 14, 33–41.
- Bowen, D.T., Frew, M.E., Hills, R., Gale, R.E., Wheatley, K., Groves, M.J., Langabeer, S.E., Kottaridis, P.D., Moorman, A.V., Burnett, A.K., et al. (2005). RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. *Blood* 106, 2113–2119.
- Braun, B.S., Tuveson, D.A., Kong, N., Le, D.T., Kogan, S.C., Rozmus, J., Le Beau, M.M., Jacks, T.E., and Shannon, K.M. (2004). Somatic activation of oncogenic Kras in hematopoietic cells initiates a rapidly fatal myeloproliferative disorder. *Proc Natl Acad Sci USA* 101, 597–602.
- Buijs, A., Sherr, S., van Baal, S., van Bezouw, S., van der Plas, D., Geurts van Kessel, A., Riegerman, P., Lekanne Deprez, R., Zwaarthoff, E., Hagemeijer, A., et al. (1995). Translocation (12;22)(p13;q11) in myeloproliferative disorders results in fusion of the ETS-like TEL gene on 12p13 to the MN1 gene on 22q11. *Oncogene* 10, 1511–1519.
- Burel, S.A., Harakawa, N., Zhou, L., Pabst, T., Tenen, D.G., and Zhang, D.E. (2001). Dichotomy of AML1-ETO functions: growth arrest versus block of differentiation. *Mol Cell Biol* 21, 5577–5590.
- Callens, C., Chevret, S., Cayuela, J.M., Cassinat, B., Raffoux, E., de Botton, S., Thomas, X., Guerci, A., Fegueux, N., Pigneux, A., et al. (2005). Prognostic implication of FLT3 and Ras gene mutations in patients with acute promyelocytic leukemia (APL): a retrospective study from the European APL Group. *Leukemia* 19, 1153–1160.
- Carapeti, M., Aguiar, R.C., Goldman, J.M., and Cross, N.C. (1998). A novel fusion between MOZ and the nuclear receptor coactivator TIF2 in acute myeloid leukemia. *Blood* 91, 3127–3133.
- Castellanos, A., Pintado, B., Weruaga, E., Arevalo, R., Lopez, A., Orfao, A., and Sanchez-Garcia, I. (1997). A BCR-ABL(p190) fusion gene made by homologous recombination causes B-cell acute lymphoblastic leukemias in chimeric mice with independence of the endogenous bcr product. *Blood* 90, 2168–2174.
- Chaffanet, M., Gressin, L., Preudhomme, C., Soenen-Cornu, V., Birnbaum, D., and Pebusque, M.J. (2000). MOZ is fused to p300 in an acute monocytic leukemia with t(8;22). *Genes Chromosomes Cancer* 28, 138–144.
- Chan, I.T., Kutok, J.L., Williams, I.R., Cohen, S., Kelly, L., Shigematsu, H., Johnson, L., Akashi, K., Tuveson, D.A., Jacks, T., et al. (2004). Conditional expression of oncogenic K-ras from its endogenous promoter induces a myeloproliferative disease. *J Clin Invest* 113, 528–538.
- Chan, I.T., Kutok, J.L., Williams, I.R., Cohen, S., Moore, S., Shigematsu, H., Ley, T.J., Akashi, K., Le Beau, M.M., and Gilliland, D.G. (2006). Oncogenic K-ras cooperates with PML-RAR alpha to induce an acute promyelocytic leukemia-like disease. *Blood* 108, 1708–1715.
- Chen, W., Li, Q., Hudson, W.A., Kumar, A., Kirchhof, N., and Kersey, J.H. (2006). A murine Mll-AF4 knock-in model results in lymphoid and myeloid deregulation and hematologic malignancy. *Blood* 108, 669–677.
- Chiarle, R., Gong, J.Z., Guasparri, I., Pesci, A., Cai, J., Liu, J., Simmons, W.J., Dhall, G., Howes, J., Piva, R., et al. (2003). NPM-ALK transgenic mice spontaneously develop T-cell lymphomas and plasma cell tumors. *Blood* 101, 1919–1927.

- Collins, S.J., Robertson, K.A., and Mueller, L. (1990). Retinoic acid-induced granulocytic differentiation of HL-60 myeloid leukemia cells is mediated directly through the retinoic acid receptor (RAR-alpha). *Mol Cell Biol* 10, 2154–2163.
- Corral, J., Forster, A., Thompson, S., Lampert, F., Kaneko, Y., Slater, R., Kroes, W.G., van der Schoot, C.E., Ludwig, W.D., Karpas, A., et al. (1993). Acute leukemias of different lineages have similar MLL gene fusions encoding related chimeric proteins resulting from chromosomal translocation. *Proc Natl Acad Sci USA* 90, 8538–8542.
- Dahia, P.L., Aguiar, R.C., Alberta, J., Kum, J.B., Caron, S., Sill, H., Marsh, D.J., Ritz, J., Freedman, A., Stiles, C., et al. (1999). PTEN is inversely correlated with the cell survival factor Akt/PKB and is inactivated via multiple mechanisms in haematological malignancies. *Hum Mol Genet* 8, 185–193.
- Daley, G.Q., Van Etten, R.A., and Baltimore, D. (1990). Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* 247, 824–830.
- Dawson, M.I., Elstner, E., Kizaki, M., Chen, D.L., Pakkala, S., Kerner, B., and Koeffler, H.P. (1994). Myeloid differentiation mediated through retinoic acid receptor/retinoic X receptor (RXR) not RXR/RXR pathway. *Blood* 84, 446–452.
- de Guzman, C.G., Warren, A.J., Zhang, Z., Gartland, L., Erickson, P., Drabkin, H., Hiebert, S.W., and Klug, C.A. (2002). Hematopoietic stem cell expansion and distinct myeloid developmental abnormalities in a murine model of the AML1-ETO translocation. *Mol Cell Biol* 22, 5506–5517.
- Deguchi, K., Ayton, P.M., Carapeti, M., Kutok, J.L., Snyder, C.S., Williams, I.R., Cross, N.C., Glass, C.K., Cleary, M.L., and Gilliland, D.G. (2003). MOZ-TIF2-induced acute myeloid leukemia requires the MOZ nucleosome binding motif and TIF2-mediated recruitment of CBP. *Cancer Cell* 3, 259–271.
- Demarest, S.J., Martinez-Yamout, M., Chung, J., Chen, H., Xu, W., Dyson, H.J., Evans, R.M., and Wright, P.E. (2002). Mutual synergistic folding in recruitment of CBP/p300 by p160 nuclear receptor coactivators. *Nature* 415, 549–553.
- DiMartino, J.F., Miller, T., Ayton, P.M., Landewe, T., Hess, J.L., Cleary, M.L., and Shilatifard, A. (2000). A carboxy-terminal domain of ELL is required and sufficient for immortalization of myeloid progenitors by MLL-ELL. *Blood* 96, 3887–3893.
- Ding, X.F., Anderson, C.M., Ma, H., Hong, H., Uht, R.M., Kushner, P.J., and Stallcup, M.R. (1998). Nuclear receptor-binding sites of coactivators glucocorticoid receptor interacting protein 1 (GRIP1) and steroid receptor coactivator 1 (SRC-1): multiple motifs with different binding specificities. *Mol Endocrinol* 12, 302–313.
- Dobson, C.L., Warren, A.J., Pannell, R., Forster, A., Lavenir, I., Corral, J., Smith, A.J., and Rabbitts, T.H. (1999). The mll-AF9 gene fusion in mice controls myeloproliferation and specifies acute myeloid leukaemogenesis. *Embo J* 18, 3564–3574.
- Domer, P.H., Fakhrazadeh, S.S., Chen, C.S., Jockel, J., Johansen, L., Silverman, G.A., Kersey, J.H., and Korsmeyer, S.J. (1993). Acute mixed-lineage leukemia t(4;11)(q21;q23) generates an MLL-AF4 fusion product. *Proc Natl Acad Sci USA* 90, 7884–7888.
- Fenrick, R., Amann, J.M., Lutterbach, B., Wang, L., Westendorf, J.J., Downing, J.R., and Hiebert, S.W. (1999). Both TEL and AML-1 contribute repression domains to the t(12;21) fusion protein. *Mol Cell Biol* 19, 6566–6574.
- Fenske, T.S., Pengue, G., Mathews, V., Hanson, P.T., Hamm, S.E., Riaz, N., and Graubert, T.A. (2004). Stem cell expression of the AML1/ETO fusion protein induces a myeloproliferative disorder in mice. *Proc Natl Acad Sci USA* 101, 15184–15189.
- Fischer, M., Schwieger, M., Horn, S., Niebuhr, B., Ford, A., Roscher, S., Bergholz, U., Greaves, M., Lohler, J., and Stocking, C. (2005). Defining the oncogenic function of the TEL/AML1 (ETV6/RUNX1) fusion protein in a mouse model. *Oncogene* 24, 7579–7591.
- Ford, A.M., Bennett, C.A., Price, C.M., Bruin, M.C., Van Wering, E.R., and Greaves, M. (1998). Fetal origins of the TEL-AML1 fusion gene in identical twins with leukemia. *Proc Natl Acad Sci USA* 95, 4584–4588.

- Glass, C.K., Rose, D.W., and Rosenfeld, M.G. (1997). Nuclear receptor coactivators. *Curr Opin Cell Biol* 9, 222–232.
- Golub, T.R., Barker, G.F., Bohlander, S.K., Hiebert, S.W., Ward, D.C., Bray-Ward, P., Morgan, E., Raimondi, S.C., Rowley, J.D., and Gilliland, D.G. (1995). Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. *Proc Natl Acad Sci USA* 92, 4917–4921.
- Golub, T.R., Barker, G.F., Lovett, M., and Gilliland, D.G. (1994). Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell* 77, 307–316.
- Grisolano, J.L., Wesselschmidt, R.L., Pelicci, P.G., and Ley, T.J. (1997). Altered myeloid development and acute leukemia in transgenic mice expressing PML-RAR alpha under control of cathepsin G regulatory sequences. *Blood* 89, 376–387.
- Heery, D.M., Kalkhoven, E., Hoare, S., and Parker, M.G. (1997). A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* 387, 733–736.
- Hess, J.L., and Hug, B.A. (2004). Fusion-protein truncation provides new insights into leukemogenesis. *Proc Natl Acad Sci USA* 101, 16985–16986.
- Hess, M., Huggins, M.B., Mudzamiri, R., and Heincz, U. (2004). Avian metapneumovirus excretion in vaccinated and non-vaccinated specified pathogen free laying chickens. *Avian Pathol* 33, 35–40.
- Higuchi, M., O'Brien, D., Kumaravelu, P., Lenny, N., Yeoh, E.J., and Downing, J.R. (2002). Expression of a conditional AML1-ETO oncogene bypasses embryonic lethality and establishes a murine model of human t(8;21) acute myeloid leukemia. *Cancer Cell* 1, 63–74.
- Honda, H., Inaba, T., Suzuki, T., Oda, H., Ebihara, Y., Tsuji, K., Nakahata, T., Ishikawa, T., Yazaki, Y., and Hirai, H. (1999). Expression of E2A-HLF chimeric protein induced T-cell apoptosis, B-cell maturation arrest, and development of acute lymphoblastic leukemia. *Blood* 93, 2780–2790.
- Horwitz, K.B., Jackson, T.A., Bain, D.L., Richer, J.K., Takimoto, G.S., and Tung, L. (1996). Nuclear receptor coactivators and corepressors. *Mol Endocrinol* 10, 1167–1177.
- Hunger, S.P., Ohyashiki, K., Toyama, K., and Cleary, M.L. (1992). Hlf, a novel hepatic bZIP protein, shows altered DNA-binding properties following fusion to E2A in t(17;19) acute lymphoblastic leukemia. *Genes Dev* 6, 1608–1620.
- Ichikawa, H., Shimizu, K., Hayashi, Y., and Ohki, M. (1994). An RNA-binding protein gene, TLS/FUS, is fused to ERG in human myeloid leukemia with t(16;21) chromosomal translocation. *Cancer Res* 54, 2865–2868.
- Ida, K., Kitabayashi, I., Taki, T., Taniwaki, M., Noro, K., Yamamoto, M., Ohki, M., and Hayashi, Y. (1997). Adenoviral E1A-associated protein p300 is involved in acute myeloid leukemia with t(11;22)(q23;q13). *Blood* 90, 4699–4704.
- Imamura, T., Kakazu, N., Hibi, S., Morimoto, A., Fukushima, Y., Ijuin, I., Hada, S., Kitabayashi, I., Abe, T., and Imashuku, S. (2003). Rearrangement of the MOZ gene in pediatric therapy-related myelodysplastic syndrome with a novel chromosomal translocation t(2;8)(p23;p11). *Genes Chromosomes Cancer* 36, 413–419.
- Jackson, E.L., Willis, N., Mercer, K., Bronson, R.T., Crowley, D., Montoya, R., Jacks, T., and Tuveson, D.A. (2001). Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes Dev* 15, 3243–3248.
- Jager, R., Hahne, J., Jacob, A., Egert, A., Schenkel, J., Wernert, N., Schorle, H., and Wellmann, A. (2005). Mice transgenic for NPM-ALK develop non-Hodgkin lymphomas. *Anticancer Res* 25, 3191–3196.
- Joh, T., Yamamoto, K., Kagami, Y., Kakuda, H., Sato, T., Yamamoto, T., Takahashi, T., Ueda, R., Kaibuchi, K., and Seto, M. (1997). Chimeric MLL products with a Ras binding cytoplasmic protein AF6 involved in t(6;11)(q27;q23) leukemia localize in the nucleus. *Oncogene* 15, 1681–1687.

- Kamps, M.P., Look, A.T., and Baltimore, D. (1991). The human t(1;19) translocation in pre-B ALL produces multiple nuclear E2A-Pbx1 fusion proteins with differing transforming potentials. *Genes Dev* 5, 358–368.
- Katsumata, M., Siegel, R.M., Louie, D.C., Miyashita, T., Tsujimoto, Y., Nowell, P.C., Greene, M.I., and Reed, J.C. (1992). Differential effects of Bcl-2 on T and B cells in transgenic mice. *Proc Natl Acad Sci USA* 89, 11376–11380.
- Kelly, L.M., Kutok, J.L., Williams, I.R., Boulton, C.L., Amaral, S.M., Curley, D.P., Ley, T.J., and Gilliland, D.G. (2000a). PML/RARalpha and FLT3-ITD induce an APL-like disease in a mouse model. *Proc Natl Acad Sci USA* 99, 8283–8288.
- Kelly, L.M., Liu, Q., Kutok, J.L., Williams, I.R., Boulton, C.L., and Gilliland, D.G. (2002b). FLT3 internal tandem duplication mutations associated with human acute myeloid leukemias induce myeloproliferative disease in a murine bone marrow transplant model. *Blood* 99, 310–318.
- Kitabayashi, I., Aikawa, Y., Nguyen, L.A., Yokoyama, A., and Ohki, M. (2001a). Activation of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion protein. *Embo J* 20, 7184–7196.
- Kitabayashi, I., Aikawa, Y., Yokoyama, A., Hosoda, F., Nagai, M., Kakazu, N., Abe, T., and Ohki, M. (2001b). Fusion of MOZ and p300 histone acetyltransferases in acute monocytic leukemia with a t(8;22)(p11;q13) chromosome translocation. *Leukemia* 15, 89–94.
- Kitabayashi, I., Yokoyama, A., Shimizu, K., and Ohki, M. (1998). Interaction and functional cooperation of the leukemia-associated factors AML1 and p300 in myeloid cell differentiation. *Embo J* 17, 2994–3004.
- Kong, X.T., Ida, K., Ichikawa, H., Shimizu, K., Ohki, M., Maseki, N., Kaneko, Y., Sako, M., Kobayashi, Y., Tojou, A., et al. (1997). Consistent detection of TLS/FUS-ERG chimeric transcripts in acute myeloid leukemia with t(16;21)(p11;q22) and identification of a novel transcript. *Blood* 90, 1192–1199.
- Koschmieder, S., Gottgens, B., Zhang, P., Iwasaki-Arai, J., Akashi, K., Kutok, J.L., Dayaram, T., Geary, K., Green, A.R., Tenen, D.G., et al. (2005). Inducible chronic phase of myeloid leukemia with expansion of hematopoietic stem cells in a transgenic model of BCR-ABL leukemogenesis. *Blood* 105, 324–334.
- Kottaridis, P.D., Gale, R.E., Frew, M.E., Harrison, G., Langabeer, S.E., Belton, A.A., Walker, H., Wheatley, K., Bowen, D.T., Burnett, A.K., et al. (2001). The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood* 98, 1752–1759.
- Kroon, E., Thorsteinsdottir, U., Mayotte, N., Nakamura, T., and Sauvageau, G. (2001). NUP98-HOXA9 expression in hemopoietic stem cells induces chronic and acute myeloid leukemias in mice. *Embo J* 20, 350–361.
- Kuefer, M.U., Look, A.T., Pulford, K., Behm, F.G., Pattengale, P.K., Mason, D.Y., and Morris, S.W. (1997). Retrovirus-mediated gene transfer of NPM-ALK causes lymphoid malignancy in mice. *Blood* 90, 2901–2910.
- Kuhn, R., Schwenk, F., Aguet, M., and Rajewsky, K. (1995). Inducible gene targeting in mice. *Science* 269, 1427–1429.
- Kulkarni, S., Heath, C., Parker, S., Chase, A., Iqbal, S., Pocock, C.F., Kaeda, J., Cwynarski, K., Goldman, J.M., and Cross, N.C. (2000). Fusion of H4/D10S170 to the platelet-derived growth factor receptor beta in BCR-ABL-negative myeloproliferative disorders with a t(5;10)(q33;q21). *Cancer Res* 60, 3592–3598.
- Kuo, Y.H., Landrette, S.F., Heilman, S.A., Perrat, P.N., Garrett, L., Liu, P.P., Le Beau, M.M., Kogan, S.C., and Castilla, L.H. (2006). Cbf beta-SMMHC induces distinct abnormal myeloid progenitors able to develop acute myeloid leukemia. *Cancer Cell* 9, 57–68.

- La Starza, R., Sambani, C., Crescenzi, B., Matteucci, C., Martelli, M.F., and Mecucci, C. (2001). AML1/MTG16 fusion gene from a t(16;21)(q24;q22) translocation in treatment-induced leukemia after breast cancer. *Haematologica* 86, 212–213.
- Lacronique, V., Boureux, A., Valle, V.D., Poirel, H., Quang, C.T., Mauchauffe, M., Berthou, C., Lessard, M., Berger, R., Ghysdael, J., et al. (1997). A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. *Science* 278, 1309–1312.
- Landis, S.H., Murray, T., Bolden, S., and Wingo, P.A. (1998). Cancer statistics, 1998. *CA Cancer J Clin* 48, 6–29.
- Lee, S.Y., Reichlin, A., Santana, A., Sokol, K.A., Nussenzweig, M.C., and Choi, Y. (1997). TRAF2 is essential for JNK but not NF-kappaB activation and regulates lymphocyte proliferation and survival. *Immunity* 7, 703–713.
- Leo, C., and Chen, J.D. (2000). The SRC family of nuclear receptor coactivators. *Gene* 245, 1–11.
- Lessard, J., and Sauvageau, G. (2003). Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature* 423, 255–260.
- Levanon, D., Goldstein, R.E., Bernstein, Y., Tang, H., Goldenberg, D., Stifani, S., Paroush, Z., and Groner, Y. (1998). Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. *Proc Natl Acad Sci USA* 95, 11590–11595.
- Li, D.M., and Sun, H. (1998). PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G1 cell cycle arrest in human glioblastoma cells. *Proc Natl Acad Sci USA* 95, 15406–15411.
- Li, S., Ilaria, R.L., Jr., Million, R.P., Daley, G.Q., and Van Etten, R.A. (1999). The P190, P210, and p230 forms of the *BCR/ABL* oncogene induce a similar chronic myeloid leukemia-like syndrome in mice but have different lymphoid leukemogenic activity. *J Exp Med* 189, 1399–1412.
- Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., et al. (1997). PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275, 1943–1947.
- Liang, J., Prouty, L., Williams, B.J., Dayton, M.A., and Blanchard, K.L. (1998). Acute mixed lineage leukemia with an inv(8)(p11q13) resulting in fusion of the genes for MOZ and TIF2. *Blood* 92, 2118–2122.
- Lin, Y., Ryan, J., Lewis, J., Wani, M.A., Lingrel, J.B., and Liu, Z.G. (2003). TRAF2 exerts its antiapoptotic effect by regulating the expression of Kruppel-like factor LKLF. *Mol Cell Biol* 23, 5849–5856.
- Liu, P., Tarle, S.A., Hajra, A., Claxton, D.F., Marlton, P., Freedman, M., Siciliano, M.J., and Collins, F.S. (1993). Fusion between transcription factor CBF beta/PEPB2 beta and a myosin heavy chain in acute myeloid leukemia. *Science* 261, 1041–1044.
- Liu, P.P., Wijmenga, C., Hajra, A., Blake, T.B., Kelley, C.A., Adelstein, R.S., Bagg, A., Rector, J., Cotelingam, J., Willman, C.L., et al. (1996). Identification of the chimeric protein product of the CBF $\beta$ -MYH11 fusion gene in inv(16) leukemia cells. *Genes Chromosomes Cancer* 16, 77–87.
- Lou, J., Cao, W., Bernardin, F., Ayyanathan, K., Rauscher, I.F., and Friedman, A.D. (2000). Exogenous cdk4 overcomes reduced cdk4 RNA and inhibition of G1 progression in hematopoietic cells expressing a dominant-negative CBF – a model for overcoming inhibition of proliferation by CBF oncoproteins. *Oncogene* 19, 2695–2703.
- Lutterbach, B., Westendorf, J.J., Linggi, B., Patten, A., Moniwa, M., Davie, J.R., Huynh, K.D., Bardwell, V.J., Lavinsky, R.M., Rosenfeld, M.G., et al. (1998). ETO, a target of t(8;21) in acute leukemia, interacts with the N-CoR and mSin3 corepressors. *Mol Cell Biol* 18, 7176–7184.
- Ma, Z., Morris, S.W., Valentine, V., Li, M., Herbrick, J.A., Cui, X., Bouman, D., Li, Y., Mehta, P.K., Nizetic, D., et al. (2001). Fusion of two novel genes, RBM15 and MKL1, in the t(1;22)(p13;q13) of acute megakaryoblastic leukemia. *Nat Genet* 28, 220–221.

- Maki, K., Yamagata, T., Asai, T., Yamazaki, I., Oda, H., Hirai, H., and Mitani, K. (2005). Dysplastic definitive hematopoiesis in AML1/EVII knock-in embryos. *Blood* 106, 2147–2155.
- Mercher, T., Coniat, M.B., Monni, R., Mauchauffe, M., Nguyen Khac, F., Gressin, L., Mugneret, F., Leblanc, T., Dastugue, N., Berger, R., et al. (2001). Involvement of a human gene related to the *Drosophila* *spen* gene in the recurrent t(1;22) translocation of acute megakaryocytic leukemia. *Proc Natl Acad Sci USA* 98, 5776–5779.
- Meyers, S., Downing, J.R., and Hiebert, S.W. (1993). Identification of AML-1 and the (8;21) translocation protein (AML-1/ETO) as sequence-specific DNA-binding proteins: the Runt homology domain is required for DNA binding and protein-protein interactions. *Mol Cell Biol* 13, 6336–6345.
- Miethling, C., Grundler, R., Fend, F., Hoepfl, J., Mugler, C., von Schilling, C., Morris, S.W., Peschel, C., and Duyster, J. (2003). The oncogenic fusion protein nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) induces two distinct malignant phenotypes in a murine retroviral transplantation model. *Oncogene* 22, 4642–4647.
- Munzert, G., Kirchner, D., Stobbe, H., Bergmann, L., Schmid, R.M., Dohner, H., and Heimpel, H. (2002). Tumor necrosis factor receptor-associated factor 1 gene overexpression in B-cell chronic lymphocytic leukemia: analysis of NF-kappa B/Rel-regulated inhibitors of apoptosis. *Blood* 100, 3749–3756.
- Murray, R.J., O'ReillyR.J., Cannell, P., French, M.A. (1999). B-cell acute lymphoblastic leukaemia in HIV infection. *Annu Conf Australas Soc HIV Med*, 11.
- Nakamura, T., Largaespada, D.A., Lee, M.P., Johnson, L.A., Ohyahiki, K., Toyama, K., Chen, S.J., Willman, C.L., Chen, I.M., Feinberg, A.P., et al. (1996). Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukaemia. *Nat Genet* 12, 154–158.
- Nakazato, H., Shiozaki, H., Zhou, M., Nakatsu, M., Motoji, T., Mizoguchi, H., Miyawaki, S., and Sato, Y. (2001). TEL/MN1 fusion in a de novo acute myeloid leukaemia-M2 patient who showed strong resistance to treatment. *Br J Haematol* 113, 1079–1081.
- Narducci, M.G., Pescarmona, E., Lazzeri, C., Signoretti, S., Lavinia, A.M., Remotti, D., Scala, E., Baroni, C.D., Stoppacciaro, A., Croce, C.M., et al. (2000). Regulation of TCL1 expression in B- and T-cell lymphomas and reactive lymphoid tissues. *Cancer Res* 60, 2095–2100.
- Narducci, M.G., Stoppacciaro, A., Imada, K., Uchiyama, T., Virgilio, L., Lazzeri, C., Croce, C.M., and Russo, G. (1997). TCL1 is overexpressed in patients affected by adult T-cell leukemias. *Cancer Res* 57, 5452–5456.
- Odero, M.D., Vizmanos, J.L., Roman, J.P., Lahortiga, I., Panizo, C., Calasanz, M.J., Zeleznik-Le, N.J., Rowley, J.D., and Novo, F.J. (2002). A novel gene, MDS2, is fused to ETV6/TEL in a t(1;12)(p36.1;p13) in a patient with myelodysplastic syndrome. *Genes Chromosomes Cancer* 35, 11–19.
- Okuda, T., Cai, Z., Yang, S., Lenny, N., Lyu, C.J., van Deursen, J.M., Harada, H., and Downing, J.R. (1998). Expression of a knocked-in AML1-ETO leukemia gene inhibits the establishment of normal definitive hematopoiesis and directly generates dysplastic hematopoietic progenitors. *Blood* 91, 3134–3143.
- Okuda, T., Takeda, K., Fujita, Y., Nishimura, M., Yagyu, S., Yoshida, M., Akira, S., Downing, J.R., and Abe, T. (2000). Biological characteristics of the leukemia-associated transcriptional factor AML1 disclosed by hematopoietic rescue of *AML1*-deficient embryonic stem cells by using a knock-in strategy. *Mol Cell Biol* 20, 319–328.
- Onodera, M., Kunisada, T., Nishikawa, S., Sakiyama, Y., Matsumoto, S., and Nishikawa, S. (1995). Overexpression of retinoic acid receptor alpha suppresses myeloid cell differentiation at the promyelocyte stage. *Oncogene* 11, 1291–1298.
- Pabst, T., Mueller, B.U., Harakawa, N., Schoch, C., Haferlach, T., Behre, G., Hiddemann, W., Zhang, D.E., and Tenen, D.G. (2001). AML1-ETO downregulates the granulocytic differentiation factor C/EBPalpha in t(8;21) myeloid leukemia. *Nat Med* 7, 444–451.

- Panagopoulos, I., Aman, P., Fioretos, T., Hoglund, M., Johansson, B., Mandahl, N., Heim, S., Behrendtz, M., and Mitelman, F. (1994). Fusion of the FUS gene with ERG in acute myeloid leukemia with t(16;21)(p11;q22). *Genes Chromosomes Cancer* 11, 256–262.
- Panagopoulos, I., Fioretos, T., Isaksson, M., Samuelsson, U., Billstrom, R., Strombeck, B., Mitelman, F., and Johansson, B. (2001). Fusion of the MORF and CBP genes in acute myeloid leukemia with the t(10;16)(q22;p13). *Hum Mol Genet* 10, 395–404.
- Papadopoulos, P., Ridge, S.A., Boucher, C.A., Stocking, C., and Wiedemann, L.M. (1995). The novel activation of ABL by fusion to an ets-related gene, TEL. *Cancer Res* 55, 34–38.
- Park, I.K., Qian, D., Kiel, M., Becker, M.W., Pihalja, M., Weissman, I.L., Morrison, S.J., and Clarke, M.F. (2003). Bmi-1 is required for maintenance of adult self-renewing hematopoietic stem cells. *Nature* 423, 302–305.
- Poiesz, B.J., Papsidero, L.D., Ehrlich, G., Sherman, M., Dube, S., Poiesz, M., Dillon, K., Ruscetti, F.W., Slamon, D., Fang, C., et al. (2001). Prevalence of HTLV-I-associated T-cell lymphoma. *Am J Hematol* 66, 32–38.
- Poirel, H., Rack, K., Delabesse, E., Radford-Weiss, I., Troussard, X., Debert, C., Leboeuf, D., Bastard, C., Picard, F., Veil-Buzyn, A., et al. (1996). Incidence and characterization of MLL gene (11q23) rearrangements in acute myeloid leukemia M1 and M5. *Blood* 87, 2496–2505.
- Raynaud, S.D., Baens, M., Grosgeorge, J., Rodgers, K., Reid, C.D., Dainton, M., Dyer, M., Fuzibet, J.G., Gratecos, N., Taillan, B., et al. (1996). Fluorescence in situ hybridization analysis of t(3; 12)(q26; p13): a recurring chromosomal abnormality involving the TEL gene (ETV6) in myelodysplastic syndromes. *Blood* 88, 682–689.
- Rego, E.M., Ruggero, D., Trieboli, C., Cattoretti, G., Kogan, S., Redner, R.L., and Pandolfi, P.P. (2006). Leukemia with distinct phenotypes in transgenic mice expressing PML/RAR alpha, PLZF/RAR alpha or NPM/RAR alpha. *Oncogene* 25, 1974–1979.
- Rhoades, K.L., Hetherington, C.J., Harakawa, N., Yergeau, D.A., Zhou, L., Liu, L.Q., Little, M.T., Tenen, D.G., and Zhang, D.E. (2000). Analysis of the role of AML1-ETO in leukemogenesis, using an inducible transgenic mouse model. *Blood* 96, 2108–2115.
- Ritchie, K.A., Aprikyan, A.A., Bowen-Pope, D.F., Norby-Slycord, C.J., Conyers, S., Bartelmez, S., Sitnicka, E.H., and Hickstein, D.D. (1999). The Tel-PDGFRbeta fusion gene produces a chronic myeloproliferative syndrome in transgenic mice. *Leukemia* 13, 1790–1803.
- Rubnitz, J.E., Camitta, B.M., Mahmoud, H., Raimondi, S.C., Carroll, A.J., Borowitz, M.J., Shuster, J.J., Link, M.P., Pullen, D.J., Downing, J.R., et al. (1999). Childhood acute lymphoblastic leukemia with the MLL-ENL fusion and t(11;19)(q23;p13.3) translocation. *J Clin Oncol* 17, 191–196.
- Salomon-Nguyen, F., Busson-Le Coniat, M., Lafage Pochitaloff, M., Mozziconacci, J., Berger, R., and Bernard, O.A. (2000a). AML1-MTG16 fusion gene in therapy-related acute leukemia with t(16;21)(q24;q22): two new cases. *Leukemia* 14, 1704–1705.
- Salomon-Nguyen, F., Della-Valle, V., Mauchauffe, M., Busson-Le Coniat, M., Ghysdael, J., Berger, R., and Bernard, O.A. (2000b). The t(1;12)(q21;p13) translocation of human acute myeloblastic leukemia results in a TEL-ARNT fusion. *Proc Natl Acad Sci USA* 97, 6757–6762.
- Satake, N., Ishida, Y., Otoh, Y., Hinohara, S., Kobayashi, H., Sakashita, A., Maseki, N., and Kaneko, Y. (1997). Novel MLL-CBP fusion transcript in therapy-related chronic myelomonocytic leukemia with a t(11;16)(q23;p13) chromosome translocation. *Genes Chromosomes Cancer* 20, 60–63.
- Sawyers, C.L., and Denny, C.T. (1994). Chronic myelomonocytic leukemia: Tel-a-kinase what Ets all about. *Cell* 77, 171–173.
- Schwaller, J., Anastasiadou, E., Cain, D., Kutok, J., Wojiski, S., Williams, I.R., LaStarza, R., Crescenzi, B., Sternberg, D.W., Andreasson, P., et al. (2001). H4(D10S170), a gene frequently rearranged in papillary thyroid carcinoma, is fused to the platelet-derived

- growth factor receptor beta gene in atypical chronic myeloid leukemia with t(5;10)(q33;q22). *Blood* 97, 3910–3918.
- Schwaller, J., Frantsve, J., Aster, J., Williams, I.R., Tomasson, M.H., Ross, T.S., Peeters, P., Van Rompaey, L., Van Etten, R.A., Ilaria, R., Jr., et al. (1998). Transformation of hematopoietic cell lines to growth-factor independence and induction of a fatal myelo- and lymphoproliferative disease in mice by retrovirally transduced TEL/JAK2 fusion genes. *Embo J* 17, 5321–5333.
- Schwaller, J., Parganas, E., Wang, D., Cain, D., Aster, J.C., Williams, I.R., Lee, C.K., Gerthner, R., Kitamura, T., Frantsve, J., et al. (2000). Stat5 is essential for the myelo- and lymphoproliferative disease induced by TEL/JAK2. *Mol Cell* 6, 693–704.
- Sherr, C.J. (2004). Principles of tumor suppression. *Cell* 116, 235–246.
- Sobulo, O.M., Borrow, J., Tomek, R., Reshmi, S., Harden, A., Schlegelberger, B., Housman, D., Doggett, N.A., Rowley, J.D., and Zeleznik-Le, N.J. (1997). MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;p13.3). *Proc Natl Acad Sci USA* 94, 8732–8737.
- Soekarman, D., von Lindern, M., Daenen, S., de Jong, B., Fonatsch, C., Heinze, B., Bartram, C., Hagemeyer, A., and Grosveld, G. (1992). The translocation (6;9)(p23;q34) shows consistent rearrangement of two genes and defines a myeloproliferative disorder with specific clinical features. *Blood* 79, 2990–2997.
- Stegmaier, K., Pendse, S., Barker, G.F., Bray-Ward, P., Ward, D.C., Montgomery, K.T., Krauter, K.S., Reynolds, C., Sklar, J., Donnelly, M., et al. (1995). Frequent loss of heterozygosity at the TEL gene locus in acute lymphoblastic leukemia of childhood. *Blood* 86, 38–44.
- Stirewalt, D.L., Kopecky, K.J., Meshinch, S., Appelbaum, F.R., Slovak, M.L., Willman, C.L., and Radich, J.P. (2001). FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. *Blood* 97, 3589–3595.
- Strasser, A., Harris, A.W., and Cory, S. (1993). E mu-bcl-2 transgene facilitates spontaneous transformation of early pre-B and immunoglobulin-secreting cells but not T cells. *Oncogene* 8, 1–9.
- Suzukawa, K., Shimizu, S., Nemoto, N., Takei, N., Taki, T., and Nagasawa, T. (2005). Identification of a chromosomal breakpoint and detection of a novel form of an MLL-AF17 fusion transcript in acute monocytic leukemia with t(11;17)(q23;q21). *Int J Hematol* 82, 38–41.
- Taki, T., Sako, M., Tsuchida, M., and Hayashi, Y. (1997). The t(11;16)(q23;p13) translocation in myelodysplastic syndrome fuses the MLL gene to the CBP gene. *Blood* 89, 3945–3950.
- Takizawa, J., Suzuki, R., Kuroda, H., Utsunomiya, A., Kagami, Y., Joh, T., Aizawa, Y., Ueda, R., and Seto, M. (1998). Expression of the TCL1 gene at 14q32 in B-cell malignancies but not in adult T-cell leukemia. *Jpn J Cancer Res* 89, 712–718.
- Tenen, D.G., Hromas, R., Licht, J.D., and Zhang, D.E. (1997). Transcription factors, normal myeloid development, and leukemia. *Blood* 90, 489–519.
- Thick, J., Metcalfe, J.A., Mak, Y.F., Beatty, D., Minegishi, M., Dyer, M.J., Lucas, G., and Taylor, A.M. (1996). Expression of either the TCL1 oncogene, or transcripts from its homologue MTCP1/c6.1B, in leukaemic and non-leukaemic T cells from ataxia telangiectasia patients. *Oncogene* 12, 379–386.
- Tomasson, M.H., Sternberg, D.W., Williams, I.R., Carroll, M., Cain, D., Aster, J.C., Ilaria, R.L., Jr., Van Etten, R.A., and Gilliland, D.G. (2000). Fatal myeloproliferation, induced in mice by TEL/PDGF $\beta$ AR expression, depends on PDGF $\beta$ AR tyrosines 579/581. *J Clin Invest* 105, 423–432.
- Torchia, J., Rose, D.W., Inostroza, J., Kamei, Y., Westin, S., Glass, C.K., and Rosenfeld, M.G. (1997). The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* 387, 677–684.
- Tsai, S., and Collins, S.J. (1993). A dominant negative retinoic acid receptor blocks neutrophil differentiation at the promyelocyte stage. *Proc Natl Acad Sci USA* 90, 7153–7157.

- Tsuzuki, S., Seto, M., Greaves, M., and Enver, T. (2004). Modeling first-hit functions of the t(12;21) TEL-AML1 translocation in mice. *Proc Natl Acad Sci USA* *101*, 8443–8448.
- Virgilio, L., Narducci, M.G., Isobe, M., Billips, L.G., Cooper, M.D., Croce, C.M., and Russo, G. (1994). Identification of the TCL1 gene involved in T-cell malignancies. *Proc Natl Acad Sci USA* *91*, 12530–12534.
- von Lindern, M., Fornerod, M., van Baal, S., Jaegle, M., de Wit, T., Buijs, A., and Grosfeld, G. (1992). The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, dek and can, and the expression of a chimeric, leukemia-specific dek-can mRNA. *Mol Cell Biol* *12*, 1687–1697.
- Wang, C.Y., Mayo, M.W., Korneluk, R.G., Goeddel, D.V., and Baldwin, A.S., Jr. (1998). NF- $\kappa$ B antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* *281*, 1680–1683.
- Westervelt, P., Lane, A.A., Pollock, J.L., Oldfather, K., Holt, M.S., Zimonjic, D.B., Popescu, N.C., DiPersio, J.F., and Ley, T.J. (2003). High-penetrance mouse model of acute promyelocytic leukemia with very low levels of PML-RAralpha expression. *Blood* *102*, 1857–1865.
- Wlodarska, I., Mecucci, C., Marynen, P., Guo, C., Franckx, D., La Starza, R., Aventin, A., Bosly, A., Martelli, M.F., Cassiman, J.J., et al. (1995). TEL gene is involved in myelodysplastic syndromes with either the typical t(5;12)(q33;p13) translocation or its variant t(10;12)(q24;p13). *Blood* *85*, 2848–2852.
- Yamamoto, Y., Kiyo, H., Nakano, Y., Suzuki, R., Kodera, Y., Miyawaki, S., Asou, N., Kuriyama, K., Yagasaki, F., Shimazaki, C., et al. (2001). Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood* *97*, 2434–2439.
- Yan, M., Burel, S.A., Peterson, L.F., Kanbe, E., Iwasaki, H., Boyapati, A., Hines, R., Akashi, K., and Zhang, D.E. (2004). Deletion of an AML1-ETO C-terminal Ncor/SMRT-interacting region strongly induces leukemia development. *Proc Natl Acad Sci USA* *101*, 17186–17191.
- Yokota, S., Kiyo, H., Nakao, M., Iwai, T., Misawa, S., Okuda, T., Sonoda, Y., Abe, T., Kahsima, K., Matsuo, Y., et al. (1997). Internal tandem duplication of the FLT3 gene is preferentially seen in acute myeloid leukemia and myelodysplastic syndrome among various hematological malignancies. A study on a large series of patients and cell lines. *Leukemia* *11*, 1605–1609.
- Yuan, Y., Zhou, L., Miyamoto, T., Iwasaki, H., Harakawa, N., Hetherington, C.J., Burel, S.A., Lagasse, E., Weissman, I.L., Akashi, K., et al. (2001). AML1-ETO expression is directly involved in the development of acute myeloid leukemia in the presence of additional mutations. *Proc Natl Acad Sci USA* *98*, 10398–10403.
- Zapata, J.M., Krajewska, M., Krajewski, S., Kitada, S., Welsh, K., Monks, A., McCloskey, N., Gordon, J., Kipps, T.J., Gascoyne, R.D., et al. (2000). TNFR-associated factor family protein expression in normal tissues and lymphoid malignancies. *J Immunol* *165*, 5084–5096.
- Zhang, J., Grindley, J.C., Yin, T., Jayasinghe, S., He, X.C., Ross, J.T., Haug, J.S., Rupp, D., Porter-Westpfahl, K.S., Wiedemann, L.M., et al. (2006). PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. *Nature* *441*, 518–522.

# **Chapter 3**

## **Murine Models of Hematopoietic Disease: Pathologic Analysis and Characterization**

**Benjamin H. Lee and Jeffery L. Kutok**

### **Contents**

3.1	Introduction . . . . .	45
3.2	General Approach to the Analysis of a Mouse . . . . .	46
3.2.1	Physical Findings . . . . .	46
3.2.2	Laboratory Findings . . . . .	47
3.2.3	Necropsy Findings . . . . .	48
3.3	Histology of Normal Murine Hematopoietic Tissue . . . . .	49
3.4	Cytology of Normal Murine Hematopoietic Cells . . . . .	52
3.5	Bethesda Classification Systems of Murine Hematopoietic Disease . . . . .	54
3.6	Ancillary Techniques for the Evaluation of Murine Hematopoietic Disease . . . . .	56
3.7	Nonlymphoid Hematopoietic Neoplasms in Mice . . . . .	58
3.7.1	Myeloproliferative Disorders . . . . .	59
3.7.2	Acute Myeloid Leukemia . . . . .	68
3.7.3	AML Associated with Recurrent Genetic Abnormalities . . . . .	69
3.8	Hematopoietic Disease Models and Molecularly Targeted Therapy . . . . .	73
3.9	Conclusions . . . . .	74

### **3.1 Introduction**

The pathologic evaluation of murine hematologic disease can be considerably more challenging than the assessment of human hematopoietic malignancies. Diagnoses in humans that may be difficult based solely on histology are often straightforward with careful review of the patient's laboratory data or after specialized ancillary testing. Unfortunately, such luxuries do not always exist in the workup of a mouse. Obtaining important clinical and laboratory data for each mouse in a cohort is often difficult and costly. Peripheral blood counts and cytologic findings in the blood and marrow play a critical role in the diagnosis of disease in both mice and humans; however, quite often, a mouse is simply

---

J.L. Kutok

Brigham and Women's Hospital, Harvard Medical School, Department of Pathology,  
Boston, MA 02115, USA  
jkutok@partners.org

fixed in formalin enabling only histopathologic review. Immunophenotyping by flow cytometry or cytochemical studies require fresh cells and are necessary to confirm morphologic impressions. This testing should be a part of all murine hematopoietic workups but needs to be planned and performed soon after the time of euthanasia. Genetic testing, such as karyotyping and Southern blotting for clonality, also require unfixed cells obtained at the time of euthanasia. These studies are difficult and infrequently performed and, additionally, may be of limited value in some murine genetic models where monoclonality may not necessarily be synonymous with aggressive disease. Finally, while the objective of the analysis is often to compare the murine findings to the corresponding human diseases, this is not always possible. The genetic backgrounds created in some experimental mice may be sufficient to induce hematologic abnormalities but may not result in phenocopies of the human diseases. In addition, variations in murine strains can contribute greatly to the observed hematologic abnormalities. With all of these inherent difficulties in mind, a recent attempt to standardize the classification of nonlymphoid and lymphoid murine hematopoietic malignancies has been undertaken (Kogan et al. 2002; Morse et al. 2002). The goals of these classification systems are to allow investigators to diagnose murine hematopoietic neoplasms as well-defined entities according to accepted criteria. They emphasize the need to incorporate peripheral blood findings, cytologic features of hematopoietic cells, histopathology of hematopoietic tissues, immunophenotypic and genetic features, and the clinical course into each diagnosis. In addition, the differences between the murine and the human diseases are reflected in the nomenclature and methods used in these classifications.

This chapter will provide an overview of the general approach to the pathologic characterization of murine hematopoietic diseases. A review of the normal histology of the murine system will be provided and attention will be paid to the pathologic differences and similarities between the murine and the human hematopoietic systems. Specific emphasis will be placed on nonlymphoid leukemias, myeloproliferative-like leukemias, and myeloid proliferations, and the application of existing classification guidelines in their diagnosis will be highlighted.

## 3.2 General Approach to the Analysis of a Mouse

### 3.2.1 Physical Findings

The hallmark of neoplastic hematopoietic disease is the infiltration or proliferation of myeloid or lymphoid cells into the hematolymphoid organs. The hematolymphoid organs include the bone marrow, spleen, lymph nodes, Peyer's patches, and thymus. The neoplastic infiltration typically leads to hematopoietic failure secondary to the replacement of normal hematopoietic

cells within the marrow, that is, lack of normal red blood cells (RBCs), white blood cells (WBCs), and platelets. In humans, the clinical presentations that result from the loss of normal hematopoietic cells include anemia (due to RBC loss), infection (due to lymphocyte and granulocyte loss), and bleeding (due to platelet loss). In mice, however, the spectrum of clinical signs may be much more limited. The sterile environment in which laboratory mice are kept reduces the infectious complications resulting from leukopenia, and spontaneous bleeding due to platelet deficiencies is uncommon. Anemia is often the most consistent indicator of hematopoietic failure, but the clinical appearance of anemia in mice can be subtle. Enlarged organs and tissues are frequent signs of disease, as mice can survive with a marked degree of tumor burden, but organomegaly often occurs later in the disease process. Sites of neoplastic cell dissemination into nonhematolymphoid organs include the liver, kidneys, lungs, and bowel. Given these issues, routine observation to identify the onset of a disease phenotype should be undertaken. Once a mouse dies, the clotting of blood and autolysis of the marrow and lymphoid organs precludes meaningful evaluation. Daily observation and weekly physical examination is, therefore, a useful practice. Observable signs of systemic disease include lethargy or inactivity, poor grooming with unkempt fur, weight loss, respiratory difficulties (often associated with a mediastinal mass), hind-limb paralysis (associated with spinal cord infiltrates), or a visible mass. Anemic mice often have pale ears and feet. On physical examination, careful palpation can reveal enlargement of the spleen, liver, or lymph nodes, particularly the nodes of the axilla or neck. It is critical to realize that the spleen is a functioning hematopoietic organ throughout the life of a mouse and is often a primary site of hematopoietic disease (much like the bone marrow). If available, small animal noninvasive imaging modalities, including high-frequency ultrasound and FDG PET/CT, may also assist in following tumor development (Tatsumi et al. 2003; Liao et al. 2005).

### **3.2.2 Laboratory Findings**

Very commonly, the composition of the peripheral blood will mirror systemic infiltrates, and monthly blood draws are suggested to monitor for abnormalities. Blood collection from the saphenous or dorsal pedal vein do not require anesthesia, whereas anesthesia is necessary for tail vein, orbital sinus, or jugular vein blood draws (Hoff 2000). Blood should not be collected from the orbital sinus more frequently than once every 2 weeks; other venous sites can be used as often as needed (Hoff 2000). Of note, packed cell volume and hemoglobin measurements have been reported to be higher from tail vein draws compared to other sites (Sakaki 1961). Automated cell differential counts can be performed in a veterinary blood analyzer with software capable of evaluating murine blood (e.g., HemaVet Vet950FS, Drew Scientific). Such analyzers are capable of recording hemoglobin, hematocrit, platelet counts, and white cell

counts and automated differentials. EDTA spray-coated tubes are recommended to avoid dilutional effects of the anticoagulant in the small blood volumes obtained. Considerable strain variation exists in normal hematologic parameters, so it is important to consult reference databases to determine whether the blood in question is outside of the normal range. The Mouse Phenome Database is one such database that provides a continually growing repository of phenotypic and genotypic data, including blood hematology values, for many commonly studied inbred strains that are easily accessed through the Mouse Phenome Database website (<http://www.jax.org/phenome>) maintained by the Jackson Laboratory (Bogue et al. 2007). Once peripheral blood is obtained, air-dried smears on glass slides should be made. Wright–Giemsa staining is preferred for confirming nucleated cell differential counts and observing morphologic abnormalities. Just prior to euthanasia, additional blood can be obtained from the anesthetized animal's heart, the posterior vena cava, the axillary vessels, or the orbital sinus (Hoff 2000). If the blood is thick or clotting occurs, a small amount of normal saline can be added for the purposes of preparing blood smears.

### ***3.2.3 Necropsy Findings***

Once the animal is euthanized, macroscopic or gross examination of the internal organs can be valuable in identifying pathology. An excellent resource for the necropsic evaluation of a mouse for hematopoietic disease is found at the National Cancer Institute (NCI)-sponsored website (<http://emice.nci.nih.gov/>) that contains the supplemental materials to Bethesda classification articles (Kogan et al. 2002; Morse et al. 2002), as well as the on-line Virtual Mouse Necropsy site (<http://geocities.com/virtualbiology/necropsy.html>). Attention should be paid to the size of the spleen, thymus, lymph nodes, and liver, and recording the weights of enlarged organs can be helpful in documenting abnormalities. The organs should be sectioned before fixation and examined for color and the presence of macroscopic lesions. A greenish or brownish hue to lymph nodes or spleen can occasionally be seen in myeloid leukemia, and white nodules or a lacy pattern observed in lymphomatous involvement of organs. The presence of abnormalities in the cut surface of the liver can be a sensitive indicator of neoplastic disease, since reactive conditions do not normally cause visible abnormalities. The sternum and hind limbs can be pale in color, as opposed to red, when the marrow is infiltrated by WBCs. An enlarged thymus is usually associated with T-lymphoblastic leukemia/lymphoma.

Sections of spleen, lymph nodes, thymus, liver, bone marrow (sternum or femur), lungs, and any tumor masses should be placed in 10% buffered formalin for 24–48 h and then processed for paraffin sections as soon as possible. Formalin is the best fixative to maintain antigenicity for subsequent immunohistochemical analysis. In addition, single-cell suspensions should be prepared

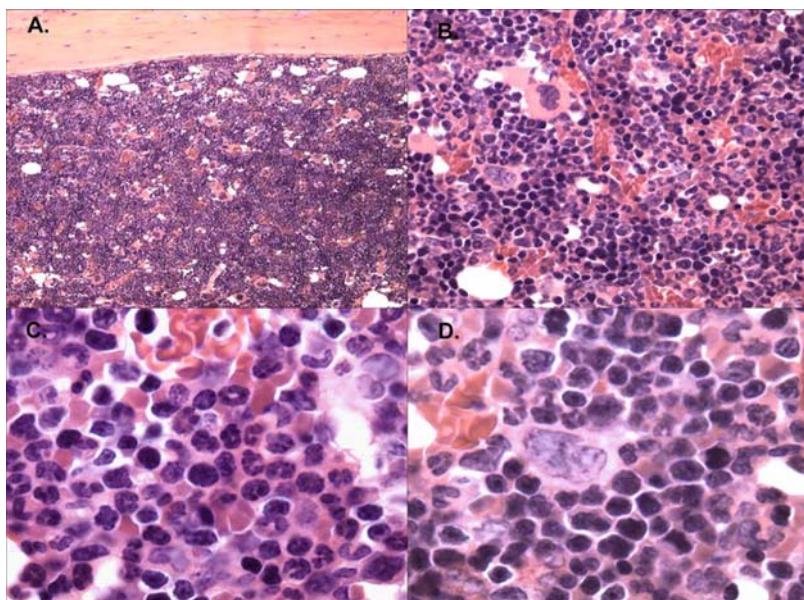
from disaggregated tissues and flushed bone marrow to be used for flow cytometric analysis, cytopsin slides (for morphology or cytochemical stains), or as a source of DNA, RNA, and protein. Unused cells can be stored in an RPMI, 20% fetal calf serum, and 10% DMSO solution at -80°C for long periods.

### 3.3 Histology of Normal Murine Hematopoietic Tissue

In order to determine whether the presence of a hematological disorder exists, careful cytologic and histologic examination must be undertaken. This requires knowledge of the normal cytology and histology of the murine system one is working with. Importantly, several distinct differences exist in the normal anatomy of the murine hematopoietic system compared to humans. It is necessary to keep these dissimilarities in mind when evaluating for hematologic disease. A detailed description of normal histology and cytology can be found in several fine atlases including the *Atlas of Mouse Hematopathology* by Fredrickson and Harris (Fredrickson and Harris 2000).

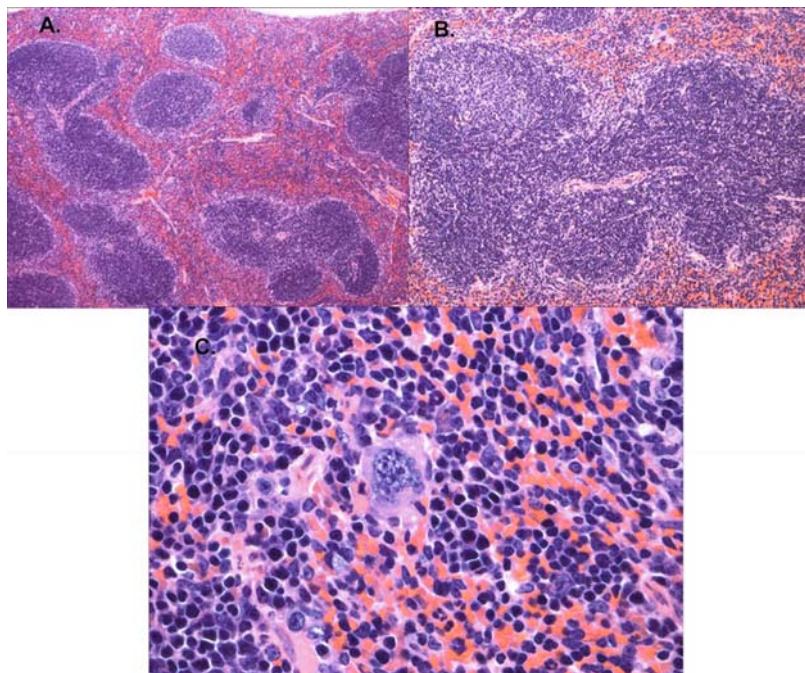
**Bone marrow.** In contrast to other mammalian species, the bone marrow of mice contains very little medullary fat and, therefore, appears quite cellular throughout life (Fig. 3.1A,B). Careful inspection of the medullary cavity does reveal a prominent network of endothelial-lined sinusoidal channels. In the normal state, these channels are open and filled with mature RBCs. When marrow hypercellularity occurs, it can be difficult to identify. One typical finding in these cases is that the sinuses become compressed and inconspicuous. In contrast, hypercellularity in humans manifests itself by the replacement of medullary fat with hematopoietic cells. Interestingly, when compared to human marrow, monocytes and lymphocytes comprise a significant proportion of murine marrow cellularity. In the mouse, granulocytes typically comprise 30–40% of the marrow nucleated cells (Fig. 3.1C), with the remaining cellularity comprised of erythroid precursors (20%) (Fig. 3.1D), monocytes (10–20%), and lymphoid cells (25–30%). In contrast, the relative proportion of cell types in human marrow is 67% granulocytes, 19% erythroid, 1% monocytes, 3% myeloblasts, and 10% lymphocytes (Bunning and McKenna 1994). Megakaryocytes are readily identifiable throughout the marrow cavity and are in similar proportions to that seen in the human (Fig. 3.1B,D).

**Spleen.** As mentioned above, the splenic red pulp is a site of primary hematopoiesis throughout the life of a mouse (Fig. 3.2). This is in complete contrast to humans, where extramedullary hematopoiesis (EMH) in the spleen is not seen under normal physiologic conditions. The amount of EMH is generally relatively small and tends to decline with the age of the mouse (Fig. 3.2B). In times of physiologic stress, however, EMH can be extremely prominent, resulting in a marked increase in splenic size. Therefore, a markedly enlarged spleen does not necessarily signify a neoplastic process but may simply be related to high demand for RBCs and/or granulocytes. The limited space for bone marrow



**Fig. 3.1** Histology of murine bone marrow. **(A)** Medium ( $200\times$ ) magnification of wild-type adult BALB/c bone marrow demonstrating normal hypercellularity (hematoxylin and eosin stain). **(B)** Higher magnification ( $400\times$ ) of the same marrow, demonstrating trilineage hematopoiesis with open sinuses filled with mature red blood cells and scattered adipocytes (hematoxylin and eosin stain). **(C)** High magnification ( $1000\times$ ) of a myeloid colony within the marrow showing maturing elements including ringed granulocyte precursors (hematoxylin and eosin stain). **(D)** High magnification ( $1000\times$ ) of an erythroid colony with darkly stained round to irregular nuclei surrounding a megakaryocyte (hematoxylin and eosin stain). (See color insert)

expansion is directly related to the increase in splenic hematopoiesis in these circumstances. In neoplastic conditions, bone marrow infiltration by leukemic or lymphomatous cells can lead to expanded reactive hematopoiesis in the spleen, or alternatively, direct expansion of neoplastic hematopoietic elements in the spleen can occur. The white pulp is supported by a network of dendritic and reticular cells organized in several distinct lymphoid zones: the T-lymphocyte-rich central zone, surrounding a central arteriole; adjacent mantle zone of small B lymphocytes; and outer marginal zone of B lymphocytes (Fig. 3.2C). The T-lymphocyte-rich zone is also referred to as the periarteriolar sheath and is composed of a predominance of CD4-positive T cells with fewer CD8-positive cells. The mantle zones contain monomorphic small IgM $^+$ IgD $^+$  B lymphocytes with little cytoplasm imparting a dark blue appearance to this zone on hematoxylin and eosin staining. The marginal zone also contains small B lymphocytes; however, these cells are IgM $^+$ IgD $^-$  and have ample cytoplasm imparting a pale appearance to this region. Of note, in contrast to mice, humans have an inner and an outer marginal zone which is surrounded by a large perifollicular zone (Meibius and Kraal 2005). Within the mantle zone region, germinal centers can



**Fig. 3.2** Histology of murine spleen. (A) Low power magnification ( $40\times$ ) of an adult wild-type BALB/c mouse showing white pulp nodules surrounded by extramedullary hematopoiesis in the red pulp (hematoxylin and eosin stain). (B) Medium power magnification ( $100\times$ ) from the same spleen, demonstrating white pulp lymphoid nodules with a germinal center (upper left) and pale marginal zones (hematoxylin and eosin stain). (C) High power magnification ( $400\times$ ) from the same spleen showing red pulp extramedullary hematopoiesis predominantly comprised of darkly staining erythroid elements and a single megakaryocyte (hematoxylin and eosin stain). (See color insert)

occasionally be seen. A so-called dark zone containing proliferating intermediate to large lymphoid cells occupies one area of the germinal center, the other half is designated the light zone, which contains an admixture of centroblasts, centrocytes, and tingible body macrophages. In the nonimmunized animals, germinal centers are small and poorly formed but can become quite prominent with immunization.

**Lymph nodes.** The lymph nodes contain B-cell follicles in the cortical region that are uniformly distributed within the subcapsular region. The follicles contain a mantle zone and may or may not have a central germinal center. The marginal zones are not visible in the follicles of the lymph node. Within the paracortical regions, between the B-cell follicles, T cells (primarily CD4+) and interdigitating follicular dendritic cells predominate. Deep to this, within the lymph node medulla, plasma cells, immunoblasts, small lymphocytes, and histiocytes can be seen.

*Peyer's patch.* Peyer's patches are foci of lymphoid tissue located in the wall of the small bowel and, to a lesser extent, in the lower intestinal tract. They are comprised of aggregates of mantle-type B cells most commonly surrounding prominent, reactive germinal centers. Smaller numbers of T cells populate the regions between the follicles, as do scattered immunoblasts and plasma cells. The Peyer's patches are evident macroscopically and are typically infiltrated by neoplastic lymphoid and, occasionally, myeloid cells.

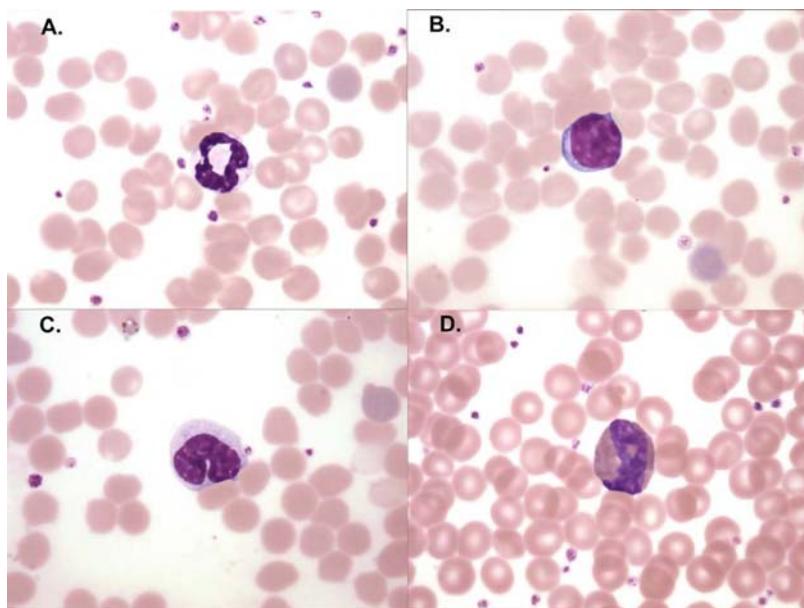
*Thymus.* Thymic architecture is relatively similar between the mouse and the humans. There is a clear histologic distinction between thymic cortex and medulla due to the decreased density in thymocytes within the medulla. In contrast to human thymic medulla, the Hassall's corpuscles are not as identifiable in the mouse thymus. Infiltration of thymic medulla by thymocytes and expansion of the thymus is typical in precursor T-cell lymphoblastic lymphoma.

### 3.4 Cytology of Normal Murine Hematopoietic Cells

The cytologic features of murine hematopoietic cells are similar to those of human hematopoietic cells, with the greatest differences observed in the granulocytic series. A brief accounting of the major cytologic features of each cell type is provided below. An outstanding more detailed description can be found elsewhere (Fredrickson and Harris 2000).

*Erythroid series.* Mature RBCs are biconcave discs with central pallor, as in humans. Reticulocytes are often abundant in peripheral blood smears, comprising 2–4% of the peripheral blood erythrocytes. These newly formed RBCs are larger than mature RBCs and have a basophilic hue with Wright–Giemsa staining secondary to the presence of RNA within the cell. Nucleated RBCs within the bone marrow show a succession from large cells with round nuclei, prominent nucleoli, and deeply basophilic cytoplasm (proerythroblast) to progressively smaller cells with round, hyperchromatic nuclei and paler cytoplasm (polychromatophilic normoblasts). In histologic sections of the spleen showing EMH, nucleated erythroid forms such as polychromatophilic normoblasts are abundant with very dark, somewhat irregular appearing nuclei and scant cytoplasm (Fig. 3.2C).

*Neutrophil series.* Neutrophils comprise approximately 5–20% of peripheral WBCs, a dramatically lower proportion of WBCs compared to humans where they comprise 40–70%. The nuclei are predominantly band-like and twisted or curled/ringed, and much less frequently are polylobate, as in the human (Fig. 3.3A). To be classified as a neutrophil, some authors suggest that, if ringed, the diameter of center of ring is greater than 50% of the diameter of nucleus or that the nucleus has fully developed segmentation (Kogan et al. 2002). A greater degree of segmentation can be observed in tissue sections of neutrophils in mice than in peripheral blood. The cytoplasm is generally



**Fig. 3.3** Cytology of murine peripheral blood cells. **(A)** Mature neutrophil from wild-type BALB/c mouse shows a hyperlobate nucleus and very fine azurophilic granules (Wright–Giemsa stain, 1000 $\times$ ). **(B)** Mature lymphocyte from wild-type BALB/c mouse shows a round nucleus and small amounts of basophilic, agranular cytoplasm (Wright–Giemsa stain, 1000 $\times$ ). **(C)** Mature monocyte from wild-type BALB/c mouse shows a kidney-shaped nucleus and pale blue-gray cytoplasm with occasional vacuoles (Wright–Giemsa stain, 1000 $\times$ ). **(D)** Mature eosinophil from wild-type BALB/c mouse shows a hyperlobate nucleus and prominent orange granules (Wright–Giemsa stain, 1000 $\times$ ). (See color insert)

plentiful and pale. In contrast to human neutrophils, the cytoplasmic granules are nearly unidentifiable with Wright–Giemsa staining. Only small violet or eosinophilic granules can be seen. Within the bone marrow, myeloblasts have oval, eccentrically or peripherally placed nuclei, with even chromatin, distinct nucleoli, and basophilic cytoplasm. Only cells with these cytologic features should be considered blasts or immature forms for the purposes of diagnosing leukemias. Promyelocytes are larger, with less prominent nucleoli and a small, central, nuclear clearing that heralds the beginning of the doughnut or ring-shaped nuclei that typify the myelocytes and metamyelocytes. Granules are also difficult to discern in the promyelocyte, particularly in relation to those seen in humans. As mentioned, the characteristic doughnut or ringed nuclei are present within the myelocytes, metamyelocytes, and bands. The earliest forms (myelocytes) have nuclei with a more doughnut-shaped appearance, which progress to a thinner ring-like form in the band cells. As the cells mature, the chromatin becomes more condensed and there are increased small, inconspicuous granules.

*Lymphocytes.* Mature lymphocytes comprise the majority (80–90%) of the peripheral blood cells, in contrast to humans where only 20–40% of the blood cells are lymphocytes. The morphology of the mature murine lymphocyte is similar to that of the human. The nucleus is dark and round with small amounts pale, agranular cytoplasm (Fig. 3.3B). Within the bone marrow, lymphoblasts are infrequent and difficult to discern from proerythroblasts. Some distinguishing characteristics of lymphoblasts compared to immature erythroid elements include more even or fine chromatin, slightly paler blue cytoplasm, and a more peripherally placed nucleus.

*Monocytes.* Monocytes are the largest cells in the circulation. They have a round to oval occasionally reniform or bean-shaped nucleus with rope-like chromatin (Fig. 3.3C). Nucleoli are not distinct, and the cytoplasm is slightly basophilic, agranular, and frequently contains vacuoles. In tissue sections stained with hematoxylin and eosin, the nuclei are pale and curved or folded with ample eosinophilic cytoplasm.

*Eosinophils and basophils.* The eosinophil has similar features to the neutrophilic band; however, the granules are prominent and red-orange in color (Fig. 3.3D). The immature eosinophilic forms have nuclear features similar to their neutrophilic counterparts. Basophils are very rarely seen. They contain large round, darkly basophilic granules throughout the cytoplasm and overlying the nucleus.

### 3.5 Bethesda Classification Systems of Murine Hematopoietic Disease

As previously mentioned, the necessity to develop a uniform classification system of murine hematopoietic neoplasms was recently recognized by the hematopathology subcommittee of the Mouse Models of Human Cancers Consortium (MMHCC) in order to meaningfully compare and contrast different murine cancer models to one another, as well as to defined human entities (<http://emice.nci.nih.gov>). Sponsored by the NCI/National Institutes of Health (NIH), the MMHC hematopathology subcommittee reported their consensus proposals for two classification systems in mice: one centered on the classification of nonlymphoid hematopoietic neoplasms (Kogan et al. 2002) and another on the classification of lymphoid neoplasms (Morse et al. 2002). Analogous to the World Health Organization (WHO) classification of human hematopoietic and lymphoid neoplasms (Jaffe et al. 2001), the Bethesda proposals sought to employ a combination of factors, including morphologic, immunophenotypic, genetic, and clinical/biologic characteristics, for the purposes of classification. In addition, they sought to recognize the differences between mouse and human biology and address the existence of gaps in the literature of human diseases that have not been well described or studied in mice. Although this chapter focuses mainly on nonlymphoid hematopoietic

**Table 3.1** Bethesda proposals for classification of nonlymphoid and lymphoid hematopoietic neoplasms in mice

Nonlymphoid	Lymphoid
<i>Nonlymphoid leukemias</i>	<i>B-cell neoplasms</i>
<ul style="list-style-type: none"> <li>• Myeloid leukemias           <ul style="list-style-type: none"> <li>– Myeloid leukemia without maturation</li> <li>– Myeloid leukemia with maturation</li> <li>– Myeloproliferative disease-like myeloid leukemia</li> <li>– Myelomonocytic leukemia</li> <li>– Monocytic leukemia</li> </ul> </li> <li>• Erythroid leukemia</li> <li>• Megakaryocytic leukemia</li> <li>• Biphenotypic leukemia</li> </ul>	<ul style="list-style-type: none"> <li>• Precursor B-cell neoplasm           <ul style="list-style-type: none"> <li>– Precursor B-cell lymphoblastic lymphoma/leukemia (pre-B LBL)</li> </ul> </li> <li>• Mature B-cell neoplasms           <ul style="list-style-type: none"> <li>– Small B-cell lymphoma (SBL)</li> <li>– Splenic marginal zone B-cell lymphoma</li> <li>– Follicular B-cell lymphoma (FBL)</li> <li>– Diffuse large B-cell lymphoma (DLBCL)               <ul style="list-style-type: none"> <li>– Morphologic variants                   <ul style="list-style-type: none"> <li>– Centroblastic (CB)</li> <li>– Immunoblastic (IB)</li> <li>– Histiocyte associated (HA)</li> </ul> </li> <li>– Subtypes                   <ul style="list-style-type: none"> <li>– Primary mediastinal (thymic) diffuse large B-cell lymphoma</li> <li>– Classical Burkitt lymphoma (BL)</li> <li>– Burkitt-like lymphoma [including mature B-cell lymphomas with lymphoblastic morphology (BLL)]</li> <li>– Plasma cell neoplasm                       <ul style="list-style-type: none"> <li>– Plasmacytoma</li> <li>– Extraskeletal plasmacytoma (PCT-E)</li> <li>– Anaplastic plasmacytoma (PCT-A)</li> <li>– <math>\beta</math>-natural killer cell lymphoma (BKNL)</li> </ul> </li> </ul> </li> </ul> </li> </ul> </li></ul>
<i>Nonlymphoid hematopoietic sarcomas</i>	<i>T-cell neoplasms</i>
<ul style="list-style-type: none"> <li>• Granulocytic sarcoma</li> <li>• Histiocytic sarcoma</li> <li>• Mast cell sarcoma</li> </ul>	<ul style="list-style-type: none"> <li>• Precursor T-cell neoplasm           <ul style="list-style-type: none"> <li>– Precursor T-cell lymphoblastic lymphoma/leukemia (pre-T LBL)</li> </ul> </li> <li>• Mature T-cell neoplasm           <ul style="list-style-type: none"> <li>– Small T-cell lymphoma (STL)</li> <li>– T-natural killer cell lymphoma (TNKL)</li> </ul> </li> <li>• T-cell neoplasm, character undetermined           <ul style="list-style-type: none"> <li>– Large cell anaplastic lymphoma (TLCA)</li> </ul> </li> </ul>
<i>Myeloid dysplasias</i>	
<ul style="list-style-type: none"> <li>• Myelodysplastic syndrome</li> <li>• Cytopenia with increased blasts</li> </ul>	
<i>Myeloid proliferations (nonreactive)</i>	
<ul style="list-style-type: none"> <li>• Myeloproliferation (genetic)</li> <li>• Myeloproliferative disease</li> </ul>	

(Adapted from Kogan et al. 2002 and Morse et al. 2002)

malignancies, Table 3.1 summarizes the broad disease categories of the Bethesda classification systems for both murine nonlymphoid and lymphoid hematopoietic neoplasms. Formal guidelines for further defining criteria and subclassification of specific entities in these general categories are contained within the aforementioned references and will be referred to in the discussion of particular disease models below. Finally, as with all classification systems, it should be recognized that while laying a solid foundation for the categorization of murine hematopoietic neoplasms, the Bethesda proposals will undoubtedly be subject to modification as investigators continue to further our understanding of hematopoietic disease in both humans and mice.

### 3.6 Ancillary Techniques for the Evaluation of Murine Hematopoietic Disease

While morphologic examination of paraffin-embedded mouse tissues, peripheral blood smears, and cytospins of single-cell suspensions is a critical initial step in characterizing hematopoietic neoplasms, valuable additional information may be obtained from a variety of ancillary diagnostic modalities. These include the use of cytochemical and immunohistochemical stains, flow cytometric immunophenotyping for lineage assignment, and less commonly, cytogenetic analysis. Preparation of murine tissues for DNA and RNA for potential molecular genetic analysis should also be considered at time of animal necropsy. It is important to realize that many of these ancillary studies require fresh and/or frozen cells that should, in some instances, be prepared under sterile conditions. For example, although not routinely performed, in vitro methylcellulose colony-forming cell (CFC) assays are often utilized in the evaluation and quantification of multipotential and lineage-restricted myeloid, erythroid, and megakaryocytic progenitors, as well as pre-B-lymphoid cells in murine models, and require sterile preparation of cells to prevent unwanted contamination of these long-term cultures. In addition, similar sterile protocols should also be applied to the preparation of hematopoietic/tumor cells that will undergo transplantation into irradiated recipient animals.

*Cytochemistry.* Cytochemistry is one of oldest laboratory methodologies used in the diagnosis and classification of human leukemias. Its use dates well before the advent of more sophisticated current diagnostic modalities including flow cytometry and cytogenetics. The application of cytochemical markers to murine samples can provide significant diagnostic information at a relatively inexpensive cost. These assays can be performed from commercially available kits (e.g., Sigma) or alternatively, recipes and protocols can be found in a number of standard hematology laboratory references (Harmening 2002). Commonly used cytochemical stains include Sudan Black B (SBB), which stains myeloid, neutrophilic, eosinophilic, and monocytic cells with variable intensity; Myeloperoxidase (MPO), which stains cells of myeloid and monocytic but not lymphoid lineage; Chloroacetate Esterase (CAE), which stains mast cells (strong), neutrophils, and occasional other cell types and Non-Specific Esterase (NSE), which variably stains cells of monocytic origin. Correlation of these studies with cytologic findings from Wright–Giemsa- or Romanowsky-stained preparations can be extremely useful for evaluating morphologically immature nonlymphoid hematopoietic neoplasms. Of note, because these staining methodologies are enzymatically based, long-term storage of unstained samples may adversely affect staining quality, and thus prompt analysis of freshly prepared samples gives optimal results.

*Immunohistochemistry and special stains.* The use of immunohistochemistry in the workup of human malignancies (hematopoietic and nonhematopoietic) is standard clinical practice; however, this technique is not commonly employed

in the analysis of murine tumors. With the refinement of immunohistochemical techniques and the development of better-characterized antibodies against murine antigens, immunohistochemistry can be invaluable in the workup of hematopoietic tumors. This is particularly true when applied to formalin-fixed tissues that commonly represent the only material that an investigator may have to work with. Prolonged fixation of tissue can adversely affect antigen retrieval with many antibodies, so care should be taken to process fixed samples as quickly as possible. Immunohistochemistry to identify protein tags, such as EGFP, HA, and FLAG, is also highly useful in evaluating the patterns of transgenic protein expression. In addition to standard hematoxylin and eosin (H&E) stains, additional useful histochemical stains can be carried out on tissue sections to evaluate hematological processes. Among these include reticulin or trichrome staining to assess for fibrosis [e.g., chronic idiopathic myelofibrosis (CIMF)], iron stains to examine iron levels (e.g., myelodysplasias), and toluidine blue or Giemsa stains, which can be used to highlight mast cells (e.g., mast cell leukemias or mast cell sarcomas). While many individual investigators are not necessarily equipped to carry out routine immunohistochemistry or special stains on murine tissues, these services are available commercially or through institutional core laboratory facilities. In addition, collaboration with pathologists is critical in the accurate interpretation of immunohistochemical results where it is important to determine appropriate immunoreactivity staining patterns (e.g., nuclear vs. cytoplasmic) and avoid misinterpretation of non-specific staining.

*Flow cytometry.* Pathologic diagnosis of hematopoietic malignancies has been greatly facilitated by the advent of flow cytometric analysis. While continuing advances in instrumentation, fluorochromes, and antibodies allow for increasingly sophisticated applications including the isolation and characterization of stem cell and multipotent progenitor subsets, standard three-color analysis is sufficient for most diagnostic immunophenotypic studies. Standard lineage markers are listed in Table 3.2 and can be utilized in a number of combinations for routine immunophenotypic studies. Current and updated recommendations for practical three-color panels for the evaluation of most hematopoietic neoplasms including nonlymphoid hematopoietic tumors are contained within the Bethesda proposals (Kogan et al. 2002; Morse et al. 2002). In addition, the frequent utilization of retroviral expression constructs, which coexpress green fluorescence protein (GFP), as well as other colors (e.g., YFP), allows for specific immunophenotypic evaluation of transduced cells by gating on GFP-positive cell populations in bone marrow transplantation (BMT) models. This makes flow cytometric analysis particularly valuable for the characterization of the transformed cells in these unique murine systems.

*Clonality.* Methods to identify monoclonality in hematopoietic tumors can be useful, particularly when there is a question of distinguishing a benign reactive process versus a malignant condition. For lymphoid neoplasms, this can be performed by the assessment of either clonal B- or T-cell gene rearrangements of the immunoglobulin heavy chain locus or T-cell receptor loci,

**Table 3.2** Commonly used markers for immunophenotypic analysis

Lineage	Antigen	Expression patterns
B cell	CD45R (B220)	B cells, abnormal T cells, NK cells
	CD19	B cells
	IgM	Mature B cells
	IgK	Mature B cells
	CD23(FceR)	Activated B cells
	CD138	Plasma cells
T cell	CD3	T cells
	CD4	Helper T cells
	CD8	Cytotoxic T cells, NK cells
	T-cell receptor (TCR)	T cells
Myeloid/monocytic	Myeloperoxidase (MPO)	Myeloid cells
	Gr-1 (Ly-6 G)	Granulocytes, monocytes
	Mac-1 (CD11b)	Granulocytes, monocytes
	F4/80 (Ly-71)	Monocytes, eosinophils
	Mac-2	Macrophages/histiocytes
	Mac-3	Macrophages/histiocytes
Erythroid	Ter119 (Ly-76)	Erythroid cells (immature to RBC)
	CD71 (transferrin receptor)	Erythroid (immature-to-mid-stage erythroid forms, expression diminishes with maturation)
Megakaryocyte	CD41	Platelets, megakaryoblasts
	CD61	Platelets, megakaryoblasts
	von Willebrand Factor (vWF)	Megakaryocytes, megakaryoblasts
Immature/early	CD34	Immature cells, endothelial cells
	Sca-1 (Ly-6A/E)	Immature cells
	c-Kit (CD117)	Immature cells, mast cells
Hematopoietic	CD31	Immature cells, endothelial cells
	CD45 (LCA)	Pan-hematopoietic marker Negative in erythroid cells

respectively, using Southern blot or polymerase chain reaction (PCR) techniques. For nonlymphoid malignancies, analysis of proviral integration sites is often performed where applicable. Less commonly, clonality may be assessed by cytogenetics through conventional G-banded karyotype analysis, fluorescence in situ hybridization (FISH), or the more complex spectral karyotyping (SKY) method, which employs multicolor chromosome-specific paints to differentiate and classify nonhomologous human or murine chromosomes.

### 3.7 Nonlymphoid Hematopoietic Neoplasms in Mice

The spectrum of hematopoietic malignancies falling under the Bethesda proposals classification system is broad and diverse. It allows for the categorization of neoplastic proliferations of all nonlymphoid hematopoietic cells including

myeloid/monocytic, erythroid, megakaryocytic, histiocytic, and mast cell lineages (i.e., leukemias and myeloproliferative processes), as well as stem cell disorders of ineffective hematopoiesis [i.e., myelodysplastic syndromes (MDSs)] (Table 3.1). Nonlymphoid leukemias are often characterized by either cytopenias or leukocytosis with a concurrent increase in nonlymphoid hematopoietic cells in both bone marrow and spleen. The proliferations that are observed may be of immature precursors (i.e., acute leukemia) or mature nonlymphoid cells [i.e., myeloproliferative disorders (MPDs)]. Distinguishing benign reactive processes that result in increases in hematopoietic cells from true neoplastic conditions can be difficult. Reactive abnormalities including leukemoid reactions in the peripheral blood and splenomegaly secondary to EMH can arise from a variety of conditions including infection, drug/toxin exposure, and nutritional deficiencies, all of which should be carefully considered before definitive characterization of a neoplastic condition is made. Useful distinguishing features between benign and neoplastic processes include the presence of monoclonality (see above), transplantability of the hematopoietic process, absence/presence of other inflammatory lesions (e.g., dermatitis), and the persistence/progression of the blood or splenic abnormalities (e.g., via serial nonlethal eye bleeds or physical exam/noninvasive imaging studies).

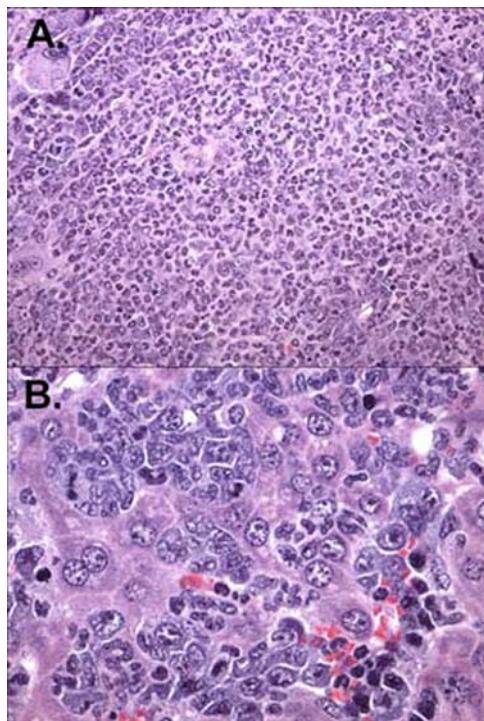
Representative examples for some disease subcategories are referred to within the Bethesda proposals (Kogan et al. 2002) and can be found in other resources in the literature (Fredrickson and Harris 2000). Our discussion will center on genetic models utilizing both transgenic and BMT platforms that have modeled leukemogenic alleles associated with human hematopoietic disease or genes important for normal hematopoiesis. The illustrations of some of their particular and salient features will serve as a useful reference for investigators as new models are evaluated.

### ***3.7.1 Myeloproliferative Disorders***

As the name implies, these proliferative stem cell disorders are characterized by increased bone marrow production of mature nonlymphoid hematopoietic elements manifesting in characteristic peripheral blood and laboratory findings. These disorders classically include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and CIMF, and more loosely include chronic eosinophilic leukemia (CEL), chronic myelomonocytic leukemia (CMML), and systemic mastocytosis (SM). Although these entities represent unique clinicopathologic disorders, the identified causative genetic mutations in all MPDs result in constitutive activation of tyrosine kinase (TK) signaling (Bartram et al. 1983; Golub et al. 1994; Longley et al. 1996; Cools et al. 2003a; Levine et al. 2005; Lee et al. 2007). Modeling these disease alleles in mice has been instrumental in understanding their contribution to both normal biology and disease pathogenesis.

*BCR-ABL*. The BCR-ABL TK fusion oncoprotein is the genetic lesion that defines CML, the prototypic disease for modern molecular oncology. It was the first genetic abnormality identified in human myeloproliferative disease and thus is undoubtedly the most studied and well characterized TK fusion protein. Numerous murine models have demonstrated that BCR-ABL expression is sufficient to initiate the development of a variety of leukemias (e.g., B-ALL, T-ALL, CML, and histiocytic sarcomas), the phenotype of which is dependent upon a number factors including promoter choice, murine strain, oncogenic isoform of BCR-ABL, and experimental conditions [for further review see, Wong and Witte (2001)]. Daley and colleagues were one of the first groups to report a murine bone marrow transplant model capable of faithfully generating a CML-like disease with characteristics of chronic phase human CML (Daley et al. 1990). In these retroviral transplant models, BCR-ABL typically induces an MPD-like myeloid leukemia characterized by leukocytosis with evidence of normal maturation but without significantly increased numbers of immature/blast forms, similar to the peripheral blood findings in human CML. In addition, peripheral basophilia, a distinct feature of human CML, is observed (Daley et al. 1990). As with human CML, marked splenomegaly is typically present. Histopathologic examination of diseased spleen tissue sections reveals effacement of normal splenic architecture with marked expansion of splenic red pulp that is comprised of an infiltrate of maturing granulocytes and concomitant reduction in lymphocytes (Fig. 3.4). This cellular expansion in the murine spleen is often accompanied by a large component of maturing erythroid precursors, which can also be observed as splenic EMH in human BCR-ABL-associated CML. The bone marrow tissue sections show a marked myeloid predominance with normal maturation and are markedly hypercellular with both compression of sinuses and leukemic cells that frequently extend beyond the medullary cavity into surrounding skeletal muscle and soft tissue. The infiltrate extensively involves the liver parenchyma contributing to the invariable hepatomegaly observed (Fig. 3.4). Myeloid infiltrates can also often be found in lymph nodes as well as mucosal-associated lymphoid tissue (e.g., Peyer's patches). Flow cytometric analysis in support of the morphologic findings for a MPD includes a significantly increased Gr1+/Mac1+ population (mature granulocyte population) in both bone marrow and spleen without an increase in the progenitor cell markers CD117 (c-kit) or CD34. As expected, there is a diminution in lymphoid populations as assessed by B- or T-cell markers (e.g., B220 and Thy1.2, respectively). Importantly, however, in time the emergence of acute leukemia (always of lymphoblastic type) is generally noted, equivalent to the blast crisis that is inevitable in human CML (Daley et al. 1990). Interestingly, the CML-like disease cannot be transplanted to secondary recipient mice, whereas the acute lymphoblastic leukemia is transplantable (Daley et al. 1990). This finding suggests that the BCR-ABL-infected stem cell may represent only a minority of stem cells, making transplantation inefficient. It is critical to note that the MPD phenotypes that are observed in these retroviral models are highly dependent on the infection conditions and, largely, the murine genetic background (Elefanti and Cory

**Fig. 3.4** Retroviral transduction of BCR–ABL in a bone marrow transplantation model. (A) Spleen from C57BL/6 recipient mouse receiving BCR–ABL transduced marrow showing effacement of the architecture by a diffuse proliferation of maturing myeloid elements. Erythropoiesis is not prominent in this case. Overall, this tumor would be classified as a myeloproliferative disorder-like myeloid leukemia (hematoxylin and eosin stains, 200 $\times$  magnification). (B) Liver parenchyma from the same animal showing an infiltrate of both maturing myeloid and erythroid elements (hematoxylin and eosin stains, 400 $\times$  magnification). (See color insert)



1992). The same is true of transgenic models of BCR–ABL-induced disease that have resulted in variable phenotypes dependent on the promoters driving BCR–ABL expression. These typically resulted in an immature lymphoid leukemia phenotype; however, transgenic mice expressing p210<sup>bcr/abl</sup> driven by the promoter of the *tec* gene that is expressed in the hematopoietic stem cell compartment has yielded a disease phenotype that is nearly identical to the MPD seen in the bone marrow transplant models (Honda et al. 1998).

**TEL–PDGFR $\beta$ .** Fusion of the PDGFR $\beta$  TK receptor to TEL was originally discovered as a consequence of a t(5;12)(q33;p13) balanced translocation identified in patients with CMML (Golub et al. 1994). Like BCR–ABL, TEL–PDGFR $\beta$  is a constitutively activated TK that also causes a fatal MPD-like myeloid leukemia in murine BMT models (Tomasson et al. 1999). This disease is characterized by marked leukocytosis with neutrophilia, splenomegaly, and EMH in hepatic and pulmonary parenchyma. The splenic red pulp is diffusely effaced by maturing myeloid elements, and these animals show a much lesser degree of erythroid hyperplasia than those of the BCR–ABL retrovirally transduced mice. Bone marrow histology reveals marked hypercellularity and a diffuse proliferation of mature myeloid forms, resembling a sea of neutrophilic forms. As with BCR–ABL-induced MPD-like myeloid leukemias, this MPD-like disease is also not transplantable into secondary recipients. Blasts in the

bone marrow and spleen are variable from animal to animal, but never comprise >20% of the total cellularity. Although human TEL–PDGFR $\beta$  CMML cases are often associated with eosinophilia, this feature is not prominent in the murine transplant models. Similar to BCR–ABL, transgenic models in which TEL–PDGFR $\beta$  is driven by a lymphoid-specific immunoglobulin enhancer-promoter induce both T- and B-cell lymphoblastic lymphomas. These animals developed diffuse lymphadenopathy and mediastinal masses, and necroscopic findings supported an immature neoplasm of lymphoid derivation (Tomasson et al. 2001).

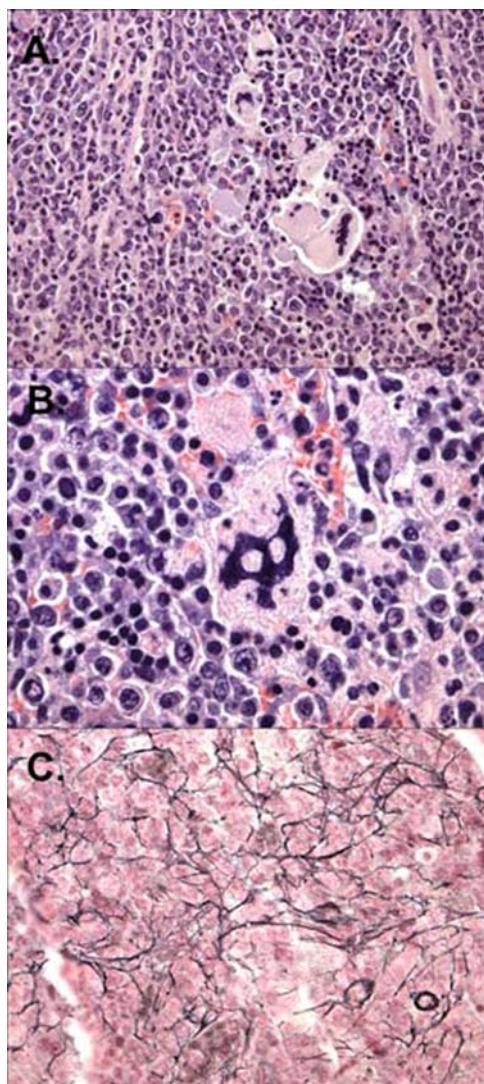
**FIP1L1–PDGFR $\alpha$ .** Hypereosinophilic syndrome (HES)/CEL is a rare hematologic disorder characterized by sustained overproduction of eosinophils in the bone marrow and peripheral eosinophilia resulting in tissue infiltration and organ damage. The genetic etiology of HES/CEL was unclear until the recent discovery of the FIP1L1–PDGFR $\alpha$  TK fusion protein associated with the disease (Cools et al. 2003a). The fusion gene that codes for this oncogenic fusion protein results as a consequence of an interstitial deletion on chromosome 4q12 and causes constitutive activation of the PDGFR $\alpha$  receptor (Cools et al. 2003a). In a murine transplant model, FIP1L1–PDGFR $\alpha$  causes a fatal MPD-like myeloid leukemia, similar to TEL–PDGFR $\beta$ -induced myeloproliferative disease (Cools et al. 2003b). Although frequent eosinophils were observed in this model (ranging from 5 to 20% in the peripheral blood), all cells within the granulocytic lineage were increased with the most significant increase in the neutrophils. In a subsequent study, Yamada and colleagues were able to demonstrate that retroviral transduction of FIP1L1–PDGFR $\alpha$  into a transgenic CD2-IL-5 mouse strain is able to induce a MPD-like disease with profound eosinophilia, indicating that FIP1L1–PDGFR $\alpha$  can cooperate with IL-5 to induce murine HES/CEL (Yamada et al. 2006). This study is a reminder that the biological context of these oncogenic fusion genes is critical to recapitulate more faithfully the human disease phenotypes. Of note, ancillary studies used to characterize and quantitate the increased eosinophil population in this report included immunoperoxidase studies for major basic protein (MBP) and flow cytometry identifying CCR3 $^+$ /CD11b $^{+/\text{low}}$  and Siglec-F $^+$ /CD11b $^{+/\text{low}}$  eosinophil populations. Interestingly, Charcot–Leyden crystals (comprised of MBP), commonly observed in human cases of HES/CEL, were not observed or reported in either study.

**JAK2V617F.** The recent discovery of a somatic activating mutation in Janus kinase 2 (*JAK2V617F*), which is present in nearly all patients with PV and a large proportion of patients with ET and primary myelofibrosis, has significantly influenced the diagnosis and classification of these similar, but clinicopathologically distinct disorders (Baxter et al. 2005; James et al. 2005; Kralovics et al. 2005; Levine et al. 2005; Levine et al. 2007). PV is a panmyelosis resulting in the accumulation of RBCs but also involves increases in platelets and WBCs and their progenitors. It is the increase in red cell mass that is the clinical hallmark of this disease, an expansion that is independent of the normal pathways that regulate erythropoiesis and not related to other identifiable causes

of secondary erythrocytosis. In retroviral bone marrow transplant models, JAK2V617F, but not wild-type JAK2, induces an MPD-like disorder (Bumm et al. 2006; Lacout et al. 2006; Wernig et al. 2006; Zaleskas et al. 2006). Significantly, in contrast to the majority of other constitutively activated TKs implicated in human MPDs that induce a predominantly neutrophilic MPD, a unique and prevailing feature of JAK2V617F expression in murine transplant models is the presence of a marked erythrocytosis, similar to the presentation in the human form of the disease. Although leukocytosis is seen with JAK2V617F expression, data from these models indicate that this feature is primarily observed in the BALB/c but not in the C57Bl/6 background, suggesting that host genetic modifiers likely play a role in disease phenotype (Wernig et al. 2006; Zaleskas et al. 2006). Moreover, while thrombocytosis is commonly observed in both PV and ET, this finding has not been prominently observed in JAK2V617F transplant models. Interestingly, Lacout and colleagues described one subset of animals in their studies with reduced JAK2V617F expression that developed a transient thrombocytosis (Lacout et al. 2006). Although thrombocytosis is not generally observed, these animals exhibit panmyelosis, including megakaryocytic hyperplasia with evidence of a megakaryocytic maturation defect as determined by ploidy analysis (Fig. 3.5A,B) (Wernig et al. 2006). Megakaryocytes from JAK2V617F-transplanted animals are large and dysplastic, frequently occurring singly or in clusters. They display deeply lobulated and hyperlobated nuclei, as well as abnormal patterns of chromatin clumping with emperipoleisis of neutrophils in megakaryocyte cytoplasm (Fig. 3.5A,B). These morphologic features are not typically observed in many of the other TK MPD mouse models and appear unique to the JAK2V617F abnormality. Myelofibrosis is a feature of all forms of MPDs in humans but is most prominent in late phase PV and CIMF. Myelofibrosis is also present in the JAK2V617F murine models and is highlighted by reticulin, silver, or trichrome stains (Fig. 3.5C). The degree of myelofibrosis also may be dependent upon the mouse strain employed (e.g., BALB/c vs. C57Bl/6) (Bumm et al. 2006; Lacout et al. 2006; Wernig et al. 2006; Zaleskas et al. 2006). Similar pathologic features from murine transplant models have been observed with other JAK2 mutant alleles including JAK2 exon 12 mutations (e.g., JAK2K539L and JAK2T875N) (Scott et al. 2007). These mutations have been described in patients with PV and idiopathic erythrocytosis, the JAK2T875N mutation being originally identified in an acute megakaryocytic leukemia (AMKL) cell line (Mercher et al. 2006). Finally, murine BMT assays modeling TEL–JAK2 fusions proteins originally observed in human cases of atypical CML, B-ALL, and T-ALL (Lacroix et al. 1997; Peeters et al. 1997) also develop a fatal mixed myeloproliferative and T-cell lymphoproliferative disorder. Interestingly, the features of MPD are more similar to TEL–PDGFR $\beta$  and FLT3–ITD (internal tandem duplication) BMT models (see below) with no evidence of polycythemia (Schwaller et al. 1998), suggesting that not all forms of constitutively active JAK2 are the same.

*MPLW515L/K.* A search for other mutations in cases of JAK2V617F-negative PV, ET, and CIMF revealed novel somatic activating alleles (MPLW515L

**Fig. 3.5** Retroviral transduction of JAK2V617F in a bone marrow transplantation model. (A) Histology of Jak2V617F-transduced BALB/c mice showing pathology in representative sections of spleen revealing marked leukocytosis consisting predominantly of maturing myeloid elements and a prominent population of megakaryocytes, including large, atypical forms occurring in occasional clusters (hematoxylin and eosin stains, 200 $\times$  magnification). (B) Large abnormal megakaryocyte from the same animal showing bizarre nuclear convolutions and emperipoleisis of neutrophils in the megakaryocyte cytoplasm. (hematoxylin and eosin stains, 400 $\times$  magnification). (C) Bone marrow from the same animal stained with reticulin stain showing markedly increased numbers of reticulin fibers, indicative of myelofibrosis (400 $\times$  magnification). (See color insert)

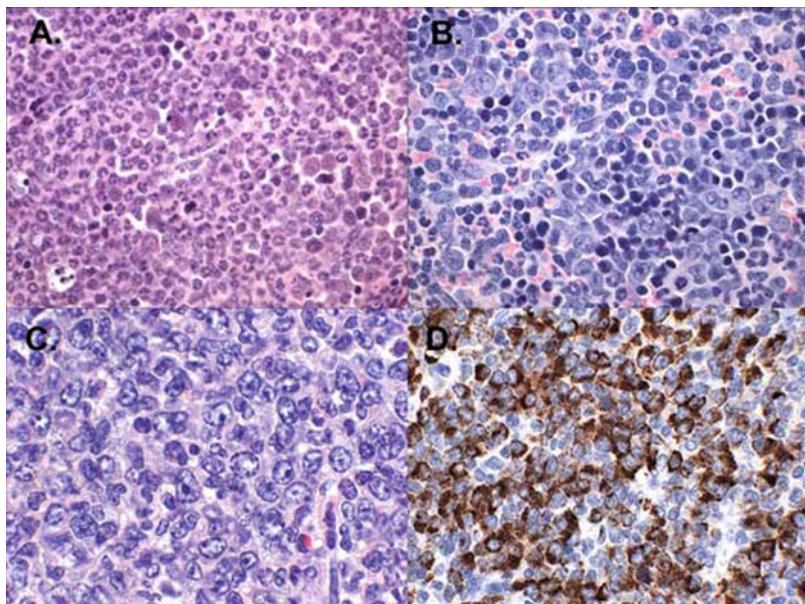


and W515K) in the thrombopoietin TK receptor (Pardanani et al. 2006; Pikman et al. 2006). Like the JAK2V617F mutation, expression of MPLW515L induces a fully penetrant fatal MPD-like myeloid leukemia in a murine bone marrow transplant model. Unlike JAK2V617F, expression of the activated MPL allele induces a marked thrombocytosis (platelet count from 1.9 to  $4.0 \times 10^{12}/\text{L}$ ) but without polycythemia (Pikman et al. 2006). Interestingly, analysis in the MPLW515L model indicated that there was no effect on megakaryocyte ploidy, in contrast to the JAK2V617F allele. Thus, despite sharing similar histopathologic features of JAK2V617F-induced myeloproliferative disease

in the BALB/c background, additional investigation will be required to fully understand these phenotypic differences.

*RAS mutations.* Downstream from many of the TKs described thus far, Ras proteins are a family of guanine nucleotide-binding proteins that cycle between an inactive GDP-bound state and an active GTP-bound state. Oncogenic *ras* alleles are common in myeloid malignancies including acute myeloid leukemia (AML), CMML, and juvenile myelomonocytic leukemia (JMML). As with BCR-ABL and activating FLT3 mutations (see below), the disease phenotype, penetrance, and latency in murine models studying leukemogenic ras mutations can be quite variable (Dunbar et al. 1991; Hawley et al. 1995; Darley et al. 1997; MacKenzie et al. 1999). Conditional activation of an oncogenic K-ras allele (K-Ras<sup>G12D</sup>) under the control of its endogenous promoter induces a rapidly fatal MPD-like myeloid leukemia characterized by leukocytosis and marked splenomegaly (Braun et al. 2004; Chan et al. 2004). Histopathologic analysis of the marrows revealed a predominantly granulocytic/monocytic proliferation, but with subsets of mice exhibiting a striking erythroid expansion in the spleen (Fig. 3.6A,B) (Braun et al. 2004; Chan et al. 2004). Livers also showed periportal and perivascular infiltration by mature myeloid forms. Overall, the hematopoietic phenotype was comparable to myeloproliferative diseases induced in murine transplant models of BCR-ABL and TEL-PDGFR $\beta$  (Braun et al. 2004; Chan et al. 2004). Interestingly, subsets of these mice also developed thymic T-cell lymphoblastic lymphomas and nodal hyperplasia, distinct from the MPD-like leukemia. Other nonhematopoietic pathological findings in this conditional K-ras<sup>G12D</sup> model included squamous papillomas involving the anal and vulvo-vaginal skin, ear, esophageal, and oral mucosa as well as adenomas of the lung (Chan et al. 2004). Similar to cooperative models with FLT3-ITD and PML-RAR $\alpha$  (see below), transgenic animals expressing both endogenous K-ras<sup>G12D</sup> and PML-RAR $\alpha$  induces an acute promyelocytic leukemia (APML) with an incomplete penetrance (Chan et al. 2006). These tumors are characterized by expansion of splenic red pulp and bone marrow by an increased proportion of immature myeloid forms most consistent with features of an acute leukemia. Immunohistochemical analysis of these cells confirmed MPO-expressing myeloid forms, similar to findings in other murine models of APML-like disease (Fig. 3.6C,D).

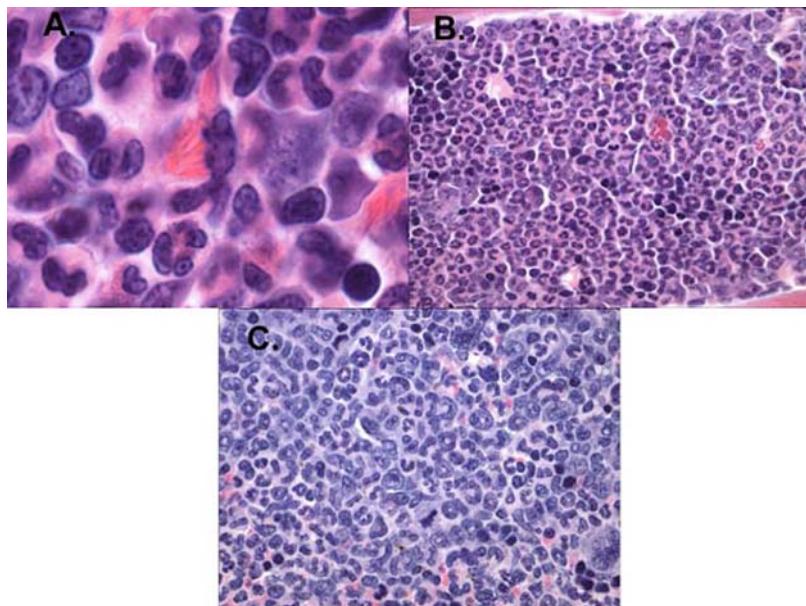
*SHP2 mutations.* The SH2-containing tyrosine phosphatase SHP2 (*PTPN11*) is essential for normal activation of the Ras-Erk signaling in most receptor TK and cytokine signaling pathways and is the first protein-tyrosine phosphatase (PTP) implicated in leukemogenesis. Germline mutations in *SHP2* cause the autosomal dominant disorder Noonan syndrome (NS), and *SHP2* mutations have been reported in approximately 35% of JMMLs as well as in smaller percentages of pediatric AML and MDS (Loh et al. 2004; Tartaglia et al. 2004), B-ALL (Tartaglia et al. 2004), and adult AML (Bentires-Alj et al. 2004). The Shp2 mutation *PTPN11*<sup>D61G</sup>, associated with NS, induces a well-tolerated mild myeloproliferative syndrome in animals heterozygous (*PTPN11*<sup>D61G/+</sup>) for the mutation in a murine knock-in model (Araki et al.



**Fig. 3.6** Conditional activation of an oncogenic K-ras allele ( $K\text{-Ras}^{G12D}$ ) in a mixed BALB/c, C57BL/6, and 129 v/Jae genetic background. (A) Bone marrow and (B) spleen sections reveal a marked maturing myeloid proliferation consistent with a myeloproliferative disorder-like myeloid leukemia (hematoxylin and eosin stains,  $400\times$  magnification). (C) When a transgenic mouse expressing PML–RAR $\alpha$  is crossed with oncogenic K-ras expressing mouse, a short latency, highly penetrant acute promyelocytic leukemia is seen. A representative leukemia within a spleen is pictured here, demonstrating round to irregular nuclei, dispersed chromatin and small amounts of eosinophilic cytoplasm (hematoxylin and eosin stains,  $400\times$  magnification). (D) Myeloperoxidase immunohistochemistry of the tumor in C is pictured, revealing intense staining for myeloperoxidase consistent with acute promyelocytic leukemia. (See color insert)

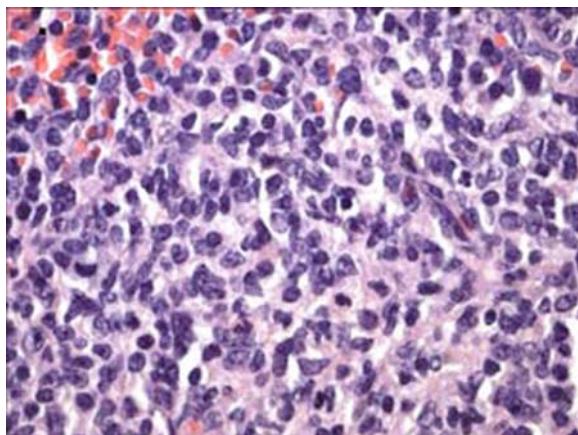
2004). BM and spleen tissue sections demonstrated a mild mature myeloid hyperplasia. Also noted in the BM of these animals were frequent pseudo-Gaucher-like cells filled with crystal-like eosinophilic material, which we have also observed in a number of other myeloproliferative mouse models (Fig. 3.7A). In retroviral transplant models, Shp2 mutations primarily associated with JMML and other hematopoietic neoplasms ( $PTPN11^{E76K}$  or  $D61Y$ ) induce a fatal MPD-like myelomonocytic leukemia (similar to that seen in JMML), as well as T-cell acute lymphoblastic leukemia/lymphoma (Mohi et al. 2005) (Fig. 3.7B,C). Importantly, the findings in these oncogenic forms of PTPN11 are similar to those seen in the activated  $K\text{-ras}^{G12D}$  knock-in mice (Braun et al. 2004; Chan et al. 2004), underscoring the importance of dysregulated RAS signaling in leukemia.

**FLT3 mutations.** Activating mutations in the FLT3 receptor TK constitute one of the most common genetic lesions in patients with AML and are comprised of two broad classes of mutations, namely ITDs mutations within the



**Fig. 3.7** Models of mutant SHP2 disease. (A) Marrow from a knock-in mouse model of the mutant SHP2 D61G associated with Noonan syndrome. These mice demonstrate a myeloid proliferation and reactive pseudo-Gaucher cells filled with crystal-like eosinophilic material (hematoxylin and eosin stains, 1000 $\times$  magnification). (B) Marrow and (C) spleen from recipient mice transplanted with retrovirally transduced bone marrow expressing the SHP2 mutations D61Y and E76K, found only in juvenile myelomonocytic leukemia (JMML) and other neoplasms. Note the prominent marrow hypercellularity and compression of the sinuses in the bone marrow with a mature myeloid (granulocytic) predominance and massive splenomegaly showing infiltration of spleen with myeloid elements (hematoxylin and eosin stains, 400 $\times$  magnification). (See color insert)

juxtamembrane domain and activation loop (AL) mutations of the TK domain. FLT3 mutations are commonly present in association with other chromosomal translocations in AML (i.e., PML–RAR $\alpha$  and AML–ETO), suggesting the need for cooperativity among genetic lesions for the development of AML. This is supported by murine BMT models in which FLT3–ITD causes a fatal MPD-like myeloid leukemia with marked neutrophilia, similar to other constitutively active TK MPD models (e.g., TEL–PDGFR $\beta$ ), but not AML (Kelly et al. 2002b). FLT3–ITD also induces a myeloproliferative syndrome with thrombocythemia in transgenic mouse models under the control of the *vav* panhematopoietic promoter (Lee et al. 2005) and myeloproliferative disease with features reminiscent of human CMML when expressed under an endogenous murine *Flt3* promoter (Lee et al. 2007) (Fig. 3.8). Histopathologic sections of spleen in the latter model system shows that the red pulp is expanded largely by maturing myeloid and erythroid elements in both hetero- and homozygous animals but also display a prominent white pulp expansion comprised largely



**Fig. 3.8** FLT3 model of disease. A myeloproliferative disease with features reminiscent of human chronic myelomonocytic leukemia (CML) is shown in this model of homozygous FLT3-ITD expressed in C57BL/6 mice under an endogenous murine *Flt3* promoter. In this representative section of spleen, splenic red pulp is expanded by both maturing myeloid and erythroid elements with enlarged white pulp comprised of intermediate-sized mononuclear cell infiltrate with irregular nuclei and ample eosinophilic cytoplasm (pictured here) that have a myelomonocytic phenotype by flow cytometry (hematoxylin and eosin stains, 400 $\times$  magnification). (See color insert)

of intermediate-sized monocytic cells with moderate amounts of pale cytoplasm. Importantly, neither of these *Flt3* model systems is sufficient to induce acute leukemia. Support for the requirement of additional mutation(s) to induce AML comes from murine models combining both the FLT3-ITD and the PML-RAR $\alpha$  fusion proteins, in which transplantation of bone marrow cells transgenic for the PML-RAR $\alpha$  fusion protein and transduced with FLT3-ITD are able to induce a short latency fatal disease resembling APML (Kelly et al. 2002a). Interestingly, under comparable BMT conditions in the BALB/c background, FLT3-AL mutations have been shown to induce longer latency acute lymphoid leukemias/lymphomas (T-ALL and B-ALL) (Grundler et al. 2005), a phenotype that has also been seen in the *vav* FLT3-ITD transgenic animal model (Lee et al. 2005), as well as in retroviral models with FLT3-ITD in a mixed B6/C3H murine background (Kelly et al. 2002a). The presence of FLT3-associated lymphoid leukemias in mice is consistent with reports of activating FLT3 mutations in human acute lymphoid malignancies (Armstrong et al. 2003; Armstrong et al. 2004; Paietta et al. 2004).

### 3.7.2 Acute Myeloid Leukemia

AML is characterized by impaired hematopoietic differentiation, in contrast to MPD where there is normal myeloid maturation. Careful examination of

peripheral blood smear, cytologic preparations, and tissue sections is required to discern between these two, often closely related, disease categories. In close parallel with the current WHO classification, the Bethesda proposals employ a percentage cutoff of at least 20% immature forms/blast (peripheral blood, spleen, or bone marrow) to warrant classification as AML (Kogan et al. 2002). Nonlymphoid immature forms/blast are typically medium to large in size with basophilic cytoplasm (occasionally with azurophilic granules), round to irregular nuclei with fine chromatin and nucleoli, whereas lymphoblasts will more often be relatively smaller in size with scant amounts of cytoplasm and nucleoli with a more central location. In our experience, Auer rods are never observed in murine myeloblasts, even in APL-like disease. Morphologic determination of immature myeloid blasts is also often aided by the recognition of any accompanying cell populations that are readily recognizable as maturing myeloid forms (e.g. ring granulocytic forms). Ancillary data in support of an acute nonlymphoid leukemia should demonstrate immunophenotypic evidence of myeloid origin (e.g., Mac-1 and MPO), expression of markers typically restricted to immature hematopoietic cells including c-kit, CD34, and Sca-1 and without evidence of lymphoid markers (e.g., B220, CD19, and Thy1.2).

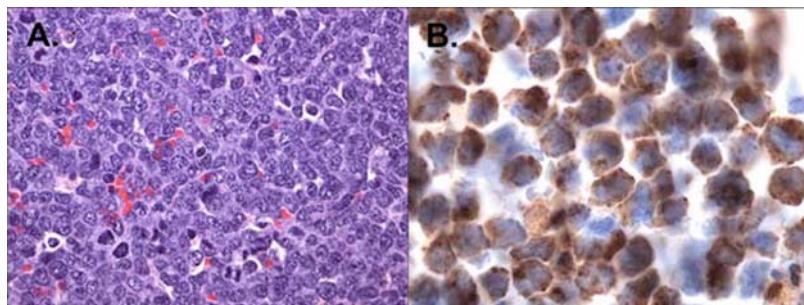
### ***3.7.3 AML Associated with Recurrent Genetic Abnormalities***

Recurrent cytogenetic abnormalities associated with human AML include PML–RAR $\alpha$ , AML1–ETO, CBF $\beta$ –MYH11, and MLL (11q23) rearrangements and currently comprises a distinct category within the WHO classification. While it is clear that these genetic alleles contribute to disease pathogenesis, data from numerous murine systems modeling these mutations suggest that in and of themselves, they are insufficient to induce AML (Brown et al. 1997; Grisolano et al. 1997; Castilla et al. 1999; Rhoades et al. 2000; Higuchi et al. 2002; Westervelt et al. 2003; Kuo et al. 2006). For example, although transgenic models of PML–RAR $\alpha$  (e.g., cathepsin G-PML–RAR $\alpha$  and hMRP8 PML–RAR $\alpha$ ) induce APL-like disease in mice, this develops after a long latency with incomplete penetrance and is preceded by a pre-leukemic phase characterized by a myeloproliferative syndrome (Brown et al. 1997; Grisolano et al. 1997). Although higher penetrance of APL-like disease is observed in a transgenic cathepsin G-PML/RAR $\alpha$  knock-in model, the long latency indicates that PML–RAR $\alpha$  expression alone is insufficient for APL development and that additional genetic events are required. Moreover, development of AML in CBF $\beta$ –MYH11 and AML1–ETO transgenic mice requires the addition of ENU mutagenesis, further supporting this notion (Castilla et al. 1999; Higuchi et al. 2002). Indeed, substantial epidemiologic evidence and data from additional murine models support this “two-hit” model of AML which requires the cooperativity of a constitutive proliferative signal

(e.g., FLT3-ITD, K-RAS, and BCR-ABL) and a class of mutations typified by transcription factor mutations or fusion proteins required to impair hematopoietic differentiation (e.g., PML-RAR $\alpha$ , MOZ-TIF2, and AML1-ETO) (Deguchi and Gilliland 2002; Kelly and Gilliland 2002). In addition to the FLT3-ITD-PML-RAR $\alpha$  (Kelly et al. 2002a) and K-Ras $G12D$ -PML-RAR $\alpha$  (Chan et al. 2006) APL models described above, cooperativity has also been demonstrated in murine BMT experiments with BCR-ABL and NUP98-HOXA9 (Dash et al. 2002), TEL-PDGFR $\beta$  and AML1-ETO (Grisolano et al. 2003), and FLT3-ITD and AML1-ETO (Schessl et al. 2005).

**MOZ-TIF2.** The MOZ-TIF2 fusion protein is associated with AML involving the inv(8)(p11q13), a relatively rare cytogenetic abnormality that is frequently associated with acute myelomonocytic leukemias. In a mouse BMT model, retrovirally transduced MOZ-TIF2 is able to induce a fatal and transplantable AML with a relatively long latency (~3–4 months), suggesting that additional cooperating mutations are required for disease development (Deguchi et al. 2003). In addition to hepatosplenomegaly, mice with MOZ-TIF2-induced AML frequently demonstrate other necroscopic findings including generalized lymphadenopathy (axillary, mesenteric, para-aortic, and femoral nodes) and leukemic infiltration into cervical lymph nodes and salivary glands that often spread into the craniofacial region. Left-shifted leukocytosis with immature myeloid and monocytic forms, as well as leukemic blasts in the peripheral blood, can be seen. More homogenous populations of immature myeloid cells and blasts and limited maturation is observed in tissue sections of diseased organs (e.g., spleens, lymph nodes, BM, and liver). Flow cytometric analysis of EGFP-gated cells (a particular advantage of this model which employed the use of an MSCV-MOZ-TIF2-IRES-EGFP retroviral vector) demonstrated that MOZ-TIF2 expressing leukemic cells were Mac-1+, variable-to-low Gr-1+, variable c-kit+, and CD34-negative without B (CD19) or T-cell (Thy1.2) markers. These immunophenotypic features are consistent with the morphologic findings of a prominent immature myeloid population with partial granulocytic differentiation (Deguchi et al. 2003). The overall pathologic features share many in common to those seen in inv(8)(p11q13) human AML.

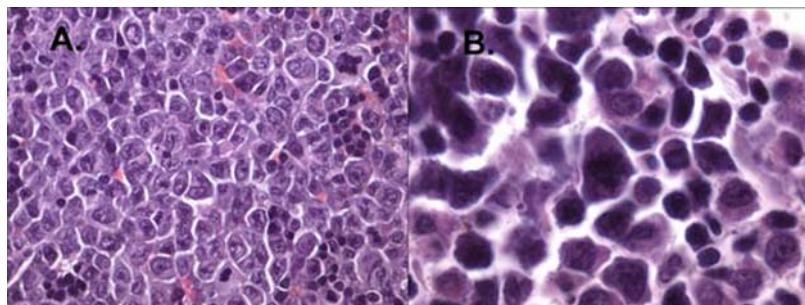
**PU.1.** The PU.1 transcription factor is a critical gene essential for normal hematopoietic development. It was first isolated as the product of the *Sfpi1* locus which was overexpressed as a result of a viral insertion leading to murine erythroleukemias (Moreau-Gachelin et al. 1988, 1996). Graded changes in PU.1 concentrations have been shown to have significant effects on hematopoietic lineage fate decisions (DeKoter and Singh 2000; Dahl et al. 2003) and has been examined in a unique PU.1 “knock-down” transgenic murine model. In this model, deletion of an important upstream regulatory element (URE) of the PU.1 gene creates a hypomorphic *Sfpi1* allele such that PU.1 expression is reduced to 20% of normal levels (Rosenbauer et al. 2004). Interestingly, these animals develop a fatal and transplantable AML with marked hepatosplenomegaly and expansion of immature myeloid cells and blasts (c-kit+, Mac1 $^{low}$ , Gr1 $^{low}$ , MPO positive) (Fig. 3.9) (Rosenbauer et al. 2004). Similar



**Fig. 3.9** Pu.1 model of acute myeloid leukemia. (A) Spleen from PU.1 knock-down C57BL/6 transgenic mouse showing a diffuse infiltrate of immature mononuclear cells with immunophenotypic features of acute myeloid leukemia (hematoxylin and eosin stains, 400 $\times$  magnification). (B) Myeloid derivation confirmed by immunohistochemistry for myeloperoxidase shown in this marrow specimen from the same mouse (1000 $\times$  magnification). (See color insert)

to PML/RAR $\alpha$  transgenic animals, AML in the PU.1 “knock-down” mice is preceded by a pre-leukemic phase characterized by a mature myeloid proliferation. Cytogenetic studies performed by FISH and SKY analysis showed monoclonality, illustrating the utility of these ancillary techniques in this regard. In addition, SKY analysis provides the potential for the identification of recurrent structural abnormalities that might cooperate with PU.1 “knock-down” in the development of AML (Rosenbauer et al. 2004).

**C/EBP $\alpha$ .** The CCAAT enhancer-binding protein alpha (C/EBP $\alpha$ ) is a hematopoietic transcription factor that is critical for the normal differentiation of myeloid progenitors. C/EBP $\alpha$  knockout animals demonstrate a lack of mature granulocytes (Zhang et al. 1997), and mutations within C/EBP $\alpha$  have been found in approximately 9% of AML patient samples (Nerlov 2004). To further highlight its crucial role in myeloid differentiation, a recent mouse transplantation model with expression of BCR-ABL into C/EBP $\alpha$ -null fetal liver cells was able to induce a fatal and transplantable acute erythroleukemia (Fig. 3.10), while BCR-ABL in fetal liver cells wild-type for C/EBP $\alpha$  produced a more typical fatal MPD-like leukemia (Wagner et al. 2006). In keeping with a diagnosis of acute erythroleukemia, the peripheral blood of BCR-ABL-C/EBP $\alpha$  $^{+/+}$  animals contained normoblasts and erythroblasts that comprised two-thirds of the nucleated cells with a significant number of proerythroblasts. Identical populations of erythroid precursors were observed in the BM and spleen. In addition to this predominant immature erythroid population, an increase in mast cells was identified and confirmed by immunohistochemistry for mast cell tryptase and toluidine blue stains. Flow cytometric analysis confirmed this diagnosis showing increased expression of the erythroid marker Ter119 and the immaturity marker c-kit and an absence of MPO by immunohistochemistry (Wagner et al. 2006). Erythroid maturation is accompanied by a decrease of CD71 expression (transferrin receptor) in Ter119 cells (Socolovsky



**Fig. 3.10** CEBP $\alpha$  knock-out model of acute erythroleukemia. (A) Spleen (400 $\times$  magnification) and (B) bone marrow (1000 $\times$  magnification) from a C57BL/6 CEBP $\alpha$  knock-out mouse transplanted with bone marrow retrovirally transduced by BCR-ABL. The infiltrate in both tissues is comprised of an immature population of large blast forms with round to irregular nuclei, containing ample basophilic cytoplasm consistent with proerythroblasts and a diagnosis of acute erythroleukemia. (See color insert)

et al. 2001), and flow cytometric analysis with these two markers demonstrated a marked increase in Ter119 $^{high}$ /CD71 $^{high}$  cells consistent with morphologic findings. Overall, this model supports the critical role for C/EBP $\alpha$  in driving differentiation down the myeloid pathway.

**CDX2 and CDX4.** The clustered homeobox (*HOX*) genes have been shown to play an important role in normal hematopoiesis, and global *HOX* gene dysregulation is found in both AML and ALL cases in humans (Frohling et al. 2007). As developmental regulators of *HOX* gene expression, the *CDX* family of nonclustered *HOX* genes (e.g., Cdx4 and Cdx1) also appears to play an important role in hematopoiesis during development (Davidson et al. 2003; Davidson and Zon 2006). Recently, the *CDX* genes have been shown to also be involved in acute leukemia. CDX2 is fused to the *ETV6* (TEL) gene on chromosome 12p13 in a patient with AML and a t(12;13)(p13;q12) translocation (Chase et al. 1999) and more recently has been shown to be expressed in leukemic cells in approximately 90% of patients with AML but not in hematopoietic stem and progenitor cells derived from normal individuals (Scholl et al. 2007). Interestingly, overexpression of full-length CDX2 alone but not the ETV6-CDX2 fusion protein in murine BMT assays induces a long latency, fully penetrant, and transplantable AML (Rawat et al. 2004; Bansal et al. 2006; Scholl et al. 2007). It is characterized by leukocytosis with frequent numbers of immature myeloid cells and blasts in the peripheral blood, BM, spleen, and liver and supported by ancillary flow cytometric findings and immunohistochemistry studies (Rawat et al. 2004; Bansal et al. 2006; Scholl et al. 2007). In a similar series of studies, expression of CDX4 in a murine BMT system also induces a long latency, transplantable, but partially penetrant AML which upon coexpression of the Hox cofactor Meis1a was rendered fully penetrant with a shorter latency of disease (Bansal et al. 2006).

### 3.8 Hematopoietic Disease Models and Molecularly Targeted Therapy

Modeling human hematopoietic mutations in mice provides invaluable preclinical platforms to assess the therapeutic efficacy of novel molecularly targeted compounds against hematopoietic neoplasms. From a histopathologic perspective, examination of hematopoietic organs (e.g., BM and spleen) from responsive drug-treated animals should reveal morphologic changes that demonstrate a return to more normal histology in these tissues, as well as a reduction/elimination of leukemic infiltrates in other often involved organs (e.g., liver and lungs). As always, additional ancillary diagnostic data including flow cytometric studies should also be employed to support these morphologic changes. Various murine models (including some of aforementioned reports) have been utilized to evaluate the therapeutic efficacy of small molecule compounds for a number of the activated TK alleles associated with human chronic MPDs (Cools et al. 2003b; Weisberg et al. 2005; Peng et al. 2007). In addition, these types of studies are particularly useful in evaluating the therapeutic efficacy of these compounds against drug-resistant alleles. Table 3.3 represents a sampling of reports examining the efficacy of targeted molecular therapeutic agents in nonlymphoid leukemia murine models. While not comprehensive, they provide a useful reference that will undoubtedly grow as novel compounds and new models emerge.

**Table 3.3** Murine models of hematopoietic disease and molecularly targeted therapy

Disease allele	Molecular compound(s) (murine disease)	Reference
BCR-ABL	Gleevec (STI571) (MPD)	(Wolff and Ilaria 2001)
	AMN107 (MPD)	(Weisberg et al. 2005)
	IPI-504, Gleevec (STI571) (MPD, B-ALL)	(Peng et al. 2007)
	Gleevec (STI571), Dasatinib (MPD, B-ALL)	(Hu et al. 2006)
	PKC412 (MPD)	(Cools et al. 2003b)
FIP1L1-PDGFR $\alpha$	AMN107 (MPD)	(Stover et al. 2005)
	Gleevec (STI571) (B-ALL)	(Tomasson et al. 1999)
	Gleevec (STI571) (MPD)	(Grisolano et al. 2003)
FLT3-ITD	AMN107 (MPD)	(Stover et al. 2005)
	PKC412 (MPD)	(Weisberg et al. 2002)
	CT53518 (MPD)	(Kelly et al. 2002c)
ZNF198-FGFR1	PKC412 (B, T-ALL)	(Lee et al. 2005)
	PKC412 (MPD)	(Chen et al. 2004)
FGFR3-TDII	PKC412 (B-ALL)	(Chen et al. 2005)
TEL-FGFR3	PKC412 (MPD)	(Chen et al. 2005)
TEL-PDGFR $\beta$ /AML1-ETO	Gleevec (STI571), SAHA, TSA (AML)	(Grisolano et al. 2003)
FLT3-ITD/PML-RAR $\alpha$	SU11657, ATRA (APL)	(Sohal et al. 2003)

### 3.9 Conclusions

Murine models of human hematopoietic neoplasms provide tremendous insights into the pathophysiology underlying these genetic disease alleles. While their expression in mice is frequently able to recapitulate many of the features of their human disease counterparts, it is important to recognize the wide variations in experimental systems that can significantly influence disease phenotype. Comprehensive pathologic evaluation is extremely valuable in making these distinctions and comparisons. While traditional histomorphologic review of diseased tissues and blood smears remains the primary method of evaluation, it is clear that with advances and more prevalent use of ancillary diagnostic techniques, our ability to evaluate murine hematopoietic tumors will continue to expand and, in turn, enhance our understanding of human hematopoietic disease.

## References

- Araki, T., Mohi, M.G., Ismat, F.A., Bronson, R.T., Williams, I.R., Kutok, J.L., Yang, W., Pao, L.I., Gilliland, D.G., Epstein, J.A., and Neel, B.G. 2004. Mouse model of Noonan syndrome reveals cell type- and gene dosage-dependent effects of Ptpn11 mutation. *Nat Med* **10**(8): 849–857.
- Armstrong, S.A., Kung, A.L., Mabon, M.E., Silverman, L.B., Stam, R.W., Den Boer, M.L., Pieters, R., Kersey, J.H., Sallan, S.E., Fletcher, J.A., Golub, T.R., Griffin, J.D., and Korsmeyer, S.J. 2003. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. *Cancer Cell* **3**(2): 173–183.
- Armstrong, S.A., Mabon, M.E., Silverman, L.B., Li, A., Gribben, J.G., Fox, E.A., Sallan, S.E., and Korsmeyer, S.J. 2004. FLT3 mutations in childhood acute lymphoblastic leukemia. *Blood* **103**(9): 3544–3546.
- Bansal, D., Scholl, C., Frohling, S., McDowell, E., Lee, B.H., Dohner, K., Ernst, P., Davidson, A.J., Daley, G.Q., Zon, L.I., Gilliland, D.G., and Huntly, B.J. 2006. Cdx4 dysregulates Hox gene expression and generates acute myeloid leukemia alone and in cooperation with Meis1a in a murine model. *Proc Natl Acad Sci USA* **103**(45): 16924–16929.
- Bartram, C.R., de Klein, A., Hagemeijer, A., van Agthoven, T., Geurts van Kessel, A., Bootsma, D., Grosveld, G., Ferguson-Smith, M.A., Davies, T., Stone, M., and et al. 1983. Translocation of c-abl oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. *Nature* **306**(5940): 277–280.
- Baxter, E.J., Scott, L.M., Campbell, P.J., East, C., Fourouclas, N., Swanton, S., Vassiliou, G.S., Bench, A.J., Boyd, E.M., Curtin, N., Scott, M.A., Erber, W.N., and Green, A.R. 2005. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* **365**(9464): 1054–1061.
- Bentires-Alj, M., Paez, J.G., David, F.S., Keilhack, H., Halmos, B., Naoki, K., Maris, J.M., Richardson, A., Bardelli, A., Sugurbaker, D.J., Richards, W.G., Du, J., Girard, L., Minna, J.D., Loh, M.L., Fisher, D.E., Velculescu, V.E., Vogelstein, B., Meyerson, M., Sellers, W.R., and Neel, B.G. 2004. Activating mutations of the noonan syndrome-associated SHP2/PTPN11 gene in human solid tumors and adult acute myelogenous leukemia. *Cancer Res* **64**(24): 8816–8820.
- Bogue, M.A., Grubb, S.C., Maddatu, T.P., and Bult, C.J. 2007. Mouse Phenome Database (MPD). *Nucleic Acids Res* **35**(Database issue): D643–649.

- Braun, B.S., Tuveson, D.A., Kong, N., Le, D.T., Kogan, S.C., Rozmus, J., Le Beau, M.M., Jacks, T.E., and Shannon, K.M. 2004. Somatic activation of oncogenic Kras in hematopoietic cells initiates a rapidly fatal myeloproliferative disorder. *Proc Natl Acad Sci USA* **101**(2): 597–602.
- Brown, D., Kogan, S., Lagasse, E., Weissman, I., Alcalay, M., Pelicci, P.G., Atwater, S., and Bishop, J.M. 1997. A PMLRAR $\alpha$  transgene initiates murine acute promyelocytic leukemia. *Proc Natl Acad Sci USA* **94**(6): 2551–2556.
- Brunnning, R.D., McKenna, R.W. 1994. Tumors of the bone marrow. Normal Bone Marrow. In: *Atlas of Tumor Pathology*. Third series. Washington, D.C.: Armed Forces Institutes of Pathology. pp. 1–18.
- Bumm, T.G., Elsea, C., Corbin, A.S., Loriaux, M., Sherbenou, D., Wood, L., Deininger, J., Silver, R.T., Druker, B.J., and Deininger, M.W. 2006. Characterization of murine JAK2V617F- positive myeloproliferative disease. *Cancer Res* **66**(23): 11156–11165.
- Castilla, L.H., Garrett, L., Adya, N., Orlic, D., Dutra, A., Anderson, S., Owens, J., Eckhaus, M., Bodine, D., and Liu, P.P. 1999. The fusion gene Cbf $\beta$ -MYH11 blocks myeloid differentiation and predisposes mice to acute myelomonocytic leukaemia. *Nat Genet* **23**(2): 144–146.
- Chan, I.T., Kutok, J.L., Williams, I.R., Cohen, S., Kelly, L., Shigematsu, H., Johnson, L., Akashi, K., Tuveson, D.A., Jacks, T., and Gilliland, D.G. 2004. Conditional expression of oncogenic K-ras from its endogenous promoter induces a myeloproliferative disease. *J Clin Invest* **113**(4): 528–538.
- Chan, I.T., Kutok, J.L., Williams, I.R., Cohen, S., Moore, S., Shigematsu, H., Ley, T.J., Akashi, K., Le Beau, M.M., and Gilliland, D.G. 2006. Oncogenic K-ras cooperates with PML-RAR alpha to induce an acute promyelocytic leukemia-like disease. *Blood* **108**(5): 1708–1715.
- Chase, A., Reiter, A., Burci, L., Cazzaniga, G., Biondi, A., Pickard, J., Roberts, I.A., Goldman, J.M., and Cross, N.C. 1999. Fusion of ETV6 to the caudal-related homeobox gene CDX2 in acute myeloid leukemia with the t(12;13)(p13;q12). *Blood* **93**(3): 1025–1031.
- Chen, J., DeAngelo, D.J., Kutok, J.L., Williams, I.R., Lee, B.H., Wadleigh, M., Duclos, N., Cohen, S., Adelsperger, J., Okabe, R., Coburn, A., Galinsky, I., Huntly, B., Cohen, P.S., Meyer, T., Fabbro, D., Roesel, J., Banerji, L., Griffin, J.D., Xiao, S., Fletcher, J.A., Stone, R.M., and Gilliland, D.G. 2004. PKC412 inhibits the zinc finger 198-fibroblast growth factor receptor 1 fusion tyrosine kinase and is active in treatment of stem cell myeloproliferative disorder. *Proc Natl Acad Sci USA* **101**(40): 14479–14484.
- Chen, J., Lee, B.H., Williams, I.R., Kutok, J.L., Mitsiades, C.S., Duclos, N., Cohen, S., Adelsperger, J., Okabe, R., Coburn, A., Moore, S., Huntly, B.J., Fabbro, D., Anderson, K.C., Griffin, J.D., and Gilliland, D.G. 2005. FGFR3 as a therapeutic target of the small molecule inhibitor PKC412 in hematopoietic malignancies. *Oncogene* **24**(56): 8259–8267.
- Cools, J., DeAngelo, D.J., Gotlib, J., Stover, E.H., Legare, R.D., Cortes, J., Kutok, J., Clark, J., Galinsky, I., Griffin, J.D., Cross, N.C., Tefferi, A., Malone, J., Alam, R., Schrier, S.L., Schmid, J., Rose, M., Vandenberghe, P., Verhoef, G., Boogaerts, M., Wlodarska, I., Kantarjian, H., Marynen, P., Coutre, S.E., Stone, R., and Gilliland, D.G. 2003a. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N Engl J Med* **348**(13): 1201–1214.
- Cools, J., Stover, E.H., Boulton, C.L., Gotlib, J., Legare, R.D., Amaral, S.M., Curley, D.P., Duclos, N., Rowan, R., Kutok, J.L., Lee, B.H., Williams, I.R., Coutre, S.E., Stone, R.M., DeAngelo, D.J., Marynen, P., Manley, P.W., Meyer, T., Fabbro, D., Neuberg, D., Weisberg, E., Griffin, J.D., and Gilliland, D.G. 2003b. PKC412 overcomes resistance to imatinib in a murine model of FIP1L1-PDGFR $\alpha$ -induced myeloproliferative disease. *Cancer Cell* **3**(5): 459–469.
- Dahl, R., Walsh, J.C., Lancki, D., Laslo, P., Iyer, S.R., Singh, H., and Simon, M.C. 2003. Regulation of macrophage and neutrophil cell fates by the PU.1:C/EBP $\alpha$  ratio and granulocyte colony-stimulating factor. *Nature immunology* **4**(10): 1029–1036.

- Daley, G.Q., Van Etten, R.A., and Baltimore, D. 1990. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* **247**(4944): 824–830.
- Darley, R.L., Hoy, T.G., Baines, P., Padua, R.A., and Burnett, A.K. 1997. Mutant N- RAS induces erythroid lineage dysplasia in human CD34+ cells. *J Exp Med* **185**(7): 1337–1347.
- Dash, A.B., Williams, I.R., Kutok, J.L., Tomasson, M.H., Anastasiadou, E., Lindahl, K., Li, S., Van Etten, R.A., Borrow, J., Housman, D., Druker, B., and Gilliland, D.G. 2002. A murine model of CML blast crisis induced by cooperation between BCR/ABL and NUP98/HOXA9. *Proc Natl Acad Sci USA* **99**(11): 7622–7627.
- Davidson, A.J., Ernst, P., Wang, Y., Dekens, M.P., Kingsley, P.D., Palis, J., Korsmeyer, S.J., Daley, G.Q., and Zon, L.I. 2003. cdx4 mutants fail to specify blood progenitors and can be rescued by multiple hox genes. *Nature* **425**(6955): 300–306.
- Davidson, A.J. and Zon, L.I. 2006. The caudal-related homeobox genes cdx1a and cdx4 act redundantly to regulate hox gene expression and the formation of putative hematopoietic stem cells during zebrafish embryogenesis. *Developmental biology* **292**(2): 506–518.
- Deguchi, K. and Gilliland, D.G. 2002. Cooperativity between mutations in tyrosine kinases and in hematopoietic transcription factors in AML. *Leukemia* **16**(4): 740–744.
- Deguchi, K., Ayton, P.M., Carapeti, M., Kutok, J.L., Snyder, C.S., Williams, I.R., Cross, N.C., Glass, C.K., Cleary, M.L., and Gilliland, D.G. 2003. MOZ-TIF2-induced acute myeloid leukemia requires the MOZ nucleosome binding motif and TIF2-mediated recruitment of CBP. *Cancer Cell* **3**(3): 259–271.
- DeKoter, R.P. and Singh, H. 2000. Regulation of B lymphocyte and macrophage development by graded expression of PU.1. *Science* **288**(5470): 1439–1441.
- Dunbar, C.E., Crosier, P.S., and Nienhuis, A.W. 1991. Introduction of an activated RAS oncogene into murine bone marrow lymphoid progenitors via retroviral gene transfer results in thymic lymphomas. *Oncogene research* **6**(1): 39–51.
- Elefanti, A.G. and Cory, S. 1992. Hematologic disease induced in BALB/c mice by a bcr-abl retrovirus is influenced by the infection conditions. *Mol Cell Biol* **12**(4): 1755–1763.
- Fredrickson, T.N. and Harris, A.W. 2000. *Atlas of Mouse Hematopathology*. Harwood Academic Publishers, Amsterdam.
- Frohling, S., Scholl, C., Bansal, D., and Huntly, B.J.P. 2007. HOX Gene Regulation in Acute Myeloid Leukemia. *Cell Cycle* **6**(18): e1–e5.
- Golub, T.R., Barker, G.F., Lovett, M., and Gilliland, D.G. 1994. Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell* **77**(2): 307–316.
- Grisolano, J.L., O’Neal, J., Cain, J., and Tomasson, M.H. 2003. An activated receptor tyrosine kinase, TEL/PDGF $\beta$ R, cooperates with AML1/ETO to induce acute myeloid leukemia in mice. *Proc Natl Acad Sci USA* **100**(16): 9506–9511.
- Grisolano, J.L., Wesselschmidt, R.L., Pelicci, P.G., and Ley, T.J. 1997. Altered myeloid development and acute leukemia in transgenic mice expressing PML-RAR alpha under control of cathepsin G regulatory sequences. *Blood* **89**(2): 376–387.
- Grundler, R., Miethling, C., Thiede, C., Peschel, C., and Duyster, J. 2005. FLT3-ITD and tyrosine kinase domain mutants induce 2 distinct phenotypes in a murine bone marrow transplantation model. *Blood* **105**(12): 4792–4799.
- Hawley, R.G., Fong, A.Z., Ngan, B.Y., and Hawley, T.S. 1995. Hematopoietic transforming potential of activated ras in chimeric mice. *Oncogene* **11**(6): 1113–1123.
- Harmening D.M. 2002. *Clinical Hematology and Fundamentals of Hemostasis*. F.A. Davis Co. Philadelphia, PA.
- Higuchi, M., O’Brien, D., Kumaravelu, P., Lenny, N., Yeoh, E.J., and Downing, J.R. 2002. Expression of a conditional AML1-ETO oncogene bypasses embryonic lethality and establishes a murine model of human t(8;21) acute myeloid leukemia. *Cancer Cell* **1**(1): 63–74.
- Hoff, J. 2000. Methods of blood collection in the mouse. *Lab Animal* **29**(10): 47–53.

- Honda, H., Hideaki, O., Suzuki, T., Takahashi, T., Witte, ON., Ozawa, K., Ishikawa, T., Yazaki, Y., Hirai, H. 1998. Development of acute lymphoblastic leukemia and myeloproliferative disorder in transgenic mice expressing p210<sup>ber/abl</sup>. A novel transgenic model for human Ph<sup>1</sup>-Positive leukemias. *Blood*, **91**(6) pp. 2067–2075.
- Hu, Y., Swerdlow, S., Duffy, T.M., Weinmann, R., Lee, F.Y., and Li, S. 2006. Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph+ leukemia in mice. *Proc Natl Acad Sci USA* **103**(45): 16870–16875.
- Jaffe, E.S., Harris, N.L., Stein, H., and Vardiman, J. 2001. *WHO Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. IARC Press, Lyons, France.
- James, C., Ugo, V., Le Couedic, J.P., Staerk, J., Delhommeau, F., Lacout, C., Garcon, L., Raslova, H., Berger, R., Bennaceur-Griscelli, A., Villeval, J.L., Constantinescu, S.N., Casadevall, N., and Vainchenker, W. 2005. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* **434**(7037): 1144–1148.
- Kelly, L.M. and Gilliland, D.G. 2002. Genetics of myeloid leukemias. *Annu Rev Genomics Hum Genet* **3**: 179–198.
- Kelly, L.M., Kutok, J.L., Williams, I.R., Boulton, C.L., Amaral, S.M., Curley, D.P., Ley, T.J., and Gilliland, D.G. 2002a. PML/RARalpha and FLT3-ITD induce an APL-like disease in a mouse model. *Proc Natl Acad Sci USA* **99**(12): 8283–8288.
- Kelly, L.M., Liu, Q., Kutok, J.L., Williams, I.R., Boulton, C.L., and Gilliland, D.G. 2002b. FLT3 internal tandem duplication mutations associated with human acute myeloid leukemias induce myeloproliferative disease in a murine bone marrow transplant model. *Blood* **99**(1): 310–318.
- Kelly, L.M., Yu, J.C., Boulton, C.L., Apatira, M., Li, J., Sullivan, C.M., Williams, I., Amaral, S.M., Curley, D.P., Duclos, N., Neuberg, D., Scarborough, R.M., Pandey, A., Hollenbach, S., Abe, K., Lokker, N.A., Gilliland, D.G., and Giese, N.A. 2002c. CT53518, a novel selective FLT3 antagonist for the treatment of acute myelogenous leukemia (AML). *Cancer Cell* **1**(5): 421–432.
- Kogan, S.C., Ward, J.M., Anver, M.R., Berman, J.J., Brayton, C., Cardiff, R.D., Carter, J.S., de Coronado, S., Downing, J.R., Fredrickson, T.N., Haines, D.C., Harris, A.W., Harris, N.L., Hiai, H., Jaffe, E.S., MacLennan, I.C., Pandolfi, P.P., Pattengale, P.K., Perkins, A.S., Simpson, R.M., Tuttle, M.S., Wong, J.F., and Morse, H.C., 3rd. 2002. Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice. *Blood* **100**(1): 238–245.
- Kralovics, R., Passamonti, F., Buser, A.S., Teo, S.S., Tiedt, R., Passweg, J.R., Tichelli, A., Cazzola, M., and Skoda, R.C. 2005. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* **352**(17): 1779–1790.
- Kuo, Y.H., Landrette, S.F., Heilman, S.A., Perrat, P.N., Garrett, L., Liu, P.P., Le Beau, M.M., Kogan, S.C., and Castilla, L.H. 2006. Cbf beta-SMMHC induces distinct abnormal myeloid progenitors able to develop acute myeloid leukemia. *Cancer Cell* **9**(1): 57–68.
- Lacout, C., Pisani, D.F., Tulliez, M., Gachelin, F.M., Vainchenker, W., and Villeval, J.L. 2006. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. *Blood* **108**(5): 1652–1660.
- Lacronique, V., Boureux, A., Valle, V.D., Poirel, H., Quang, C.T., Mauchauffe, M., Berthou, C., Lessard, M., Berger, R., Ghysdael, J., and Bernard, O.A. 1997. A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. *Science* **278**(5341): 1309–1312.
- Lee, B.H., Tothova, Z., Levine, R.L., Anderson, K., Buza-Vidas, N., Cullen, D.E., McDowell, E.P., Adelsperger, J., Fröhling, S., Huntly, B.J., Beran, M., Jacobsen, S.E., and Gilliland, D.G. 2007. FLT3 mutations confer enhanced proliferation and survival properties to multipotent progenitors in a murine model of chronic myelomonocytic leukemia. *Cancer Cell* **12**(4): 367–380.
- Lee, B.H., Williams, I.R., Anastasiadou, E., Boulton, C.L., Joseph, S.W., Amaral, S.M., Curley, D.P., Duclos, N., Huntly, B.J., Fabbro, D., Griffin, J.D., and Gilliland, D.G.

2005. FLT3 internal tandem duplication mutations induce myeloproliferative or lymphoid disease in a transgenic mouse model. *Oncogene* **24**(53): 7882–7892.
- Levine, R.L., Wadleigh, M., Cools, J., Ebert, B.L., Wernig, G., Huntly, B.J., Boggon, T.J., Wlodarska, I., Clark, J.J., Moore, S., Adelsperger, J., Koo, S., Lee, J.C., Gabriel, S., Mercher, T., D'Andrea, A., Frohling, S., Dohner, K., Marynen, P., Vandenberghe, P., Mesa, R.A., Tefferi, A., Griffin, J.D., Eck, M.J., Sellers, W.R., Meyerson, M., Golub, T.R., Lee, S.J., and Gilliland, D.G. 2005. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* **7**(4): 387–397.
- Levine, R.L., Pardanani, A., Tefferi, A., and Gilliland, D.G. 2007. Role of JAK2 in the pathogenesis and therapy of myeloproliferative disorders. *Nat Rev Cancer* **7**(9): 673–683.
- Liao, A.H., Li, C.H., Li, P.C., and Cheng, W.F. 2005. Non-Invasive Imaging of Small-Animal Tumors: High-Frequency Ultrasound vs. MicroPET. *Conf Proc IEEE Eng Med Biol Soc* **6**: 5695–5698.
- Loh, M.L., Vattikuti, S., Schubbert, S., Reynolds, M.G., Carlson, E., Lieuw, K.H., Cheng, J.W., Lee, C.M., Stokoe, D., Bonifas, J.M., Curtiss, N.P., Gotlib, J., Meshinchi, S., Le Beau, M.M., Emanuel, P.D., and Shannon, K.M. 2004. Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. *Blood* **103**(6): 2325–2331.
- Longley, B.J., Tyrrell, L., Lu, S.Z., Ma, Y.S., Langley, K., Ding, T.G., Duffy, T., Jacobs, P., Tang, L.H., and Modlin, I. 1996. Somatic c-KIT activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. *Nat Genet* **12**(3): 312–314.
- MacKenzie, K.L., Dolnikov, A., Millington, M., Shouman, Y., and Symonds, G. 1999. Mutant N-ras induces myeloproliferative disorders and apoptosis in bone marrow repopulated mice. *Blood* **93**(6): 2043–2056.
- Mebius, R.E. and Kraal, G. 2005. Structure and function of the spleen. *Nat Rev* **5**(8): 606–616.
- Mercher, T., Wernig, G., Moore, S.A., Levine, R.L., Gu, T.L., Frohling, S., Cullen, D., Polakiewicz, R.D., Bernard, O.A., Boggon, T.J., Lee, B.H., and Gilliland, D.G. 2006. JAK2T875N is a novel activating mutation that results in myeloproliferative disease with features of megakaryoblastic leukemia in a murine bone marrow transplantation model. *Blood* **108**(8): 2770–2779.
- Mohi, M.G., Williams, I.R., Dearolf, C.R., Chan, G., Kutok, J.L., Cohen, S., Morgan, K., Boulton, C., Shigematsu, H., Keilhack, H., Akashi, K., Gilliland, D.G., and Neel, B.G. 2005. Prognostic, therapeutic, and mechanistic implications of a mouse model of leukemia evoked by Shp2 (PTPN11) mutations. *Cancer Cell* **7**(2): 179–191.
- Moreau-Gachelin, F., Tavitian, A., and Tambourin, P. 1988. Spi-1 is a putative oncogene in virally induced murine erythroleukemias. *Nature* **331**(6153): 277–280.
- Moreau-Gachelin, F., Wendling, F., Molina, T., Denis, N., Titeux, M., Grimer, G., Briand, P., Vainchenker, W., and Tavitian, A. 1996. Spi-1/PU.1 transgenic mice develop multistep erythroleukemias. *Mol Cell Biol* **16**(5): 2453–2463.
- Morse, H.C., 3rd, Anver, M.R., Fredrickson, T.N., Haines, D.C., Harris, A.W., Harris, N.L., Jaffe, E.S., Kogan, S.C., MacLennan, I.C., Pattengale, P.K., and Ward, J.M. 2002. Bethesda proposals for classification of lymphoid neoplasms in mice. *Blood* **100**(1): 246–258.
- Nerlov, C. 2004. C/EBPalpha mutations in acute myeloid leukaemias. *Nat Rev Cancer* **4**(5): 394–400.
- Paietta, E., Ferrando, A.A., Neuberg, D., Bennett, J.M., Racevskis, J., Lazarus, H., Dewald, G., Rowe, J.M., Wiernik, P.H., Tallman, M.S., and Look, A.T. 2004. Activating FLT3 Mutations in CD117/KIT Positive T-Cell Acute Lymphoblastic Leukemias. *Blood* **104**(2): 558–60.
- Pardanani, A.D., Levine, R.L., Lasho, T., Pikman, Y., Mesa, R.A., Wadleigh, M., Steensma, D.P., Elliott, M.A., Wolanskyj, A.P., Hogan, W.J., McClure, R.F., Litzow, M.R., Gilliland, D.G., and Tefferi, A. 2006. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood* **108**(10): 3472–3476.

- Peeters, P., Raynaud, S.D., Cools, J., Wlodarska, I., Grosgeorge, J., Philip, P., Monpoux, F., Van Rompaey, L., Baens, M., Van den Berghe, H., and Marynen, P. 1997. Fusion of TEL, the ETS-variant gene 6 (ETV6), to the receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. *Blood* **90**(7): 2535–2540.
- Peng, C., Brain, J., Hu, Y., Goodrich, A., Kong, L., Grayzel, D., Pak, R., Read, M., and Li, S. 2007. Inhibition of heat shock protein 90 prolongs survival of mice with BCR-ABL-T315I-induced leukemia and suppresses leukemic stem cells. *Blood* **110**(2): 678–685.
- Pikman, Y., Lee, B.H., Mercher, T., McDowell, E., Ebert, B.L., Gozo, M., Cuker, A., Wernig, G., Moore, S., Galinsky, I., Deangelo, D.J., Clark, J.J., Lee, S.J., Golub, T.R., Wadeigh, M., Gilliland, D.G., and Levine, R.L. 2006. MPLW515L Is a Novel Somatic Activating Mutation in Myelofibrosis with Myeloid Metaplasia. *PLoS Med* **3**(7): e270.
- Rawat, V.P., Cusan, M., Deshpande, A., Hiddemann, W., Quintanilla-Martinez, L., Humphries, R.K., Bohlander, S.K., Feuring-Buske, M., and Buske, C. 2004. Ectopic expression of the homeobox gene Cdx2 is the transforming event in a mouse model of t(12;13)(p13;q12) acute myeloid leukemia. *Proc Natl Acad Sci USA* **101**(3): 817–822.
- Rhoades, K.L., Hetherington, C.J., Harakawa, N., Yergeau, D.A., Zhou, L., Liu, L.Q., Little, M.T., Tenen, D.G., and Zhang, D.E. 2000. Analysis of the role of AML1-ETO in leukemogenesis, using an inducible transgenic mouse model. *Blood* **96**(6): 2108–2115.
- Rosenbauer, F., Wagner, K., Kutok, J.L., Iwasaki, H., Le Beau, M.M., Okuno, Y., Akashi, K., Fiering, S., and Tenen, D.G. 2004. Acute myeloid leukemia induced by graded reduction of a lineage-specific transcription factor, PU.1. *Nat Genet* **36**(6): 624–630.
- Sakaki K. 1961. Hematological comparison of the mouse blood taken from the eye and the tail. *Exp Anim* **10**:14–19.
- Schessl, C., Rawat, V.P., Cusan, M., Deshpande, A., Kohl, T.M., Rosten, P.M., Spiekermann, K., Humphries, R.K., Schnittger, S., Kern, W., Hiddemann, W., Quintanilla-Martinez, L., Bohlander, S.K., Feuring-Buske, M., and Buske, C. 2005. The AML1-ETO fusion gene and the FLT3 length mutation collaborate in inducing acute leukemia in mice. *J Clin Invest* **115**(8): 2159–2168.
- Scholl, C., Bansal, D., Dohner, K., Eiwen, K., Huntly, B.J., Lee, B.H., Rucker, F.G., Schlenk, R.F., Bullinger, L., Dohner, H., Gilliland, D.G., and Frohling, S. 2007. The homeobox gene CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis. *J Clin Invest* **117**(4): 1037–1048.
- Schwaller, J., Frantsve, J., Aster, J., Williams, I.R., Tomasson, M.H., Ross, T.S., Peeters, P., Van Rompaey, L., Van Etten, R.A., Ilaria, R., Jr., Marynen, P., and Gilliland, D.G. 1998. Transformation of hematopoietic cell lines to growth-factor independence and induction of a fatal myelo- and lymphoproliferative disease in mice by retrovirally transduced TEL/JAK2 fusion genes. *Embo J* **17**(18): 5321–5333.
- Scott, L.M., Tong, W., Levine, R.L., Scott, M.A., Beer, P.A., Stratton, M.R., Futreal, P.A., Erber, W.N., McMullin, M.F., Harrison, C.N., Warren, A.J., Gilliland, D.G., Lodish, H.F., and Green, A.R. 2007. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med* **356**(5): 459–468.
- Socolovsky, M., Nam, H., Fleming, M.D., Haase, V.H., Brugnara, C., and Lodish, H.F. 2001. Ineffective erythropoiesis in Stat5a(–/–)5b(–/–) mice due to decreased survival of early erythroblasts. *Blood* **98**(12): 3261–3273.
- Sohal, J., Phan, V.T., Chan, P.V., Davis, E.M., Patel, B., Kelly, L.M., Abrams, T.J., O'Farrell, A.M., Gilliland, D.G., Le Beau, M.M., and Kogan, S.C. 2003. A model of APL with FLT3 mutation is responsive to retinoic acid and a receptor tyrosine kinase inhibitor, SU11657. *Blood* **101**(8): 3188–3197.
- Stover, E.H., Chen, J., Lee, B.H., Cools, J., McDowell, E., Adelsperger, J., Cullen, D., Coburn, A., Moore, S.A., Okabe, R., Fabbro, D., Manley, P.W., Griffin, J.D., and Gilliland, D.G. 2005. The small molecule tyrosine kinase inhibitor AMN107 inhibits TEL- PDGFRbeta and FIP1L1-PDGFRalpha in vitro and in vivo. *Blood* **106**(9): 3206–3213.

- Tartaglia, M., Martinelli, S., Cazzaniga, G., Cordeddu, V., Iavarone, I., Spinelli, M., Palmi, C., Carta, C., Pession, A., Arico, M., Maseri, G., Bassi, G., Sorcini, M., Gelb, B.D., and Biondi, A. 2004. Genetic evidence for lineage-related and differentiation stage-related contribution of somatic PTPN11 mutations to leukemogenesis in childhood acute leukemia. *Blood* **104**(2): 307–313.
- Tatsumi, T., Huang, J., Gooding, W.E., Gambotto, A., Robbins, P.D., Vujanovic, N.L., Alber, S.M., Watkins, S.C., Okada, H., and Storkus, W.J. 2003. Intratumoral delivery of dendritic cells engineered to secrete both interleukin (IL)-12 and IL-18 effectively treats local and distant disease in association with broadly reactive T-cell-type immunity. *Cancer Res* **63**(19): 6378–6386.
- Tomasson, M.H., Williams, I.R., Hasserjian, R., Udomsakdi, C., McGrath, S.M., Schwaller, J., Drucker, B., and Gilliland, D.G. 1999. TEL/PDGFBetaR induces hematologic malignancies in mice that respond to a specific tyrosine kinase inhibitor. *Blood* **93**(5): 1707–1714.
- Tomasson, M.H., Williams, I.R., Li, S., Kutok, J., Cain, D., Gillessen, S., Dranoff, G., Van Etten, R.A., and Gilliland, D.G. 2001. Induction of myeloproliferative disease in mice by tyrosine kinase fusion oncogenes does not require granulocyte-macrophage colony-stimulating factor or interleukin-3. *Blood* **97**(5): 1435–1441.
- Wagner, K., Zhang, P., Rosenbauer, F., Drescher, B., Kobayashi, S., Radomska, H.S., Kutok, J.L., Gilliland, D.G., Krauter, J., and Tenen, D.G. 2006. Absence of the transcription factor CCAAT enhancer binding protein alpha results in loss of myeloid identity in bcr/abl-induced malignancy. *Proc Natl Acad Sci USA* **103**(16): 6338–6343.
- Weisberg, E., Boulton, C., Kelly, L.M., Manley, P., Fabbro, D., Meyer, T., Gilliland, D.G., and Griffin, J.D. 2002. Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412. *Cancer Cell* **1**(5): 433–443.
- Weisberg, E., Manley, P.W., Breitenstein, W., Bruggen, J., Cowan-Jacob, S.W., Ray, A., Huntly, B., Fabbro, D., Fendrich, G., Hall-Meyers, E., Kung, A.L., Mestan, J., Daley, G.Q., Callahan, L., Catley, L., Cavazza, C., Azam, M., Neuberg, D., Wright, R.D., Gilliland, D.G., and Griffin, J.D. 2005. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell* **7**(2): 129–141.
- Wernig, G., Mercher, T., Okabe, R., Levine, R.L., Lee, B.H., and Gilliland, D.G. 2006. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. *Blood* **107**(11): 4274–4281.
- Westervelt, P., Lane, A.A., Pollock, J.L., Oldfather, K., Holt, M.S., Zimonjic, D.B., Popescu, N.C., DiPersio, J.F., and Ley, T.J. 2003. High-penetrance mouse model of acute promyelocytic leukemia with very low levels of PML-RARalpha expression. *Blood* **102**(5): 1857–1865.
- Wolff, N.C. and Ilaria, R.L., Jr. 2001. Establishment of a murine model for therapy-treated chronic myelogenous leukemia using the tyrosine kinase inhibitor ST1571. *Blood* **98**(9): 2808–2816.
- Wong, S. and Witte, O.N. 2001. Modeling Philadelphia chromosome positive leukemias. *Oncogene* **20**(40): 5644–5659.
- Yamada, Y., Rothenberg, M.E., Lee, A.W., Akei, H.S., Brandt, E.B., Williams, D.A., and Cancelas, J.A. 2006. The FIP1L1-PDGFRα fusion gene cooperates with IL-5 to induce murine hypereosinophilic syndrome (HES)/chronic eosinophilic leukemia (CEL)-like disease. *Blood* **107**(10): 4071–4079.
- Zaleskas, V.M., Krause, D.S., Lazarides, K., Patel, N., Hu, Y., Li, S., and Van Etten, R.A. 2006. Molecular pathogenesis and therapy of polycythemia induced in mice by JAK2 V617F. *PLoS ONE* **1**: e18.
- Zhang, D.E., Zhang, P., Wang, N.D., Hetherington, C.J., Darlington, G.J., and Tenen, D.G. 1997. Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein alpha-deficient mice. *Proc Natl Acad Sci USA* **94**(2): 569–574.

# **Chapter 4**

# **Mechanisms of DNA Double-Strand Break Repair in Hematopoietic Homeostasis and Oncogenesis**

**Sarah A. Maas, Lura Brianna Caddle, and Kevin D. Mills**

## **Contents**

4.1	Introduction . . . . .	82
4.2	Sources of DNA Double-Strand Breaks . . . . .	88
4.2.1	Endogenous Sources of DNA Breakage . . . . .	88
4.2.2	Exogenous Sources of DNA Breakage . . . . .	88
4.3	DNA Double-Strand Break Repair Pathways . . . . .	89
4.3.1	Homologous Recombination . . . . .	89
4.3.1.1	Gene Conversion . . . . .	89
4.3.1.2	Synthesis-Dependent Strand Annealing . . . . .	91
4.3.1.3	Single-Strand Annealing . . . . .	91
4.3.1.4	Homologous Recombination and Genomic Instability . . . . .	93
4.3.1.5	Homologous Recombination Genes and Proteins . . . . .	94
4.3.1.6	Homologous Recombination and Cancer . . . . .	98
4.3.2	Nonhomologous End Joining . . . . .	100
4.3.2.1	V(D)J Recombination . . . . .	101
4.3.2.2	RAG Endonuclease . . . . .	102
4.3.2.3	Class Switch Recombination and Somatic Hypermutation . . . . .	103
4.3.2.4	Mechanics of Nonhomologous End Joining . . . . .	104
4.3.2.5	Nonhomologous End Joining Genes and Proteins . . . . .	105
4.3.2.6	Nonhomologous End Joining and Telomere Maintenance . . . . .	108
4.3.2.7	Nonhomologous End Joining and Cancer . . . . .	109
4.4	Perspective . . . . .	111

## **List of Abbreviations**

AID	Activation-induced cytidine deaminase
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ART	Artemis
AT	Ataxia telangiectasia
ATM	Ataxia telangiectasia mutated

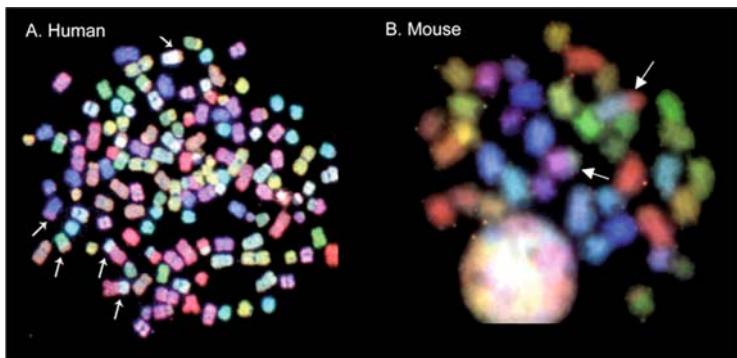
---

S.A. Maas and K.D. Mills  
The Jackson Laboratory, Bar Harbor, ME 04609, USA  
sarah.maas@jax.org, kevin.mills@jax.org

ATR	ATM and Rad3 related
B-ALL	B-cell acute lymphoblastic leukemia
BFB	Breakage–fusion–bridge
CHO	Chinese hamster ovary
CML	Chronic myelogenous leukemia
CSR	Class switch recombination
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
DSB	DNA double-strand break
FA	Fanconi anemia
GC	Gene conversion
H2AX	Histone H2A variant X
HR	Homologous recombination
ICL	Interstrand crosslinking
Ig	Immunoglobulin
IgH	Immunoglobulin heavy chain
IR	Ionizing radiation
Lig4	Ligase IV
MDS	Myelodysplastic syndrome
MEF	Mouse embryonic fibroblast
MMC	Mitomycin-C
MRN	Mre11-Rad50-Nbs1 complex
NHEJ	Nonhomologous end joining
Ph	Philadelphia
Pre-T LBL	Pre-T cell lymphoblastic leukemia
RAG	Recombination-activating gene
RPA	Replication protein A
RS	Recombination signal sequences
RS-SCID	Radiation-sensitive SCID
SCE	Sister chromatid exchange
SCID	Severe combined immunodeficiency
SDSA	Synthesis-dependent strand annealing
SHM	Somatic hypermutation
SSA	Single-strand annealing
ssDNA	Single-stranded DNA
T-ALL	T-cell acute lymphoblastic leukemia or lymphoma
TCR	T-cell receptor
TOPOIII	Topoisomerase III
XLF	XRCC4-like factor

## 4.1 Introduction

Chromosomal instability is a hallmark of cancer associated with many, if not all, tumor types (reviewed in Aplan, 2006; Ferguson and Alt, 2001; Mills et al., 2003) (Fig. 4.1). Structural and numerical chromosomal abnormalities often



**Fig. 4.1 Genomic instability observed in human and mouse tumor cells.** (A) Spectral karyotype analysis of a human bladder cancer cell line. The aneuploid J82 cell line contains multiple chromosomal aberrations. White arrows indicate translocations. Adapted from Padilla-Nash et al. (2006). (B) Spectral karyotype analysis of a mouse pro-B cell lymphoma deficient for *Dclre1c* and *Trp53*. White arrows indicate translocations. (See color insert)

play key roles in tumorigenesis and can occur as principle lesions in tumor precursor cells. These molecular alterations to the genome occur as a consequence of various exogenous or endogenous insults, producing chromosomal translocations, interstitial deletions, gene amplifications, and other aberrations. While extensive and nonrecurrent chromosomal lesions are associated with many different cancer types, specific characteristic lesions are often associated with given tumors, especially those of hematologic origin (Agarwal et al., 2006; Aplan, 2006; Ferguson and Alt, 2001).

Hematologic malignancies, affecting lymphoid, myeloid, and erythroid cells, frequently occur with characteristic chromosomal rearrangements. By contrast with solid tumors, hematologic malignancies frequently, but not invariably, incur simple, reciprocal translocations, which are often recognized for their diagnostic and prognostic value. In the past two decades, a vast body of research has shown that many of these chromosomal rearrangements, especially those occurring in hematologic malignancies, lead either to the deregulation of a specific cellular proto-oncogene or to the formation of a novel fusion oncogene (reviewed in Aplan, 2006). Analysis of karyotypic instability in malignant hematological cells and tissues has, in some cases, directly led to the identification of new oncogenes and more recently opened the possibility of developing better and more highly specific therapeutic modalities targeting tumor-specific genetic alterations.

The catalogue of recognized chromosomal translocations that characterize specific tumor types continues to grow. The prototypical example of such a characteristic rearrangement is the Philadelphia (Ph) chromosome, associated with chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL) (de Klein et al., 1982). The Ph chromosome encodes for a chimeric protein comprising part of the breakpoint cluster region (*BCR*) gene, located

on chromosome (Chr) 22 (region q11), and the Abelson gene (*ABL*) on Chr 9 (region q34). This chimeric BCR–ABL protein acts as a constitutive tyrosine kinase that increases cellular proliferation, eventually resulting in tumorigenesis.

Subsequent to the identification of the Ph chromosome, it has become clear that hematologic cancers can arise with a broad range of analogous reciprocal chromosomal translocations. In neoplasias derived from B or T lymphocytes, these translocations often result from mistakes or failures in V(D)J recombination, immunoglobulin (Ig) class switch recombination (CSR), or somatic hypermutation (SHM), processes of somatic rearrangement in developing/maturing Ig or T-cell receptor (*TCR*) genes (reviewed in Aplan, 2006; Chaudhuri and Alt, 2004; Jung and Alt, 2004; Kuppers and Dalla-Favera, 2001; Mills et al., 2003; Weinstock et al., 2006b). Burkitt's lymphoma is typically characterized by a marker t(8;14) translocation. This chromosomal aberration juxtaposes the potent oncogene *Myc* with strong regional enhancers within the Ig heavy chain (IgH) locus. Other lymphoid malignancies, such as B-cell acute lymphoblastic leukemia (B-ALL) and pre-T lymphoblastic leukemia (pre-T LBL), typically show characteristic translocations involving the Ig loci, and it has become apparent that these genomic regions are especially prone to translocation due to gene rearrangements that occur as a necessary step in the developmental program of lymphoid maturation. Lymphoid translocations typically result in oncogene amplification due to potent enhancer elements within the Ig and TCR loci rather than fusion proteins (Table 4.1).

While such translocations, usually afflicting the Ig or TCR loci, characterize numerous lymphoid malignancies, myeloid neoplasias can incur a variety of specific translocations that do not appear to be the direct consequence of DNA double-strand breaks (DSBs) introduced during a developmental program. Instead, myeloid malignancies often exhibit a different type of chromosomal translocation. Although recurrent, balanced translocations are also observed in myeloid malignancies, these translocations typically create fusion genes that encode for constitutively active chimeric proteins (Table 4.1).

Recurrent translocations observed in leukemias and lymphomas often produce novel gene products that are candidate targets for highly specific drugs. For example, the highly effective small molecule tyrosine kinase inhibitor imatinib, which targets the BCR–ABL fusion protein, has proven one of the most effective therapies now available to Ph+ CML patients (Druker et al., 2001). Molecular analysis of the BCR–ABL fusion protein exemplifies how knowledge of recurrent translocations and their gene products can be used both to understand and improve upon current chemotherapeutic mechanisms and, potentially, to develop effective new therapeutic modalities. Importantly, molecular characterization of recurrent chromosomal abnormalities associated with specific types of hematologic malignancies may also facilitate better assignment of patients into particular risk categories, permitting specifically targeted customized therapeutic regimes.

Patients with the same gross cancer diagnosis often respond dramatically differently to the same chemotherapy regimen, a likely consequence of both

**Table 4.1** Some commonly observed recurrent translocations associated with lymphoid and myeloid leukemias

Diagnosis	Translocation	Activated genes	Mechanism of activation	References
ALL-Burkitts lymphoma	t(8;14)(q24;q32)	<i>MYC</i> , <i>IGH</i>	Translocation to <i>IGH</i>	Aplan (2006), Mitelman Database
ALL	t(8;22)(q24;q11)	<i>MYC</i>	Translocation to <i>IGL</i>	Aplan (2006)
ALL	t(2;8)(p12;q24)	<i>MYC</i>	Translocation to <i>IGK</i>	Aplan (2006)
ALL	t(12;21)(p12;q22)	<i>TEL-AML</i>	Gene fusion	Aplan (2006)
ALL	t(1;19)(q21;p13)	<i>E2A-PBX1</i>	Gene fusion	Aplan (2006)
ALL	t(17;19)(q22;p13)	<i>E2A-HLF</i>	Gene fusion	Aplan (2006)
ALL	t(1;11)(p32;q23)	<i>MLL</i> , <i>MLL/EPS15</i>	Gene fusion	Hashimoto et al., (2001), Mitelman Database
ALL	inv(7)(p15;q34)	<i>TRB@/HOXA</i>	Gene fusion	Mitelman Database
ALL	t(11;19)(q23;p13)	<i>MLL</i> , <i>MLL/MLLT1</i>	Gene fusion	Mitelman Database
ALL-Ph+ chromosome	t(9;22)(q34;q11)	<i>BCR-ABL</i>	Gene fusion	Mitelman Database
ALL	t(12;21)(p13;q22)	<i>ETV6/RUNX1 (TEL/AML1)</i>	Gene fusion	Frost et al. (2004), Mitelman Database
ALL	inv(14)(q11;q32)	<i>IGH/TRA@, TCL1A</i>	Gene fusion	Sugimoto et al. (1999)
ALL	t(4;11)(q21; q23)	<i>MLL-AF4</i>	Gene fusion	Aplan (2006)
ALCL	t(2;5)(p23;q35)	<i>NPM-ALK</i>	Gene fusion	Aplan (2006)
ALCL	t(8;14)(q24;q11)	<i>MYC</i>	Translocation to <i>TRA@/D@</i>	Aplan (2006)
ALCL	t(7;19)(q35;p13)	<i>LYL1</i>	Translocation to <i>TRB@</i>	Aplan (2006)
ALCL	t(1;14)(p32;q11)	<i>SCL</i>	Translocation to <i>TRA@/D@</i>	Aplan (2006)
ALCL	t(14;21)(q11;q22)	<i>OLIG2</i>	Translocation to <i>TRA@/D@</i>	Aplan (2006)
ALCL	t(11;14)(p15;q11)	<i>LMO1(RBTN1)</i>	Translocation to <i>TRA@/D@</i>	Aplan (2006)
ALCL	t(11;14)(p13;q11)	<i>LMO2(RBTN2)</i>	Translocation to <i>TRA@/D@</i>	Aplan (2006)
ALCL	t(10;14)(q24;q11)	<i>HOX11</i>	Translocation to <i>TRA@/D@</i>	Aplan (2006)
ALCL	t(5;14)(q35;q32)	<i>HOX11L2 (TLX3)</i>	unknown	Aplan (2006) and Nagel et al. (2003)
ALCL	t(10;11)(p13;q21)	<i>CALM-<i>AF10</i></i>	Gene fusion	Aplan (2006)
ALCL	t(4;11)(q21;p15)	<i>NUP98-RAP1GDS1</i>	Gene fusion	Aplan (2006)

Table 4.1 (continued)

Diagnosis	Translocation	Activated genes	Mechanism of activation	References
ALCL	t(14;18)(q32;q21)	<i>IGH/BCL2</i>	Translocation to <i>IGH</i>	Mitelman Database
CLL	t(14;19)(q32;q13)	<i>IGH/CEBPA</i>	Translocation to <i>IGH</i>	Frost et al. (2004)
CML—Ph+ chromosome	t(9;22)(q34;q11)	<i>BCR-ABL</i>	Gene fusion	Aplan (2006), Mitelman Database
CML	t(9;11)(p22;q23)	<i>AF9-MLL</i>	Gene fusion	Aplan (2006)
APL	t(15;17)(q21;q21)	<i>PML-RARA</i>	Gene fusion	Aplan (2006)
APL	t(11;17)(q23;q21)	<i>P1ZF-RARA</i>	Gene fusion	Aplan (2006)
AML	t(4;11)(q21;q23)	<i>HRAS-MLL</i>	Gene fusion	Aplan (2006)
AML	t(11;v)(q23,v)	<i>MLL</i>	Gene fusion	Aplan (2006)
AML or CMMI	t(12;v)(p1.3;v)	<i>ETV6</i>	Gene fusion	Aplan (2006)
AML	t(11;v)(p1.5;v)	<i>NUP98</i>	Gene fusion	Aplan (2006)
AML	t(8;21)(q22;q22)	<i>AML1-ETO</i>	Gene fusion	Aplan (2006)
AML	inv(16)(p1.3;q22)	<i>CBFB-MYH11</i>	Gene fusion	Aplan (2006)
AML	t(16;21)(p11;q22)	<i>FUS-ERG</i>	Gene fusion	Aplan (2006)
AML	t(5;14)(q33;q32)	<i>CEV14-PDGFRB</i>	Gene fusion	Aplan (2006)
AML	t(8;22)(p11;q13)	<i>P300-MOZ</i>	Gene fusion	Aplan (2006)
AML	t(6;9)(p23;q34)	<i>DEK,NUP214</i>	Gene fusion	Aplan (2006)
AMKL	t(1;22)(p1.3;q1.3)	<i>RBM15,MKL</i>	Gene fusion	Aplan (2006)
AML	t(3;21)(q26;q22)	<i>AML1-EV11</i>	Gene fusion	Aplan (2006)

Mitelman Database of Chromosome Aberrations in Cancer (Cancer Genome Anatomy Project), <http://cgap.nci.nih.gov/Chromosomes/Mitelman>. It is important to note that the revised nomenclature for T-cell receptor A-D loci (formerly known as *TCRA-D*; now known as *TR4-D@*) has been used in creating this table.

individual genetic variation and tumor-specific properties. Therefore, better knowledge of characteristic chromosomal abnormalities, and their pathologic effects, will permit the use of more individualized treatment strategies that provide maximal therapeutic effect with minimal side effects. For example, acute myeloid leukemia (AML) patients exhibiting either an inverted Chr 16 or t(8;21) translocation generally respond well to high doses of the drug cytarabine, while AML patients with other cytogenetic abnormalities do not (Bloomfield et al., 1998). Similarly, Ph+ CML patients respond favorably to imatinib, showing high rates of remission, while Ph- CML patients, who present with similar symptoms, generally respond poorly (Druker et al., 2001). Characterization of such molecular anomalies will not only permit the design of highly specific therapeutics that have fewer widespread side effects but will also allow customization of treatment regimens specifically tailored to individual patients' particular needs.

Understanding the mechanisms of recurrent chromosomal abnormalities could also aid in better understanding particular risk factors associated with specific types of lymphoid and other hematologic malignancies and could help in designing better diagnostic and prognostic tests. Exposure to environmental clastogens likely results in DNA damage that can, in turn, drive the development of hematologic malignancies (Takahashi and Ohnishi, 2005 and references therein). The extent and types of DNA damage have been, in many cases, extensively analyzed at the molecular level, and results from these exhaustive molecular studies have suggested that different toxins may produce unique DNA damage profiles that could potentially be exploited for diagnostic or therapeutic effect, if the precise series of downstream consequences of such chromosomal damage can be ascertained in sufficient detail. In these cases, understanding the contributions of individual toxins to specific recurrent genome instability and mutations could be a key facet in the development of early detection schemes, individualized treatment methods, and most importantly, effective prevention strategies.

Genomic instability and translocations that lead to tumorigenesis can result from a variety of sources. One type of genomic insult, which leads to such instability, is the misrepair of DSBs (Agarwal et al., 2006; Helleday et al., 2007; Sonoda et al., 2006; Valerie and Povirk, 2003; Weinstock et al., 2006b). Both exogenous and endogenous sources can contribute to the introduction of DSBs throughout the genome. To effectively and efficiently repair damage from varied internal and external sources, mammalian cells appear to rely on two major DSB repair pathways: homologous recombination (HR) and non-homologous end joining (NHEJ). Misregulation of either pathway can result in unrepaired DSBs, and these can produce chromosomal translocations with the potential for subsequent tumorigenesis. Molecular characterization of recurrent translocations and other genomic instability present in these mouse models is the underpinning for further dissection of the mechanisms that lead to genome instability, translocations, and ultimately, tumorigenesis in humans. In this chapter, the roles of HR and NHEJ both in normal cellular function and

as contributing factors to hematologic malignancies in both humans and mouse models will be discussed.

## 4.2 Sources of DNA Double-Strand Breaks

### 4.2.1 Endogenous Sources of DNA Breakage

While DSBs can arise in B and T lymphocytes as a result of the developmentally programmed Ig and *TCR* gene rearrangement (discussed in detail in Section 3.2.), nonprogrammed DSBs can also occur, in many cell types, either randomly or in genomic regions that are especially prone to breakage, as the result of normal cellular processes. In some cases, single-strand DNA nicks, as consequences of base excision repair, chemical or radiation damage, or other means, can produce DSBs, especially when a replication fork encounters and attempts to replicate past the nick (reviewed in Mills et al., 2003; Sonoda et al., 2006; Valerie and Povirk, 2003).

Unprogrammed DSBs can occur throughout the genome but may be especially likely in regions with particularly unstable sequence or chromatin structures, such as chromosomal fragile sites (Arlt et al., 2006). Fragile sites are generally classified as regions of DNA that exhibit gaps or breaks on metaphase chromosomes after partial inhibition of DNA synthesis. Common fragile sites are found throughout the genome and are stable in normal cells. These regions are generally A and T rich, which is thought to contribute to increased DNA flexibility, leading to lower resistance to mechanical stress. Breakage at fragile sites is associated with chromosomal rearrangements and translocations that contribute to oncogenesis (reviewed in Arlt et al., 2006). The breakage at fragile sites that leads to such rearrangements is hypothesized to result from the stalling of replication forks at the repetitive sequence of these regions.

### 4.2.2 Exogenous Sources of DNA Breakage

DSBs can also result from exogenous sources of damage (Migliore and Coppede, 2002; Pages and Fuchs, 2002). One of the most common types of exogenous insults is damage that results from ionizing radiation (IR). IR induces a large range of types of DNA lesions, and it is hypothesized that multiple pathways cooperate to repair the damage resulting from exposure to IR, including the DSB repair mechanisms discussed below. In addition, DNA damage can result from exposure to a wide range of environmental toxins. Many toxins found in the environment, such as heavy metals and components of tobacco smoke, have been shown to induce damage that results in DSB formation (reviewed in Migliore and Coppede, 2002; Pages and Fuchs, 2002; Takahashi

and Ohnishi, 2005). DSBs resulting from such exogenous damage may also contribute, in as yet poorly defined ways, to lesions that lead to hematopoietic cancers.

## 4.3 DNA Double-Strand Break Repair Pathways

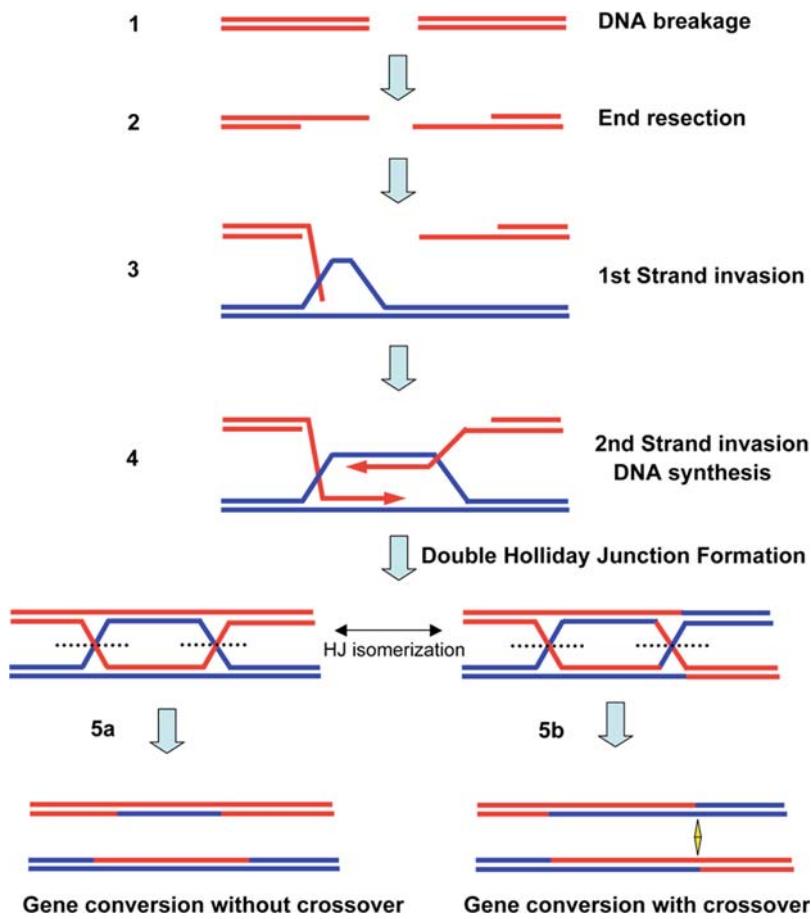
### 4.3.1 *Homologous Recombination*

The first major pathway of DSB repair is HR [for detailed reviews of HR and DSB repair see Dudas and Chovanec (2004), Helleday et al. (2007), Karran (2000), Pastink et al. (2001)]. HR catalyzes the conservative, usually error-free, repair of DSBs using an undamaged template DNA double-strand as a donor for sequence replication and replacement. Homology-mediated DSB repair can occur either in an error-free fashion, when recombination uses an undamaged sister chromatid template, or can result in nucleotide changes following repair, when recombination from a nonidentical template occurs.

Homology-mediated DSB repair is first initiated by 5'- to 3'-resection of double-stranded DNA ends by the action of the Mre11/Rad50/Nbs1 complex [(Bannister and Schimenti, 2004; Farah et al., 2005; Kobayashi et al., 2004; Krishna et al., 2007; Szostak et al., 1983); reviewed in (Dudas and Chovanec, 2004) and (Helleday et al., 2007)]. This produces a 3'-overhanging single DNA strand that becomes inserted into the undamaged homologous template to form a nucleoprotein filament containing heteroduplex DNA. This heteroduplex formation results in the displacement of a single DNA strand, termed a D-loop, at the site of the donor invasion, and a cruciform Holliday junction at the transition between the heteroduplex and the homoduplex DNA (Helleday et al., 2007). The annealed 3'-end in the heteroduplex is then used as a primer for de novo DNA synthesis to replace missing sequences. At this point, HR can be divided into distinct subpathways depending on the details in resolution of the Holliday junction and replication intermediates. Major subtypes of HR include gene conversion (GC) without crossing over, GC with an associated crossover event, and synthesis-dependent strand annealing (SDSA) (Dudas and Chovanec, 2004; Helleday et al., 2007). Another distinct mode of homology-driven break repair that does not involve DNA synthesis or Holliday junction formation, termed single-strand annealing (SSA), leads to deletion of DNA sequences flanking the DSB (Helleday et al., 2007; Karran, 2000; Pastink et al., 2001).

#### 4.3.1.1 Gene Conversion

A favored mode of HR-mediated DSB repair in mammalian cells is conservative, homology-mediated repair from an undamaged sister chromatid template. This process can result in GC either with or without sister chromatid exchange (SCE) (Fig. 4.2). GC from a sister chromatid can occur in the late-S or G2 phase



**Fig. 4.2 Schematic of gene conversion (GC).** Following DNA double-strand breakage (1), single-strand end resection in the 3' → 5'-direction produces single-stranded 3'-overhanging ends (2). These then serve as donors for strand invasion and heteroduplex formation, resulting in D-loop intermediates (3). Second-strand invasion and DNA synthesis leads to bi-directional replication and migration of the resultant Holliday junctions (4). Finally, isomerization of the Holliday junction, followed by cleavage and re-ligation of the Holliday junction joint molecules, produces a gene conversion product, either with (5b) or without (5a) crossing-over

of the cell cycle when a replicate DNA double strand is present and available. This results in restoration of the DNA double strand without the loss or alteration of DNA sequences in the vicinity of the DSB. This mode of HR repair is thought to occur through the formation of a double Holliday junction intermediate (Helleday et al., 2007; Szostak et al., 1983). By this mechanism, both DNA ends resulting from the DSB invade the undamaged homologous template to form two cruciform Holliday junctions connecting the donor and target strands. Each strand invasion then primes new DNA synthesis and,

through the activities of an incompletely understood group of proteins which likely includes RecQ family helicases and topoisomerase III (TOPOIII), the Holliday junctions can be translated away from the point of initial branch formation (Bachrati et al., 2006; Cheok et al., 2005; Hickson, 2003; Plank et al., 2006).

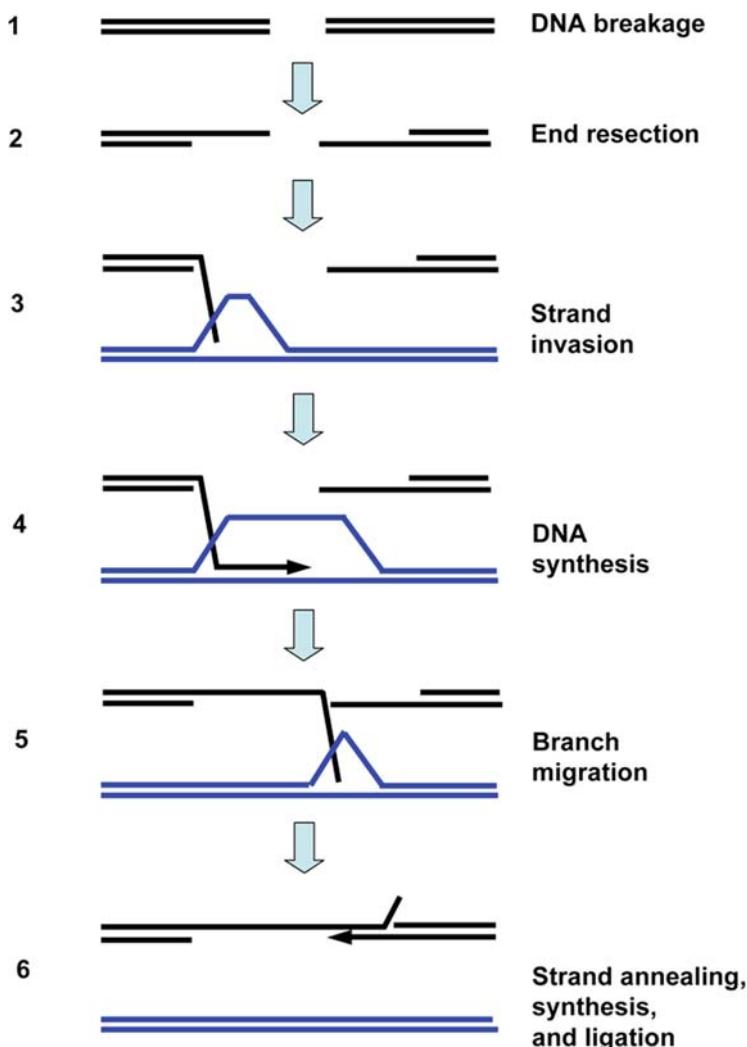
In addition to linear translation along the length of the two-participant DNA strands, Holliday junctions may also isomerize to produce either of two structurally equivalent structures. Finally, the double Holliday junction becomes resolved via nucleolytic cleavage, by an as yet unidentified resolvase, to produce the intact, repaired DNA double strands (Sharples, 2001). Depending on the isomerization state of the Holliday junction at the time of resolution, this can generate either a noncrossover product or a recombinant crossover product (reviewed in Helleday et al., 2007). Because the Holliday junction isomers are energetically equivalent and should thus both occur at the same frequency, it is expected that crossover and noncrossover events should be detected at the same rate (reviewed in Helleday et al., 2007). Curiously, however, crossover events of DSB repair appear to occur less frequently than noncrossover events, at least in some experimental contexts (reviewed in Helleday et al., 2007). Thus, it is possible that additional factors influence the asymmetric resolution of double Holliday junction intermediates. Indeed, there is some evidence that the *BLM* gene product, a member of the RecQ helicase family, functions to prevent crossover events (Cheok et al., 2005; Hickson, 2003; Khakhar et al., 2003; Raynard et al., 2006; Wu and Hickson, 2003).

#### 4.3.1.2 Synthesis-Dependent Strand Annealing

Another conservative HR-mediated repair pathway is SDSA (Fig. 4.3). As with GC, SDSA is initiated by DNA resection, strand invasion, and D-loop formation. In this mode of HR repair, a single Holliday junction, rather than a double Holliday junction, is produced (Fig. 4.3; see also Helleday et al., 2007). Branch migration then occurs in the direction of DNA synthesis, probably by the same mechanism as double Holliday junction branch migration leading to GC. Continued sliding of the junction beyond the end of the newly synthesized single strand results in its release from the heteroduplex, re-formation of the target homoduplex DNA, and production of a 3'-single strand that can be annealed to its complementary sequence in the originally broken duplex. SDSA is completed when any overhanging single-stranded flaps are removed by endonucleolytic cleavage, and the staggered DNA nicks are ligated to restore the intact double strand.

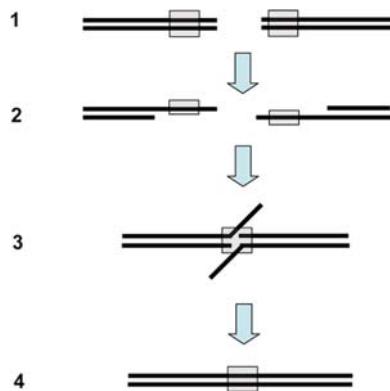
#### 4.3.1.3 Single-Strand Annealing

SSA is a distinct, nonconservative subtype of homology-mediated DSB repair that does not lead to recombination but results in the deletion of nucleotides flanking the DSB (Agarwal et al., 2006; Helleday et al., 2007) (Fig. 4.4). SSA



**Fig. 4.3 Schematic of synthesis-dependent strand annealing (SDSA).** Following DNA breakage (1), 3' → 5'-end resection (2), and first-strand invasion (3), as depicted for gene conversion in Fig. 4.2, unidirectional DNA synthesis (4) and branch migration (5) generates a newly replicated stretch of DNA terminated by sequence complementary to a portion of DNA adjacent to the original break (5). D-loop melting and annealing of complementary DNA ends (6) then promotes fill-in synthesis and re-ligation to restore the intact DNA double strand

occurs between short, directly repeated sequences located on either side of the DSB. Helicase-dependent DNA unwinding and exonucleolytic degradation produce complementary single-DNA strands that are subsequently annealed via RAD52 activity (Bachrati and Hickson, 2003; Hickson, 2003; Khakhar et al., 2003; Sharma et al., 2004). The resulting double-stranded DNA product



**Fig. 4.4 Schematic of single-strand annealing (SSA).** Following DNA breakage at a position flanked by short stretches of homologous sequence shown as shaded boxes (1), 3' → 5'-end resection exposes single-stranded 3'-overhangs with embedded complementary DNA sequence (2). Annealing of complementary sequences produces unpaired “flaps” of noncomplementary single-stranded DNA (3). These are trimmed by a flap endonuclease, and ligation of the DNA double-strand break (DSB) or staggered single-strand nicks restores the DNA double strand, accompanied by loss of a single repeat and flanking DNA sequence (4)

contains two staggered single-strand nicks and may also include overhanging, noncomplementary single-stranded DNA (ssDNA). The overhanging ends are removed by endonucleolytic cleavage directed against the junction of the double-stranded and ssDNA, and the nick is sealed via ligation (Gottlich et al., 1998). The end product of SSA is repair of the DSB with loss of a variable amount of flanking sequence (Fig. 4.4).

#### 4.3.1.4 Homologous Recombination and Genomic Instability

Chromosomal rearrangements can result when a DNA break fails to rejoin with its cognate end and instead mis-joins with another target chromosome. This is generally prevented because homology-driven repair using an undamaged sister chromatid template is strongly favored in post-replication cells, resulting both in conservative repair of the DSB without sequence alteration and averting recombination with ectopic targets. However, DSBs may occasionally become repaired using templates other than the identical sister chromatid. When HR targets the homologous chromosome rather than the identical chromatid, GC leading to loss of the original sequence and replacement with the sequence from the recombination target may occur. Loss of heterozygosity can result from targeting of the two nonidentical sequences that can, in turn, act as the molecular basis for tumor formation when a tumor suppressor gene is affected.

Alternatively, HR may target homologous sequences resident on nonhomologous chromosomes (Elliott and Jasin, 2002; Weinstock et al., 2006a, b). Most mammalian genomes are replete with highly repetitive DNA sequence elements,

such as LINEs, SINEs, or Alu sequences, that may serve as HR substrates between nonhomologous chromosomes (reviewed in Agarwal et al., 2006). Indeed, instability within, and possibly recombination between, Alu sequences located within the major breakpoint cluster region of the *BCR* and *ABL* genes has been implicated in the formation of the *BCR-ABL* fusion tyrosine kinase gene driving Ph<sup>+</sup> leukemias and lymphomas (Chissoe et al., 1995; Jeffs et al., 1998; 2001; Papadopoulos et al., 1990; Zhang et al., 1995). Moreover, elegant work by Maria Jasin and colleagues has shown that even limited homologous sequence may be sufficient to promote interchromosomal HR resulting in translocations (Richardson and Jasin, 2000; Richardson et al., 1998; 1999; Tremblay et al., 2000). Given that translocations appear to frequently produce novel fusion genes, this latter mechanism may constitute a major mode of chromosomal instability leading to cancer, especially in blood cancers where reciprocal translocations are common.

#### 4.3.1.5 Homologous Recombination Genes and Proteins

The eukaryotic HR machinery includes a number of DNA damage sensor and early response genes, such as members of the *Rad51* gene family, the *Rad52* epistasis group, *RecQ* family helicases, and the breast cancer susceptibility genes *Brca1* and *Brca2*, as well as numerous other accessory factors.

##### Damage Sensing

The first step in DSB repair, by either DSB repair pathway, is DNA damage sensing and signal transduction. The earliest steps of DNA break response initiate a signal through the MRE11–RAD50–NBS1 (MRN) complex and the ataxia telangiectasia-mutated (ATM) protein. The MRN complex likely plays several crucial roles in DNA break repair, including structural bridging of DNA ends to permit rejoicing of cognate ends by NHEJ machinery, upstream activation of ATM, and execution of ATM-dependent downstream effector functions (Dudas and Chovanec, 2004). Both *Rad50* and *Nbs1* are essential genes in mammals, and thus genetic manipulation of these genes has proven difficult although specific mutant alleles of each gene have provided information as to their molecular function (Dudas and Chovanec, 2004). Introduction of hypomorphic alleles of *Rad50* or *Nbs1* results in decreased ATM kinase activity, implicating the MRN complex in full ATM activation in response to damage. However, the precise mechanism for this activity upstream of ATM remains unclear.

ATM represents another key factor involved in early DNA damage response and the coordination of downstream pathways. ATM, together with the *Atm*- and *Rad3-related* gene product ATR, and the DNA-dependent protein kinase catalytic subunit, DNA-PKcs, defines a family of PI3-kinase-related kinases that are critical for responding to and repairing genotoxic stress of many types. ATM is thought to exist as a homodimer that can undergo autophosphorylation

and subsequent dissociation following damage recognition (McGowan and Russell, 2004). The dissociated, active monomers then target additional downstream effectors, likely including MRN components, p53, and histone H2A variant X (H2AX). In mammalian cells, ATM is activated in this fashion early in the response to as few as two DNA DSBs. One key piece of evidence implicating ATM as a regulator of HR is the observation that mice doubly deficient for *Atm* and for the HR gene *Rad52* exhibit a mild protection from cancer (Treuner et al., 2004). This intriguing finding suggests that, in the absence of *Atm*, a compensatory HR-mediated DSB repair pathway may carry the risk of oncogenic translocations. Moreover, it has been recently shown that NHEJ and HR cooperate to maintain post-replicative genome stability (Couedel et al., 2004; Mills et al., 2004). Such cooperative DSB repair may be under the control or coordination of ATM. Interestingly, one recent study also found that the ATM protein plays a direct role in stabilizing the DNA DSBs that are an obligate predecessor of V(D)J recombination (Bredemeyer et al., 2006). It was suggested that the combined cell cycle checkpoint, pro-apoptotic, and DSB repair functions of ATM collaborate to ensure efficient and correct V(D)J recombination and to eliminate those cells that fail in this process.

ATR, together with its binding partner ATRIP, appears to be key checkpoint factor responsive to stalled or collapsed replication forks, as well as other damage incurred during the replicative or post-replicative phases of the cell cycle (Cortez et al., 2001; Costanzo et al., 2003; McGowan and Russell, 2004; Shechter et al., 2004; Zou et al., 2002). While the precise mode of ATR activation is not fully elucidated, it is likely stimulated upon binding, through ATRIP, to replication protein A (RPA) complexed to ssDNA. Such RPA–ssDNA complexes are both replication-associated and HR-associated, in the latter case forming at an early stage of the recombination reaction. Although ATR is an essential factor, thus precluding the analysis of knockout mice, cells containing hypomorphic alleles or siRNA-mediated knockdown of ATR exhibit significant defects in cellular proliferation and are uniquely susceptible to replication stress or stalling. Cells with impaired ATR function are highly prone to spontaneous breakage of common chromosomal fragile sites and show a hyper-response to the replication inhibitor aphidicolin, a DNA polymerase inhibitor (Casper et al., 2002).

### Rad51 Family

In vertebrates, RAD51 is an essential HR factor constituting the core factor in most, if not all, HR reactions. In mammals, the *Rad51* gene family comprises at least six members: *Rad51*, *Rad51L1*, *Rad51L2*, *Rad51L3*, *Xrcc2*, and *Xrcc3*. *Rad51* encodes a single-strand DNA-binding factor that forms a nucleoprotein filament with HR donor sequences necessary for heteroduplex formation. In the initial phase of an HR reaction, RPA binds 3'-resected DNA ends (see above) to form a precursor nucleoprotein filament. In the second phase of the reaction, RPA is displaced by RAD51, and the newly formed RAD51–DNA

filament then promotes DNA strand transfer and heteroduplex formation with the complementary target sequence, producing a D-loop. RAD51 is thus essential for initiation and probably for completion of HR. In mice, null alleles of *Rad51* result in very early embryonic lethality (Lim and Hasty, 1996; Tsuzuki et al., 1996). In the chicken DT40 cell line, deficiency for *Rad51* results in profound defects in HR, accompanied by decreased proliferation, spontaneous chromosomal instability, and dramatic sensitivity to a range of DNA-damaging agents, including DSB-inducing agents (Sonoda et al., 1998).

The precise roles of the other *Rad51* family members in HR are less certain. All family members show single-strand DNA binding and DNA-stimulated ATPase activities (Braybrooke et al., 2000; Lio et al., 2003; Masson et al., 2001a, b; Sigurdsson et al., 2001), but these activities are, in some cases, dispensable for HR function. Data from numerous in vitro experiments have suggested that various complexes, comprising subsets of the *Rad51* family members, can stimulate RAD51-dependent strand pairing/transfer, and at least RAD51L2 may also facilitate dissociation of double-stranded DNA (Kurumizaka et al., 2001; 2002; Lio et al., 2003; Liu et al., 1998; 2002; 2007; Masson et al., 2001a; Sigurdsson et al., 2001). Finally, some evidence has implicated the RAD51-like proteins in Holliday junction resolution although this role remains controversial [for example, see Liu et al. (2004)].

### The Rad52 Epistasis Group

*Rad52*, the archetypal member of the *Rad52* epistasis group, is a centrally important HR factor in yeast, but does not appear to play a critical function in HR reactions in higher eukaryotes (Dudas and Chovanec, 2004). The basis for this difference is not yet understood, but one possibility, for which there is some evidence, is the existence of additional, as yet unidentified, *Rad52* homologs that are more closely functionally related to the yeast *Rad52*. Other members of the *Rad52* epistasis group include *Rad54*, *Rad55*, and *Rad59* (reviewed in Heyer et al., 2006). RAD52 functions to promote annealing of complementary stretches of ssDNA and apparently acts as an accessory factor mediating RAD51-dependent heteroduplex formation by promoting the loading of RAD51 onto DNA at the target recombination site (Benson et al., 1998; New et al., 1998; Shinohara and Ogawa, 1998; Sung, 1997). It has also been suggested, largely based on analysis of yeast mutants, that RAD52 antagonizes the HR-repressive effects of RPA at sites of damage and stabilizes RAD51 nucleoprotein complexes, thereby shifting the DNA-binding equilibrium toward RAD51 and away from RPA.

A second member of the *Rad52* epistasis group, *Rad54*, encodes a *Snf2* family chromatin remodeling factor central to the *Rad52* epistasis group of proteins, with DNA-dependent ATPase activity (Heyer et al., 2006). Upon binding to DNA, RAD54 translocates along the double strand, via ATP hydrolysis. During the presynapsis phase of an HR reaction, RAD54 appears to promote the replacement of RPA with RAD51 in the presynaptic nucleoprotein filament.

Next, during synapsis, RAD54 interacts with RAD51 to stimulate joint molecule formation critical for D-loop formation and strand exchange. Finally, RAD54 appears to participate in branch migration and may also function to disassemble the RAD51 nucleoprotein filament although this latter role is somewhat controversial (Heyer et al., 2006). Despite RAD54 being a central player in RAD51-dependent HR, *Rad54* knockout mice are viable and are not, on their own, especially cancer prone. Cells derived from *Rad54* null mice proliferate nearly as well as their normal counterparts but exhibit substantial sensitivity to DNA-damaging agents such as IR or mitomycin-C (MMC)-induced interstrand cross linking. In the latter case, *Rad54*-deficient cells show normal rates of spontaneous SCE but are severely compromised in MMC-induced SCE. For a comprehensive review of *Rad54* function, see Heyer et al. (2006).

### RecQ Helicase Family

The *RecQ* helicase family constitutes a highly conserved set of DNA-directed helicases that may be found in prokaryotes and eukaryotes. The human genome contains at least five genes encoding *RecQ* family members: *BLM*, *RECQL*, *RECQL*, *RECQL*, *RECQL*, and *WRN*. Mutations in *BLM*, *RECQL*, and *WRN* have been associated with Bloom's, Rothmund-Thompson, and Werner's syndromes, respectively. Each of these disorders exhibits a dramatic cancer predisposition and some aspects of premature aging. This latter feature has generated tremendous interest in the RecQ helicase family as a potential link between cancer and aging.

#### BLM

The BLM helicase, which interacts with TOPOIII, is capable of unwinding short stretches of naked duplex DNA but is highly stimulated by association with RPA. A number of potential roles in HR have been ascribed to the BLM helicase, but the details of its *in vivo* molecular functions still remain unclear. It is thought that BLM suppresses hyper-SCE perhaps by disruption of D-loop recombination intermediates. Indeed, BLM appears to favor specific structures, including D-loops and Holliday junctions, and has been shown to promote Holliday junction branch migration. As noted above, crossing over appears to be generally suppressed during homology-mediated recombination, and BLM may participate in this suppression. In this context, another possible function for BLM is to modulate the outcome of Holliday junction resolution. This is likely accomplished by BLM-dependent processing of double Holliday junctions to catenated structures that can be fully resolved via TOPOIII $\alpha$ . BLM-mediated crossover suppression may also involve SDSA. Recent data have suggested that in addition to disrupting recombination by D-loop binding, BLM can also channel these HR intermediates into the SDSA pathway by dissolution of the heteroduplex to permit re-annealing of the newly synthesized strand with its original complementary strand, at least in *Drosophila* (Weinert and Rio, 2007).

## WRN

Unlike other members of the RecQ helicase family, WRN contains both the classical helicase activity and a 3'- to 5'-exonuclease activity that target multiple DNA or RNA–DNA hybrid structures (see Dudas and Chovanec, 2004; Helleday et al., 2007; Hickson, 2003). Like BLM, the helicase activity of WRN appears to also be highly structure specific. The observation that cells from Werner's syndrome patients show defects in recombination intermediate resolution suggests that WRN functions in HR, probably by influencing DSB repair pathway choice. In this context, WRN can bind to NBS1, a member of the MRN complex, and to the Ku70/Ku80 heterodimer, a core NHEJ complex.

In addition to its role in HR, WRN is thought to function in a nonessential fashion in DNA replication. Such a role is suggested by WRN association with a series of replication factors, including PCNA and RPA. Moreover, WRN appears to stimulate the replication-associated flap endonuclease activity of FEN1 and may thus promote the processing of normal Okazaki fragments. Finally, some evidence indicates that WRN also plays a specialized role in telomere replication by disruption of G-quadruplex stretches (Dudas and Chovanec, 2004; Helleday et al., 2007; Hickson, 2003).

### 4.3.1.6 Homologous Recombination and Cancer

#### Ataxia Telangiectasia

AT is a rare autosomal recessive disorder caused by defects in *Atm* (described above) that produces progressive cerebellar degeneration with a characteristic ataxic gait, stereotypical oculocutaneous telangiectasia, and several growth and developmental abnormalities (reviewed in Matei et al., 2006; Taylor and Byrd, 2005). Another signature feature of AT is a marked, but variable, immunodeficiency, leading to frequent upper respiratory infections. AT is also associated with a severe sensitivity to IR and a very high risk of cancers, especially of lymphoid origin (reviewed in Matei et al., 2006; Taylor and Byrd, 2005).

The immunological defects associated with AT result from a range of both cellular and humoral immune defects that produce a variable, sometimes severe, lymphopenia (Waldmann et al., 1983). AT produces marked thymic hypoplasia and a shift in the representation of specific T-cell subsets (reviewed in Matei et al., 2006). It has been suggested that the low peripheral T-cell numbers are the combined result of low thymic output, perhaps as a result of the hypoplastic state, and a gross T-cell survival defect in the periphery (Matei et al., 2006). AT patients also exhibit humoral immunity defects, presenting as reductions in specific Ig isotypes. Initial models attributed the humoral defect principally to a failure of T-cell help, but more recent studies have also implicated *Atm* in IgH CSR (Lumsden et al., 2004; Reina-San-Martin et al., 2004).

In addition to the T cell, and to a lesser extent, associated B-cell developmental defects, AT patients also show a dramatic predisposition to T-cell malignancies, suggesting, perhaps, a common molecular origin for both

features. Relative to nonaffected individuals, AT patients exhibit a roughly 200-fold higher risk of lymphoid cancers, especially early in life. Consistent with the immunological defects, T-cell tumors in AT patients are approximately four-fold more frequent than B-cell tumors. During childhood, the major AT-associated tumor type is T-cell acute lymphoblastic leukemia or lymphoma (T-ALL), with a shift toward pro-lymphocytic leukemia as patients enter adulthood (Taylor et al., 1996). These disease features, together with the observation that leukemias arising in adult AT patients tend to have a more mature T-cell phenotype, suggest that ATM acts as a lymphoid tumor suppressor at many thymic and post-thymic stages of T-cell development (reviewed in Matei et al., 2006).

### ATM and Sporadic Cancers

#### *Fanconi Anemia*

Fanconi anemia (FA) is a rare cancer predisposition syndrome that features a progressive anemia, chromosomal instability, and developmental abnormalities (reviewed in Tischkowitz and Dokal, 2004). FA has been associated with genes defining at least 12 FA complementation groups: A, B, C, D1, D2, E, F, G, I, J, L, and M (FANCA–FANCM) (Dokal, 2000). These genes have been identified largely through functional cloning in FA cell lines but also by candidate gene screening and positional cloning approaches. Cells from FA patients, like other HR-deficient cells, show dramatic hypersensitivity to DNA interstrand cross-linking (ICL) agents like MMC, but the precise role of FA gene products in mediating ICL repair is, as yet, unclear (Tischkowitz and Dokal, 2004). *FANCD1* is allelic with the breast cancer susceptibility gene *BRCA2*, and the *FANCD2* protein interacts with DNA damage sensor/checkpoint factors ATM and ATR, suggesting that the FA pathway may intersect multiple modes of DNA DSB repair, including HR.

Clinically, FA results in early aplastic anemia with a propensity for total bone marrow failure, skeletal abnormalities, skin pigmentation defects, and a host of less frequent developmental deficiencies. FA patients also show a dramatic predisposition to cancers, mainly of hematopoietic origin. One study found that 23% of FA patients develop some type of blood-related malignancy, with nearly 70% of these malignancies being either myelodysplastic syndrome (MDS) or AML (Mathew, 2006). Less frequently, FA patients may develop solid tumors of the liver, head and neck, vulva, or cervix. Some studies have also investigated somatic inactivation or mutation of FA genes in sporadic AML cases, finding monoallelic mutations in approximately 5–10% of sporadic cases.

### Variant Alleles of Rad51-Family Genes

Given the apparently central roles that RAD51 and RAD51-like proteins play in the maintenance of genome integrity via HR-mediated DSB repair, it is

reasonable to expect that the *Rad51*-family genes should also act as tumor suppressors. While there has thus far been a paucity of evidence for a tumor-suppressive role in model systems, there is some indication that naturally occurring variants of *Rad51*-like genes can modulate cancer susceptibility (Auranen et al., 2005; Figueiredo et al., 2004; Han, et al., 2004; Kuschel et al., 2002; Rafii et al., 2002; Rodriguez-Lopez et al., 2004; Thacker, 2005; Wang et al., 2001). However, in most cases, a relatively small sample size has precluded a definitive evaluation of the cancer risk conferred by the variant allele. A number of studies have identified a variant allele of *Xrcc2*, in which Arg188 is converted to His (*Xrcc2*<sup>R188H</sup>), as potentially increasing the susceptibility to breast cancer (Kuschel et al., 2002; Rafii et al., 2002). Similarly, a variant of *Rad51L3* (*Rad51L3*<sup>E233G</sup>) may be weakly associated with increased breast cancer risk (Rodriguez-Lopez et al., 2004). Although the *Xrcc3*<sup>T241M</sup> variant was initially reported to correlate with increased melanoma risk, this association remains controversial (Araujo et al., 2002; Rafii et al., 2002). Thus far, none of the variant alleles of *Rad51*-like genes have been reported to increase the risk or susceptibility to hematologic malignancies.

### Xrcc2 Deletions

In addition to potentially deleterious alleles of *Xrcc2* that may predispose to some cancers, somatic rearrangements such as deletion or translocation may alter or inactivate *Xrcc2* specifically in cancer cells. The human *Xrcc2* gene is located on chromosome 7q36, a genomic location frequently affected by structural lesions in a broad range of cancers. A survey of Recurrent Aberrations in the Mitelman Database of Chromosome Aberrations in Cancer (Cancer Genome Anatomy Project; <http://cgap.nci.nih.gov/Chromosomes/Mitelman>) reveals 84 case reports of copy number gains at 7q36, with 51 of these occurring in hematopoietic malignancies, and 80 case reports of deletions affecting 7q36, with 76 of these occurring in hematopoietic malignancies. While these lesions are likely to encompass numerous genes and the specifically relevant gene(s) have not been definitively identified, it is possible that either gains or losses of *Xrcc2* could contribute to oncogenic genome instability by altering the stoichiometry of HR complexes.

#### 4.3.2 Nonhomologous End Joining

The second DSB repair pathway, NHEJ, is a pathway that repairs DSBs irrespective of DNA end sequence. Unlike homology-mediated repair pathways, NHEJ does not rely on DNA homology to repair DSBs; therefore, DSBs repaired by the NHEJ pathway may contain errors. It is generally believed that NHEJ is the repair pathway utilized most during G1 in mammalian cells, likely due to the lack of homologous sister chromatids during this phase of the cell

cycle (reviewed in Mills et al., 2003; Valerie and Povirk, 2003). In addition, NHEJ is the major repair pathway of DSBs introduced through a variety of endogenous sources and also contributes to the repair of a vast number of DSBs that occur as a result of exogenous insults. DSBs that escape repair by NHEJ, whether they are introduced as part of a developmental program or through exposure to endogenous or exogenous insults, can often lead to translocations and subsequent oncogenesis. We discuss these mechanisms in the context of hematopoietic cancers below.

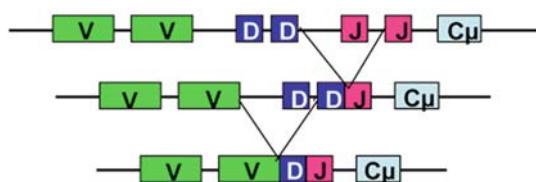
#### 4.3.2.1 V(D)J Recombination

V(D)J recombination is the process by which antigen receptors in maturing B and T lymphocytes are assembled (reviewed in Bassing et al., 2002; Jung and Alt, 2004) (see Fig. 4.5 for overview). Somatic DNA rearrangements of variable (V), diversity (D), and joining (J) gene segments within the Ig or TCR loci generate mature, functional antibody receptors. An initial step in antibody receptor gene rearrangement is the obligatory introduction of DSBs at specific locations within these genes to allow in frame recombination of these different antibody receptor segments. Targeted recombination of V(D)J component parts occurs in a precisely ordered fashion through recognition of specific recombination signal (RS) sequences that flank the constituent gene segments by the recombination-activating gene (RAG) endonuclease.

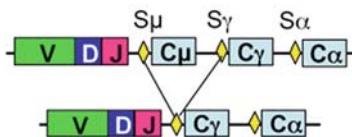
The RS elements are comprised of conserved palindromic heptamer (CACAGTG) and nonamer (ACAAAAAACC) consensus sequences. These are separated by nonconserved spacers of either 12 or 23 base pairs, referred to as 12RS or 23RS sequences (Max et al., 1979; Sakano et al., 1979). Importantly, recombination strongly favors usage of a 12RS sequence and a 23RS sequence, with 12/12 or 23/23 recombination events generally prevented (this phenomenon is referred to as 12/23 restriction). In developing B cells, *IgH* genes are assembled via a highly organized process where D to J rearrangements occur first (DJ), followed by V to DJ joining (VDJ). The IgH locus is arranged such that the J and V segments are flanked by 23RS sequences, while the D segments are flanked by 12RS sequences (Early et al., 1980). This arrangement permits ordered D to J followed by V to DJ recombination, since this recombination pattern follows the 12/23 restriction.

Functional IgH rearrangement then prompts a signal for progenitor B cells to proceed in the B-lymphoid differentiation pathway. Processes that lead to differentiation include allelic exclusion, proliferative expansion, and differentiation to the pre-B-cell stage. In pre-B cells, the Ig light chain loci, Ig $\kappa$ ? and Ig $\lambda$ , also undergo functional gene rearrangements through 12RS/23RS recombination. Functional light chain rearrangements generated through proper recombination events permit further B-cell differentiation. Similar to B-lineage development, the proper joining of TCR locus gene segments through 12RS/23RS recombination permits progression of pre-T cells to mature T lymphocytes.

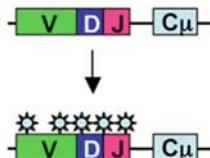
### A. V(D)J recombination



### B. Class switch recombination



### C. Somatic Hypermutation



**Fig. 4.5 Schematic of types of somatic alterations that occur during lymphocyte development.** (A) During V(D)J recombination, rearrangements between V (green rectangles), D (blue rectangles), and J (pink rectangles) gene segments create mature, functional antibody receptors in B and T cells. These rearrangements occur in a precise order, with D to J joins occurring first followed by V to DJ joins. (B) Class switch recombination changes the isotype of the immunoglobulin constant region through constant region (light blue rectangle) rearrangements. Small sequence elements known as switch regions (yellow diamonds) permit recombination between different constant gene segments. (C) In somatic hypermutation, single or multiple mutations are introduced throughout the recombined V(D)J segment (blue sunbursts) to change antibody affinity of a functional receptor. Adapted from Kuppers and Dalla-Favera (2001). (See color insert)

#### 4.3.2.2 RAG Endonuclease

The RAG endonuclease consists of an obligate heterodimeric protein complex encoded by two extremely tightly linked genes, *Rag1* and *Rag2* (Oettinger et al., 1990; Schatz et al., 1989). The genes encoding RAG1/2 are expressed in early progenitor lymphocytes, and expression levels oscillate throughout lymphocyte differentiation and maturation, as RAG1/2 activity is necessary for V(D)J recombination at different stages of B- and T-lineage cell development. It was further shown that another round of RAG expression may occur in mature germinal center B lymphocytes following antigen stimulation (Hikida et al., 1996). It is thought that expression of RAG1/2 in mature lymphocytes contributes to altered antibody specificity through a process termed receptor

editing. RAG2 activity is restricted to cells in G0/G1 phases of the cell cycle through phosphorylation-mediated degradation (Lee and Desiderio, 1999). Mice deficient in either RAG1 or RAG2 show a complete block in lymphocyte development at the progenitor stage with no other apparent physical or morphological defects, illustrating the specificity of RAG1/2 in lymphocyte development (Mombaerts et al., 1992; Shinkai et al., 1992).

RAG1/2 recognizes and binds to recombining 12RS and 23RS sequences and introduces a nick adjacent to the RS sequences to initiate coordinated cleavage. The 3'-hydroxyl group, exposed by the nick, catalyzes a transesterification reaction on an adjacent DNA strand through nucleophilic attack, generating two blunt, 5'-phosphorylated RS ends, which can be ligated, and two covalently sealed hairpin coding ends, which must be opened before joining can occur (McBlane et al., 1995). Interestingly, these two types of ends have distinct features. The RS ends become precisely fused due to the exact sequence homology in the ligated ends, while the ends of the coding segments result in imprecise joining once ligation occurs. Asymmetric nicking by RAG in the coding joins can result in the addition of palindromic nucleotide extensions called P-nucleotides (McCormack et al., 1989). The RAG complex remains stably bound to the four free DNA ends in a post-cleavage synaptic complex (Fugmann et al., 2000). It has subsequently been shown that a catalytic DDE amino acid triad in RAG1 is necessary for the formation of hairpins through direct transesterification (Landree et al., 1999; Swanson, 2001). Mice containing an engineered mutation that deletes the C-terminal portion of RAG2, leaving only the highly conserved core region, exhibit a partial arrest in lymphocyte development with decreased overall numbers of B and T cells (Akamatsu et al., 2003). The specificity of sequence sites recognized by the RAG1/2 endonuclease complex, combined with the spatial and temporal expression pattern, contributes to the infrequency of aberrant cleavage by RAG and, therefore, the precise regulation of V(D)J recombination.

#### 4.3.2.3 Class Switch Recombination and Somatic Hypermutation

Once mature B cells have been stimulated by antigen, two additional somatic alterations to Ig loci may occur to modify antigen-binding affinity and antibody effector function (summarized in Fig. 4.5). Differentiating B lymphocytes first express one IgH constant region ( $C\mu$ ). However, upon antigen stimulation, CSR can occur as the mature B-cell exchanges the  $C\mu$  constant region exon for one of seven alternate downstream exons. This switching, in turn, allows the production of antibodies of a different class and effector function.

Each of the C genes in the IgH locus is preceded by specific DNA sequence elements termed switch regions, consisting of highly repetitive, G-rich, non-template DNA. There is no known consensus target for switch regions. CSR occurs through a deletional recombination mechanism that occurs between two switch regions. CSR differs from V(D)J recombination in that the RAG endonuclease is not required for recombination of switch regions. SHM, on the other

hand, introduces mutations in the V region of both heavy and light chain loci, and these mutations act to increase antigen-binding affinity to enable selection of B cells that produce higher affinity antibodies.

Although the exact mechanism of transcription of switch regions during CSR is not well characterized, it is hypothesized that the switch regions, which exhibit G-rich sequences on the nontemplate strand, are susceptible to unique chromatin conformations that permit transcription of these regions (reviewed in Chaudhuri and Alt, 2004). Interestingly, the switch region transcripts remain stably associated with the template DNA strand, forming a DNA–RNA hybrid, while the displaced nontemplate DNA strand exists as a ssDNA loop. This entire structure is called an R-loop (Reaban and Griffin, 1990; Reaban et al., 1994; Tian and Alt, 2000).

CSR and SHM both require the expression and activity of activation-induced cytidine deaminase (AID) (reviewed in Chaudhuri and Alt, 2004; Longerich et al., 2006). AID is expressed in activated mature B-lymphocytes and specifically targets *Ig* genes. AID deaminates cytosine residues within the Ig loci, converting them to uracils. These uracils are either replicated to introduce corresponding single-nucleotide mutations (in SHM) or are removed (in CSR). Removal of uracil mismatches creates nicks in the DNA strand which, when in close proximity to other nicks in switch regions, leads to DSBs and subsequent recombination between switch regions. On the other hand, in SHM, single mutations can lead to changes in antibody affinity or specificity. At least in SHM, AID requires the cooperative activity of RPA for proper targeting to Ig regions undergoing SHM (Chaudhuri et al., 2004). Mice deficient in AID and humans with hypomorphic AID mutations show no apparent phenotypes except the inability for B cells to undergo CSR and SHM. Mice engineered to overexpress AID were predicted to show increased rates of tumorigenesis because of increased cytosine deamination and subsequent DSB induction; however, thymic lymphomas and epithelial tumors isolated from these mice did not show identifiable chromosomal translocations. Instead, oncogenic transformation in these tumors was found to be due to somatic mutation.

#### 4.3.2.4 Mechanics of Nonhomologous End Joining

The molecular components required for DSB repair by the NHEJ pathway were first identified through analysis of yeast and mammalian cell lines deficient in DNA repair. Analysis of IR-sensitive Chinese hamster ovary (CHO) cell lines led to the identification of several complementation groups (Taccioli et al., 1998; 1994). Furthermore, examination of V(D)J recombination in these cells determined that RAG-induced DSBs introduced during V(D)J recombination were likely repaired by components of the NHEJ pathway.

Conserved core NHEJ components are found in both yeast and vertebrates, while noncore components are found only in vertebrates. Known core NHEJ factors include Ku70 and Ku80, DNA Ligase IV (LIG4), and XRCC4 while noncore components include Artemis (ART), DNA-PKcs, and a recently

identified factor, Cernunnos. It is important to note that these factors are required for both V(D)J recombination in B- and T-lymphocyte development as well as for repair of DSBs that occur throughout the genome as a result of endogenous damage or clastogenic insult.

#### 4.3.2.5 Nonhomologous End Joining Genes and Proteins

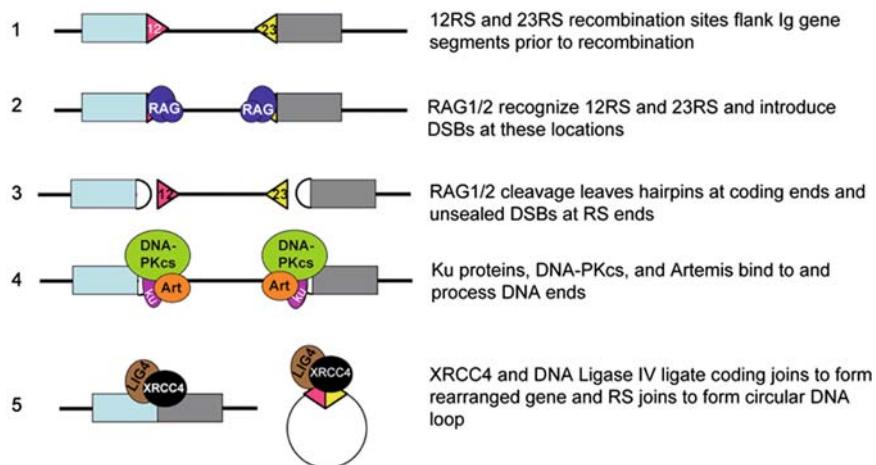
The core components conserved in all eukaryotes are required for both RS join formation subsequent to DSB initiation by RAG as well as for repair of randomly occurring DSBs throughout the genome, while the nonconserved proteins may play additional roles independent of NHEJ (reviewed in Mills et al., 2003; see Fig. 4.6). Further experimentation with IR-sensitive CHO lines led to the characterization of the roles of each NHEJ protein in DSB repair. In vertebrates, Ku70 and Ku80 are predicted, based on the crystal structure, to form a ring-shaped complex that can encompass and shield the broken DNA end until other NHEJ components are recruited to facilitate repair. Binding of the Ku complex to DNA-PKcs permits activation of DNA-PKcs through autophosphorylation, and this heterotrimeric complex is then called the DNA-PK holoenzyme. The activated DNA-PK complex is the first NHEJ component to respond to a DSB, but the precise role this protein complex plays in joining broken DNA ends has not been completely characterized. Possible roles for DNA-PK include protecting broken ends from aberrant enzymatic or endonuclease activity, keeping broken ends close together until ligation and acting as a scaffold to allow other NHEJ factors to bind to and complete DSB repair. However, Ku-deficient ES cells show different joining impairment and IR sensitivity as compared with DNA-PKcs-deficient ES cells, illustrating a DNA-PKcs-independent function for Ku proteins (Gao et al., 1998a).

DNA-PKcs is thought to be important in other cellular applications beyond NHEJ although these functions are not well understood. One of the important roles of DNA-PKcs in NHEJ repair is to recruit and bind to the ART protein. ART was first identified through analysis of two types of human severe combined immunodeficiency (SCID) disorders, SCIDA and RS SCID. ART is homologous to  $\beta$ -lactamases, proteins that repair interstrand DNA crosslinks and is thought to be necessary for end processing of DSBs, including the opening of hairpins that form during the process of NHEJ repair. Both DNA-PK and ART are primarily required for coding, and not RS, joining during V(D)J recombination (Callebaut et al., 2002).

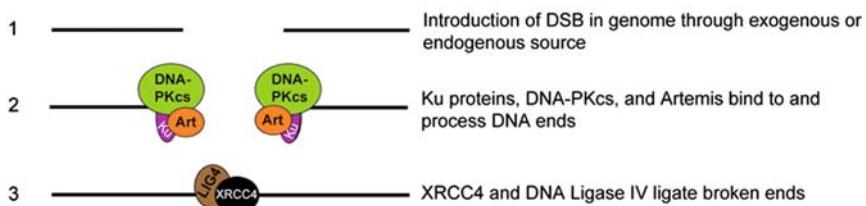
XRCC4 and DNA LIG4 form a heterodimeric complex that is required for ligation of repaired ends. Deficiencies in either protein result in identical phenotypes, including the inability to form coding and RS joins during V(D)J recombination, illustrating the indispensable NHEJ-specific functions of these proteins.

Recently, a seventh component of the NHEJ pathway, Cernunnos (also known as XLF for XRCC4-like factor), was identified independently by two groups (Ahnesorg et al., 2006; Buck et al., 2006). Knockdown of the gene encoding the

### A. NHEJ and V(D)J Recombination



### B. NHEJ and General DNA Double Strand Break Repair



**Fig. 4.6 Roles of nonhomologous end joining (NHEJ) in repair of programmed and unprogrammed exogenously induced DNA double-strand breaks (DSBs). (A)** NHEJ is required for gene rearrangements that occur during V(D)J recombination. The RAG1/2 endonuclease complex recognizes recombination signal (RS) sequences that flank Ig gene segments to be recombined (steps 1 and 2). RAG cleavage induces covalently sealed hairpin formation at the DNA ends of the coding segments and leaves the DNA fragments containing RS sequence ends unsealed (step 3). DNA-PKcs, the Ku70/Ku80 complex (Ku), and Artemis (Art) recognize the DSB and process the coding hairpin ends to prepare for ligation (step 4). In the last step, both coding joins and RS sequence end joins are ligated separately, leaving a rearranged coding gene segment and a circular loop containing the RS sequences and intervening DNA (step 5). **(B)** NHEJ is also required to repair DSBs throughout the genome that occurs as a result of unprogrammed endogenous or exogenous insults (step 1). The NHEJ factors and mechanism of DSB repair for unprogrammed DSBs are identical to those utilized in programmed DSB repair (steps 2 and 3).

Cernunnos protein, *NHEJ1*, in human cell lines leads to radiosensitivity and impaired NHEJ-mediated DSB repair similarly to that observed in other NHEJ-deficient cell lines (Ahnesorg et al., 2006). In addition, murine ES cells engineered to lack Cernunnos activity do not undergo V(D)J recombination and show defects in forming both coding joins and RS joins (Zha et al., 2007). Furthermore, Cernunnos-deficient ES cells show increased IR sensitivity and have intrinsic defects in DSB repair, providing further evidence that Cernunnos/XLF is an important component of NHEJ-mediated DSB repair (Zha et al., 2007). Immunoprecipitation experiments have since established that Cernunnos interacts directly with the XRCC4/LIG4 complex (Callebaut et al., 2006).

### Nonhomologous End Joining Deficient Mouse Models

Mice deficient for each of the NHEJ components, with the exception of Cernunnos, have been identified or engineered. These mouse models have proved valuable tools in understanding the role of NHEJ in different cell types and how similar NHEJ deficiencies can result in human disease. Mice deficient in DNA-PKcs, Ku70, Ku80, or ART show severe immune deficiencies due to the lack of NHEJ during V(D)J recombination that results in the absence of mature B- and T-cell lymphocytes and show increased sensitivity to IR (Bosma and Carroll, 1991; Gao et al., 1998a; Gu et al., 1997; Kurimasa et al., 1999; Nussenzweig et al., 1996; Ouyang et al., 1997; Rooney et al., 2002; Taccioli et al., 1998). In addition, it was found that classical SCID mice have a mutation within DNA-PKcs (Bosma and Carroll, 1991). A deficiency in either Ku70 or Ku80 results in small mice with additional defects, including cell proliferation defects, premature senescence, and increased neuronal apoptosis, although mice deficient in either Ku protein are viable (Gu et al., 1997; Nussenzweig et al., 1996; Ouyang et al., 1997). Embryos null for *Lig4* or *Xrcc4*, which exhibit late embryonic lethality, show more severe phenotypes, including impaired lymphocyte development (as determined in cells or by *in vitro* analysis of fetal liver cultures isolated from null embryos) and severe neuronal apoptosis (Barnes et al., 1998; Frank et al., 1998; Gao et al., 1998b). Mouse embryonic fibroblasts (MEFs) isolated from *Xrcc4*-/- or *Lig4*-/- embryos show marked genomic instability, including increased sensitivity to irradiation and chromosomal translocations.

Cells containing unrepaired DSBs normally undergo apoptosis mediated by the checkpoint protein p53, encoded by the *Trp53* gene in mice and *TP53* gene in humans. Interestingly, *Trp53* deficiency rescues viability in *Lig4*- and *Xrcc4*-deficient mice and prevents the neuronal apoptosis observed in the single mutant mice, suggesting that the neurodevelopmental failure and the embryonic lethality may be linked. However, NHEJ repair in developing lymphocytes in these doubly deficient mice is still compromised, producing a SCID phenotype and eventually leading to the development of progenitor (pro) B-cell lymphomas through translocations involving the IgH locus and the oncogene *c-myc*. Similarly, mice doubly null for the gene encoding ART, *Dclre1c*, and

*Trp53* also develop pro-B-cell lymphomas (Rooney et al., 2004). Interestingly, mice doubly null for both *Ku80* and *Lig4* are viable (Karanjawala et al., 2002). This unexpected result suggests that loss of *Ku80* function may initiate other DSB repair pathways, since the embryonic lethality observed in the NHEJ-specific factor *Lig4* single-deficient mice is rescued when combined with *Ku80* deficiency.

As described, deficiencies in NHEJ proteins often result in B-lineage lymphomagenesis, since the DSBs introduced during V(D)J recombination cannot be properly repaired and often act as substrates for translocations that contribute to oncogenesis. Based on this outcome, NHEJ factors have been proposed to function as tumor suppressors in developing lymphocytes. However, recent work has established that one NHEJ component, ART, plays an important tumor suppressor role in additional somatic tissues beyond developing lymphocytes, at least in a *Trp53* compromised context (Woo et al., 2007). This result provides convincing evidence to support a role for NHEJ in widespread tumor suppression, independent of its role in V(D)J recombination.

#### 4.3.2.6 Nonhomologous End Joining and Telomere Maintenance

Eukaryotic cells, which contain linear chromosomes, have developed a process to readily distinguish the ends of normal chromosomes from DSBs within the genome that require repair. Telomeres are structural components comprised of short repeated DNA elements and scaffolding proteins, such as shelterin and TRF2, which terminate the ends of linear chromosomes and protect against structural abnormalities such as chromosome fusion and attrition (reviewed in Murnane and Sabatier, 2004). Telomeres are established and maintained through the activity of telomerase, a specialized reverse transcriptase required for replication of the highly repetitive telomere sequences that cap eukaryotic chromosomes. In normal cells, expression and activity of telomerase diminishes over time. However, transfection of normal human cells with telomerase results in cessation of telomere shortening and immortalization, providing evidence that telomere shortening and attrition directly correlate with cell aging and senescence (Bodnar et al., 1998). Although normal hematopoietic stem cells exhibit typical telomere shortening, they are one of the few somatic cell types to exhibit prolonged telomerase activity, and the significance of this finding is not well understood.

Surprisingly, mice engineered to lack telomerase activity are viable. However, each subsequent generation of telomerase-deficient mice shows progressively severe defects in chromosome maintenance, apparent as increased genome instability in cells, which could confer tumorigenesis when transplanted into nude mice (Blasco et al., 1997). When a telomerase deficiency is combined with *Trp53* deficiency, normally unstable DNA intermediates and structures such as chromosome end-to-end fusions and breakage–fusion–bridge (BFB) cycles are maintained through subsequent cell cycles. Although at early stages a *p53* deficiency rescues the adverse effects observed in telomere-deficient mice,

mice doubly deficient for these genes subsequently show increased rates of genome instability that result in tumorigenesis (Chin et al., 1999).

Chromosome ends that lack proper telomeres can become a substrate for NHEJ repair. Although the exact roles NHEJ factors may play in telomere maintenance remain to be fully determined, recent work has established that at least two NHEJ components play important roles in protecting telomere structure and function. During NHEJ repair, the ring-shaped Ku70/Ku80 complex binds to free DNA ends (Walker et al., 2001). Apparently contradictory evidence suggests that this complex also binds to telomere components and can either promote normal telomere maintenance and prevent end-to-end fusions (Hsu et al., 1999; 2000; Samper et al., 2000) or can facilitate fusion of dysfunctional telomeres (Celli et al., 2006). In a further paradox, binding of the Ku70/Ku80 complex to telomeres appears to protect chromosome ends with dysfunctional telomeres from being repaired by other DNA repair mechanisms such as homology-mediated recombination (Celli et al., 2006). LIG4, another NHEJ-specific factor, prevents end-to-end chromosome fusions in mice with dysfunctional telomeres. Together, these results implicate NHEJ factors in normal telomere maintenance although the precise mechanism by which this occurs remains elusive.

#### 4.3.2.7 Nonhomologous End Joining and Cancer

Several human disorders are associated with hypomorphic or null alleles of different *NHEJ* genes. Many such alleles result in human SCID phenotypes as a consequence of improper V(D)J function. Mutant alleles of the human *DCLRE1C* gene, encoding the ART protein, cause SCID-A and RS-SCID, and there is some evidence to suggest that hypomorphic *DCLRE1C* alleles are correlated with increased predisposition to lymphoma (Ege et al., 2005; Moshous et al., 2001; 2003a, b; Nicolas et al., 1998). Another condition commonly associated with mutant alleles of both *DCLRE1C* and *RAG1/2* is Omenn syndrome. Hypomorphic missense mutations in the *RAG* genes have been identified in several families and were found to severely restrict, but not entirely eliminate, RAG function, permitting some lymphocyte development and subsequent activity in Omenn syndrome patients (Villa et al., 1998). However, the severity of SCID-like symptoms varies between patients, and it is unclear whether these differences can be attributed directly to RAG function or whether some of this variability is a result of other polymorphic modifier genes. A subset of Omenn syndrome patients do not have mutations within the *RAG* genes, indicating that mutations in other genes, including *DCLRE1C*, may cause Omenn-like symptoms (Gennery et al., 2005).

Mutations in core NHEJ components resulting in human disorders are far less common. LIG4 syndrome, an extremely rare autosomal recessive disorder caused by mutations within the *LIG4* gene, is characterized by immunodeficiency and associated microcephaly, growth retardation, and developmental delay (O'Driscoll et al., 2001). However, only a few cases of LIG4 syndrome

have been reported. Of note, not all of the patients who exhibit these symptoms have mutations within any known NHEJ factor, hinting at the possibility of as yet unidentified factors required for this pathway. Finally, the gene encoding for Cernunnos was originally identified in patients who exhibited phenotypes reminiscent of the ones observed in other NHEJ- or DSB repair-deficient disorders, including microcephaly, mild to severe SCID phenotypes, and radiosensitivity (Buck et al., 2006).

In addition to SCID phenotypes, many other lymphocyte disorders in humans have been attributed to genomic lesions similar to those observed in NHEJ-deficient mouse models (reviewed in Franco et al., 2006). In both *Dclre1c/Trp53*-and *Lig4/Trp53*-deficient mice, progenitor B-cell lymphomas develop with characteristic fusions between *Igh* and *Myc* loci as a result of the inability to properly undergo V(D)J recombination (Rooney et al., 2002; 2004; Zhu et al., 2002). Similarly, many human lymphoid tumors carry translocations involving the fusion of Ig or TCR loci to proto-oncogenes. In addition, lesions that lead to hematopoietic malignancies have been identified in the chromosomal locations associated with each gene encoding a member of the NHEJ pathway, suggesting that mutations or translocations in these genes may cause hematologic malignancies. Analysis of mice deficient in various NHEJ components could provide insight into similar malignancies in humans.

### Susceptibility of Tumorigenesis During V(D)J Recombination and CSR

DSBs are intermediate products of both V(D)J and CSR reactions, which can serve as substrates for chromosomal rearrangements and translocations if left unrepaired (reviewed in Aplan, 2006; Weinstock et al., 2006b). Hematopoietic malignant transformations resulting from these processes are likely due to the high frequency of specific gene alterations and rearrangements that occur throughout development and maturation of these cell types. Translocations that occur in human hematopoietic malignancies are usually simple reciprocal translocations, often involving either the *Ig* or the *TCR* loci, which bring the translocated proto-oncogene in close proximity to strong enhancers within these loci. This process makes oncogenic transformation a rapid step that does not result in high levels of general genome instability in these tumors.

Burkitt's lymphoma is characterized by a recurrent t(8;14) translocation. As in the mouse models of NHEJ deficiency, the translocation breakpoint brings regions of the *IgH* locus on Chr 14 in close proximity with Chr 8, containing the *Myc* locus. The initial breaks within *IgH* likely result from mistakes during V(D)J recombination. Sequencing of translocation junctions of human patients diagnosed with endemic Burkitt's lymphoma revealed that many of these tumors contain breakpoints within V or J regions of *IgH*, indicating the translocation occurred in the initial steps of V(D)J recombination.

Translocation data retrieved from the Mitelman breakpoint database identified 90 human patients diagnosed with mature B-cell neoplasms and 40 diagnosed with ALLs that showed a characteristic translocation containing

*IgH* and the potent oncogene *BCL2*. Analysis of tumors from patients diagnosed with follicular lymphoma, a type of tumor arising in mature B cells, also showed translocation breakpoints between *IgH* and *BCL2* (reviewed in Koppers and Dalla-Favera, 2001; Mills et al., 2003). Molecular examination of the breakpoint sequences of these tumors revealed that many of these joins are often within an RS sequence near a D region. Furthermore, these joins show germline sequence at the 5'-end of the D region involved in the breakpoint, suggesting that these breaks were introduced during de novo V(D)J formation and not mediated through DSBs introduced at already rearranged V(D)J joins. There is some evidence to suggest that the oncogene partner chosen in translocations involving *IgH* may be dependent on the developmental stage of B-cell development. Mantle-zone lymphomas, which typically show t(11;14) (*BCL1/IgH*) translocations, show J joins similar to those in normal B cells, while follicular lymphomas with t(14;18) translocations and *BCL2* amplifications show preferential usage of the most downstream J and D genes, indicating that the cells may have been undergoing a secondary D to J rearrangement (Jager et al., 2000; Welzel et al., 2001).

Another type of Burkitt's lymphoma, sporadic Burkitt's lymphoma, is associated with a similar t(8;14) translocation. However, in this case, the translocation breakpoint maps to a different region of the *IgH* locus, often occurring within the switch regions. This finding indicates that these tumors generally develop from RAG-induced DSBs that are introduced during CSR. Many different lymphoid tumor types, including B-cell chronic lymphocytic leukemia, diffuse large cell lymphoma, extranodal lymphoma, and multiple myeloma, have translocation breakpoints within the switch regions on Chr 14 (reviewed in Koppers and Dalla-Favera, 2001). Together, these results indicate that misrepair of DSBs introduced at any stage of lymphoid development can lead to the development of lymphoid tumors.

#### 4.4 Perspective

The genome is at nearly constant risk of damage from a barrage of internal and external insults. Such damage is a substantial threat to genetic integrity, carrying the risk of gene loss, mutation, or chromosomal rearrangements including translocations. Each of these events can lead to cell death or neoplastic transformation. To preserve genomic integrity and resist the potentially deleterious downstream effects of DNA damage, eukaryotic cells employ a wide range of efficient and highly coordinated DNA damage response and repair mechanisms. To effect the repair of DSBs, mammalian cells rely on two predominant, complementary pathways: HR and NHEJ. The combined, and sometimes overlapping, action of these two pathways generally ensures the rapid resolution of DSBs and the restoration of the intact genome. The apparent importance of these pathways in repairing broken DNA suggested that such repair

should thus be critical to prevent the kinds of chromosomal abnormalities so frequently noted in a broad range of cancers. Indeed, there is rapidly burgeoning evidence, garnered to a large extent from highly valuable mouse models, pointing to a tumor-suppressive role for various DSB repair factors. In this context, development of new and better models of DNA damage response and repair defects, and particularly those that faithfully recapitulate human cancer phenotypes, will significantly impact our mechanistic understanding of neoplasia. To date, many of the mouse models of DSB response/repair have relied on gene-targeted inactivation of the gene of interest. While this approach has been highly informative and provided key insights into the function of specific genes, another important avenue for modeling cancer, including blood cancers, will be the identification or generation of mice that reflect tumor-specific alleles, naturally occurring allelic variants, specific polymorphisms, and complex multi-gene interactions. With the rapidly expanding molecular, genetic, and informatic tools that can be brought to bear on cancer models of all types, these challenges become increasingly tractable to solve. With this perspective in mind, important new discoveries concerning the roles of DSB repair in preventing or promoting blood cancers hold the promise of significant clinical impacts, now and in the future.

**Acknowledgments** We thank Dr. Sophie La Salle for critical review of this manuscript.

## References

- Agarwal S, Tafel AA, Kanaar R (2006). DNA double-strand break repair and chromosome translocations. *DNA Repair (Amst)* 5: 1075–81.
- Ahnesorg P, Smith P, Jackson SP (2006). XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell* 124: 301–13.
- Akamatsu Y, Monroe R, Dudley DD, Elkin SK, Gartner F, Talukder SR et al. (2003). Deletion of the RAG2 C terminus leads to impaired lymphoid development in mice. *Proc Natl Acad Sci U S A* 100: 1209–14.
- Aplan PD (2006). Causes of oncogenic chromosomal translocation. *Trends Genet* 22: 46–55.
- Araujo FD, Pierce AJ, Stark JM, Jasin M (2002). Variant XRCC3 implicated in cancer is functional in homology-directed repair of double-strand breaks. *Oncogene* 21: 4176–80.
- Arlt MF, Durkin SG, Ragland RL, Glover TW (2006). Common fragile sites as targets for chromosome rearrangements. *DNA Repair (Amst)* 5: 1126–35.
- Auranen A, Song H, Waterfall C, Dicioccio RA, Kuschel B, Kjaer SK et al. (2005). Polymorphisms in DNA repair genes and epithelial ovarian cancer risk. *Int J Cancer* 117: 611–8.
- Bachrati CZ, Borts RH, Hickson ID (2006). Mobile D-loops are a preferred substrate for the Bloom's syndrome helicase. *Nucleic Acids Res* 34: 2269–79.
- Bachrati CZ, Hickson ID (2003). RecQ helicases: suppressors of tumorigenesis and premature aging. *Biochem J* 374: 577–606.
- Bannister LA, Schimenti JC (2004). Homologous recombinational repair proteins in mouse meiosis. *Cytogenet Genome Res* 107: 191–200.
- Barnes DE, Stamp G, Rosewell I, Denzel A, Lindahl T (1998). Targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. *Curr Biol* 8: 1395–8.

- Bassing CH, Swat W, Alt FW (2002). The mechanism and regulation of chromosomal V(D)J recombination. *Cell* 109 Suppl: S45–55.
- Benson FE, Baumann P, West SC (1998). Synergistic actions of Rad51 and Rad52 in recombination and DNA repair. *Nature* 391: 401–4.
- Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA et al. (1997). Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* 91: 25–34.
- Bloomfield CD, Lawrence D, Byrd JC, Carroll A, Pettenati MJ, Tantravahi R et al. (1998). Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. *Cancer Res* 58: 4173–9.
- Bohdnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB et al. (1998). Extension of life-span by introduction of telomerase into normal human cells. *Science* 279: 349–52.
- Bosma MJ, Carroll AM (1991). The SCID mouse mutant: definition, characterization, and potential uses. *Annu Rev Immunol* 9: 323–50.
- Braybrooke JP, Spink KG, Thacker J, Hickson ID (2000). The RAD51 family member, RAD51L3, is a DNA-stimulated ATPase that forms a complex with XRCC2. *J Biol Chem* 275: 29100–6.
- Bredemeyer AL, Sharma GG, Huang CY, Helmink BA, Walker LM, Khor KC et al. (2006). ATM stabilizes DNA double-strand-break complexes during V(D)J recombination. *Nature* 442: 466–70.
- Buck D, Malivert L, de Chasseval R, Barraud A, Fondaneche MC, Sanal O et al. (2006). Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell* 124: 287–99.
- Callebaut I, Malivert L, Fischer A, Mornon JP, Revy P, de Villartay JP (2006). Cernunnos interacts with the XRCC4 x DNA-ligase IV complex and is homologous to the yeast nonhomologous end-joining factor Nej1. *J Biol Chem* 281: 13857–60.
- Callebaut I, Moshous D, Mornon JP, de Villartay JP (2002). Metallo-beta-lactamase fold within nucleic acids processing enzymes: the beta-CASP family. *Nucleic Acids Res* 30: 3592–601.
- Casper AM, Nghiêm P, Arlt MF, Glover TW (2002). ATR regulates fragile site stability. *Cell* 111: 779–89.
- Celli GB, Denchi EL, de Lange T (2006). Ku70 stimulates fusion of dysfunctional telomeres yet protects chromosome ends from homologous recombination. *Nat Cell Biol* 8: 885–90.
- Chaudhuri J, Alt FW (2004). Class-switch recombination: interplay of transcription, DNA deamination and DNA repair. *Nat Rev Immunol* 4: 541–52.
- Chaudhuri J, Khuong C, Alt FW (2004). Replication protein A interacts with AID to promote deamination of somatic hypermutation targets. *Nature* 430: 992–8.
- Cheok CF, Bachrati CZ, Chan KL, Ralf C, Wu L, Hickson ID (2005). Roles of the Bloom's syndrome helicase in the maintenance of genome stability. *Biochem Soc Trans* 33: 1456–9.
- Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ et al. (1999). p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell* 97: 527–38.
- Chissoe SL, Bodenteich A, Wang YF, Wang YP, Burian D, Clifton SW et al. (1995). Sequence and analysis of the human ABL gene, the BCR gene, and regions involved in the Philadelphia chromosomal translocation. *Genomics* 27: 67–82.
- Cortez D, Guntuku S, Qin J, Elledge SJ (2001). ATR and ATRIP: partners in checkpoint signaling. *Science* 294: 1713–6.
- Costanzo V, Shechter D, Lupardus PJ, Cimprich KA, Gottesman M, Gautier J (2003). An ATR- and Cdc7-dependent DNA damage checkpoint that inhibits initiation of DNA replication. *Mol Cell* 11: 203–13.
- Couedel C, Mills KD, Barchi M, Shen L, Olshen A, Johnson RD et al. (2004). Collaboration of homologous recombination and nonhomologous end-joining factors for the survival and integrity of mice and cells. *Genes Dev* 18: 1293–304.

- de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D et al. (1982). A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. *Nature* 300: 765–7.
- Dokal I (2000). The genetics of Fanconi's anaemia. *Baillieres Best Pract Res Clin Haematol* 13: 407–25.
- Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM et al. (2001). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 344: 1031–7.
- Dudas A, Chovanec M (2004). DNA double-strand break repair by homologous recombination. *Mutat Res* 566: 131–67.
- Early P, Huang H, Davis M, Calame K, Hood L (1980). An immunoglobulin heavy chain variable region gene is generated from three segments of DNA: VH, D and JH. *Cell* 19: 981–92.
- Ege M, Ma Y, Manfras B, Kalwak K, Lu H, Lieber MR et al. (2005). Omenn syndrome due to ARTEMIS mutations. *Blood* 105: 4179–86.
- Elliott B, Jasin M (2002). Double-strand breaks and translocations in cancer. *Cell Mol Life Sci* 59: 373–85.
- Farah JA, Cromie G, Steiner WW, Smith GR (2005). A novel recombination pathway initiated by the Mre11/Rad50/Nbs1 complex eliminates palindromes during meiosis in *Schizosaccharomyces pombe*. *Genetics* 169: 1261–74.
- Ferguson DO, Alt FW (2001). DNA double strand break repair and chromosomal translocation: lessons from animal models. *Oncogene* 20: 5572–9.
- Figueiredo JC, Knight JA, Briollais L, Andrulis IL, Ozcelik H (2004). Polymorphisms XRCC1-R399Q and XRCC3-T241M and the risk of breast cancer at the Ontario site of the Breast Cancer Family Registry. *Cancer Epidemiol Biomarkers Prev* 13: 583–91.
- Franco S, Alt FW, Manis JP (2006). Pathways that suppress programmed DNA breaks from progressing to chromosomal breaks and translocations. *DNA Repair (Amst)* 5: 1030–41.
- Frank KM, Sekiguchi JM, Seidl KJ, Swat W, Rathbun GA, Cheng HL et al. (1998). Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. *Nature* 396: 173–7.
- Frost BM, Forestier E, Gustafsson G, Nygren P, Hellebostad M, Jonsson OG et al. (2004). Translocation t(12;21) is related to in vitro cellular drug sensitivity to doxorubicin and etoposide in childhood acute lymphoblastic leukemia. *Blood* 104: 2452–7.
- Fugmann SD, Lee AI, Shockett PE, Villey IJ, Schatz DG (2000). The RAG proteins and V(D)J recombination: complexes, ends, and transposition. *Annu Rev Immunol* 18: 495–527.
- Gao Y, Chaudhuri J, Zhu C, Davidson L, Weaver DT, Alt FW (1998a). A targeted DNA-PKcs-null mutation reveals DNA-PK-independent functions for KU in V(D)J recombination. *Immunity* 9: 367–76.
- Gao Y, Sun Y, Frank KM, Dikkes P, Fujiwara Y, Seidl KJ et al. (1998b). A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. *Cell* 95: 891–902.
- Gennery AR, Hodges E, Williams AP, Harris S, Villa A, Angus B et al. (2005). Omenn's syndrome occurring in patients without mutations in recombination activating genes. *Clin Immunol* 116: 246–56.
- Gottlich B, Reichenberger S, Feldmann E, Pfeiffer P (1998). Rejoining of DNA double-strand breaks in vitro by single-strand annealing. *Eur J Biochem* 258: 387–95.
- Gu Y, Seidl KJ, Rathbun GA, Zhu C, Manis JP, van der Stoep N et al. (1997). Growth retardation and leaky SCID phenotype of Ku70-deficient mice. *Immunity* 7: 653–65.
- Han J, Colditz GA, Samson LD, Hunter DJ (2004). Polymorphisms in DNA double-strand break repair genes and skin cancer risk. *Cancer Res* 64: 3009–13.
- Hashimoto K, Nakagawa Y, Morikawa H, Niki M, Egashira Y, Hirata I et al. (2001). Co-overexpression of DEAD box protein rck/p54 and c-myc protein in human colorectal adenomas and the relevance of their expression in cultured cell lines. *Carcinogenesis* 22: 1965–70.

- Helleday T, Lo J, van Gent DC, Engelward BP (2007). DNA double-strand break repair: From mechanistic understanding to cancer treatment. *DNA Repair (Amst)* 6: 923–35.
- Heyer WD, Li X, Rolfsmeier M, Zhang XP (2006). Rad54: the Swiss Army knife of homologous recombination? *Nucleic Acids Res* 34: 4115–25.
- Hickson ID (2003). RecQ helicases: caretakers of the genome. *Nat Rev Cancer* 3: 169–78.
- Hikida M, Mori M, Takai T, Tomochika K, Hamatani K, Ohmori H (1996). Reexpression of RAG-1 and RAG-2 genes in activated mature mouse B cells. *Science* 274: 2092–4.
- Hsu HL, Gilley D, Blackburn EH, Chen DJ (1999). Ku is associated with the telomere in mammals. *Proc Natl Acad Sci U S A* 96: 12454–8.
- Hsu HL, Gilley D, Galande SA, Hande MP, Allen B, Kim SH et al. (2000). Ku acts in a unique way at the mammalian telomere to prevent end joining. *Genes Dev* 14: 2807–12.
- Jager U, Bocska S, Le T, Mitterbauer G, Bolz I, Chott A et al. (2000). Follicular lymphomas' BCL-2/IgH junctions contain templated nucleotide insertions: novel insights into the mechanism of t(14;18) translocation. *Blood* 95: 3520–9.
- Jeffs AR, Benjes SM, Smith TL, Sowerby SJ, Morris CM (1998). The BCR gene recombines preferentially with Alu elements in complex BCR-ABL translocations of chronic myeloid leukaemia. *Hum Mol Genet* 7: 767–76.
- Jeffs AR, Wells E, Morris CM (2001). Nonrandom distribution of interspersed repeat elements in the BCR and ABL1 genes and its relation to breakpoint cluster regions. *Genes Chromosomes Cancer* 32: 144–54.
- Jung D, Alt FW (2004). Unraveling V(D)J recombination; insights into gene regulation. *Cell* 116: 299–311.
- Karanjawala ZE, Adachi N, Irvine RA, Oh EK, Shibata D, Schwarz K et al. (2002). The embryonic lethality in DNA ligase IV-deficient mice is rescued by deletion of Ku: implications for unifying the heterogeneous phenotypes of NHEJ mutants. *DNA Repair (Amst)* 1: 1017–26.
- Karran P (2000). DNA double strand break repair in mammalian cells. *Curr Opin Genet Dev* 10: 144–50.
- Khakhar RR, Cobb JA, Bjergbaek L, Hickson ID, Gasser SM (2003). RecQ helicases: multiple roles in genome maintenance. *Trends Cell Biol* 13: 493–501.
- Kobayashi J, Antoccia A, Tauchi H, Matsura S, Komatsu K (2004). NBS1 and its functional role in the DNA damage response. *DNA Repair (Amst)* 3: 855–61.
- Krishna S, Wagener BM, Liu HP, Lo YC, Sterk R, Petrini JH et al. (2007). Mre11 and Ku regulation of double-strand break repair by gene conversion and break-induced replication. *DNA Repair (Amst)* 6: 797–808.
- Kuppers R, Dalla-Favera R (2001). Mechanisms of chromosomal translocations in B cell lymphomas. *Oncogene* 20: 5580–94.
- Kurimasa A, Kumano S, Boubnov NV, Story MD, Tung CS, Peterson SR et al. (1999). Requirement for the kinase activity of human DNA-dependent protein kinase catalytic subunit in DNA strand break rejoicing. *Mol Cell Biol* 19: 3877–84.
- Kurumizaka H, Ikawa S, Nakada M, Eda K, Kagawa W, Takata M et al. (2001). Homologous-pairing activity of the human DNA-repair proteins Xrc3.Rad51C. *Proc Natl Acad Sci U S A* 98: 5538–43.
- Kurumizaka H, Ikawa S, Nakada M, Enomoto R, Kagawa W, Kinebuchi T et al. (2002). Homologous pairing and ring and filament structure formation activities of the human Xrc2\*Rad51D complex. *J Biol Chem* 277: 14315–20.
- Kuschel B, Auranen A, McBride S, Novik KL, Antoniou A, Lipscombe JM et al. (2002). Variants in DNA double-strand break repair genes and breast cancer susceptibility. *Hum Mol Genet* 11: 1399–407.
- Landree MA, Wibbenmeyer JA, Roth DB (1999). Mutational analysis of RAG1 and RAG2 identifies three catalytic amino acids in RAG1 critical for both cleavage steps of V(D)J recombination. *Genes Dev* 13: 3059–69.

- Lee J, Desiderio S (1999). Cyclin A/CDK2 regulates V(D)J recombination by coordinating RAG-2 accumulation and DNA repair. *Immunity* 11: 771–81.
- Lim DS, Hasty P (1996). A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol Cell Biol* 16: 7133–43.
- Lio YC, Mazin AV, Kowalczykowski SC, Chen DJ (2003). Complex formation by the human Rad51B and Rad51C DNA repair proteins and their activities in vitro. *J Biol Chem* 278: 2469–78.
- Liu N, Lamerdin JE, Tebbs RS, Schild D, Tucker JD, Shen MR et al. (1998). XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol Cell* 1: 783–93.
- Liu N, Schild D, Thelen MP, Thompson LH (2002). Involvement of Rad51C in two distinct protein complexes of Rad51 paralogs in human cells. *Nucleic Acids Res* 30: 1009–15.
- Liu Y, Masson JY, Shah R, O'Regan P, West SC (2004). RAD51C is required for Holliday junction processing in mammalian cells. *Science* 303: 243–6.
- Liu Y, Tarsounas M, O'Regan P, West SC (2007). Role of RAD51C and XRCC3 in genetic recombination and DNA repair. *J Biol Chem* 282: 1973–9.
- Longerich S, Basu U, Alt F, Storb U (2006). AID in somatic hypermutation and class switch recombination. *Curr Opin Immunol* 18: 164–74.
- Lumsden JM, McCarty T, Petinot LK, Shen R, Barlow C, Wynn TA et al. (2004). Immunoglobulin class switch recombination is impaired in Atm-deficient mice. *J Exp Med* 200: 1111–21.
- Masson JY, Stasiak AZ, Stasiak A, Benson FE, West SC (2001a). Complex formation by the human RAD51C and XRCC3 recombination repair proteins. *Proc Natl Acad Sci U S A* 98: 8440–6.
- Masson JY, Tarsounas MC, Stasiak AZ, Stasiak A, Shah R, McIlwraith MJ et al. (2001b). Identification and purification of two distinct complexes containing the five RAD51 paralogs. *Genes Dev* 15: 3296–307.
- Matei IR, Guidos CJ, Danska JS (2006). ATM-dependent DNA damage surveillance in T-cell development and leukemogenesis: the DSB connection. *Immunol Rev* 209: 142–58.
- Mathew CG (2006). Fanconi anaemia genes and susceptibility to cancer. *Oncogene* 25: 5875–84.
- Max EE, Seidman JG, Leder P (1979). Sequences of five potential recombination sites encoded close to an immunoglobulin kappa constant region gene. *Proc Natl Acad Sci U S A* 76: 3450–4.
- McBlane JF, van Gent DC, Ramsden DA, Romeo C, Cuomo CA, Gellert M et al. (1995). Cleavage at a V(D)J recombination signal requires only RAG1 and RAG2 proteins and occurs in two steps. *Cell* 83: 387–95.
- McCormack WT, Tjoelker LW, Carlson LM, Petryniak B, Barth CF, Humphries EH et al. (1989). Chicken IgL gene rearrangement involves deletion of a circular episome and addition of single nonrandom nucleotides to both coding segments. *Cell* 56: 785–91.
- McGowan CH, Russell P (2004). The DNA damage response: sensing and signaling. *Curr Opin Cell Biol* 16: 629–33.
- Migliore L, Coppede F (2002). Genetic and environmental factors in cancer and neurodegenerative diseases. *Mutat Res* 512: 135–53.
- Mills KD, Ferguson DO, Alt FW (2003). The role of DNA breaks in genomic instability and tumorigenesis. *Immunol Rev* 194: 77–95.
- Mills KD, Ferguson DO, Essers J, Eckersdorff M, Kanaar R, Alt FW (2004). Rad54 and DNA Ligase IV cooperate to maintain mammalian chromatin stability. *Genes Dev* 18: 1283–92.
- Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, Papaioannou VE (1992). RAG-1-deficient mice have no mature B and T lymphocytes. *Cell* 68: 869–77.
- Moshous D, Callebaut I, de Chasseval R, Corneo B, Cavazzana-Calvo M, Le Deist F et al. (2001). Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* 105: 177–86.

- Moshous D, Callebaut I, de Chasseval R, Poinsignon C, Villey I, Fischer A et al. (2003a). The V(D)J recombination/DNA repair factor artemis belongs to the metallo-beta-lactamase family and constitutes a critical developmental checkpoint of the lymphoid system. *Ann N Y Acad Sci* 987: 150–7.
- Moshous D, Pannetier C, Chasseval Rd R, Deist Fl F, Cavazzana-Calvo M, Romana S et al. (2003b). Partial T and B lymphocyte immunodeficiency and predisposition to lymphoma in patients with hypomorphic mutations in Artemis. *J Clin Invest* 111: 381–7.
- Murnane JP, Sabatier L (2004). Chromosome rearrangements resulting from telomere dysfunction and their role in cancer. *Bioessays* 26: 1164–74.
- Nagel S, Kaufmann M, Drexler HG, MacLeod RA (2003). The cardiac homeobox gene NKX2-5 is deregulated by juxtaposition with BCL11B in pediatric T-ALL cell lines via a novel t(5;14)(q35.1;q32.2). *Cancer Res* 63: 5329–34.
- New JH, Sugiyama T, Zaitseva E, Kowalczykowski SC (1998). Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. *Nature* 391: 407–10.
- Nicolas N, Moshous D, Cavazzana-Calvo M, Papadopoulos D, de Chasseval R, Le Deist F et al. (1998). A human severe combined immunodeficiency (SCID) condition with increased sensitivity to ionizing radiations and impaired V(D)J rearrangements defines a new DNA recombination/repair deficiency. *J Exp Med* 188: 627–34.
- Nussenzweig A, Chen C, da Costa Soares V, Sanchez M, Sokol K, Nussenzweig MC et al. (1996). Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. *Nature* 382: 551–5.
- O'Driscoll M, Cerosaletti KM, Girard PM, Dai Y, Stumm M, Kysela B et al. (2001). DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. *Mol Cell* 8: 1175–85.
- Orettinger MA, Schatz DG, Gorka C, Baltimore D (1990). RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science* 248: 1517–23.
- Ouyang H, Nussenzweig A, Kurimasa A, Soares VC, Li X, Cordon-Cardo C et al. (1997). Ku70 is required for DNA repair but not for T cell antigen receptor gene recombination *in vivo*. *J Exp Med* 186: 921–9.
- Padilla-Nash HM, Barenboim-Stapleton L, Difilippantonio MJ, Ried T (2006). Spectral karyotyping analysis of human and mouse chromosomes. *Nat Protoc* 1: 3129–42.
- Pages V, Fuchs RP (2002). How DNA lesions are turned into mutations within cells? *Oncogene* 21: 8957–66.
- Papadopoulos PC, Greenstein AM, Gaffney RA, Westbrook CA, Wiedemann LM (1990). Characterization of the translocation breakpoint sequences in Philadelphia-positive acute lymphoblastic leukemia. *Genes Chromosomes Cancer* 1: 233–9.
- Pastink A, Eeken JC, Lohman PH (2001). Genomic integrity and the repair of double-strand DNA breaks. *Mutat Res* 480–481: 37–50.
- Plank JL, Wu J, Hsieh TS (2006). Topoisomerase IIIalpha and Bloom's helicase can resolve a mobile double Holliday junction substrate through convergent branch migration. *Proc Natl Acad Sci U S A* 103: 11118–23.
- Rafii S, O'Regan P, Xinarianos G, Azmy I, Stephenson T, Reed M et al. (2002). A potential role for the XRCC2 R188H polymorphic site in DNA-damage repair and breast cancer. *Hum Mol Genet* 11: 1433–8.
- Raynard S, Bussen W, Sung P (2006). A double Holliday junction dissolvosome comprising BLM, topoisomerase IIIalpha, and BLAP75. *J Biol Chem* 281: 13861–4.
- Reaban ME, Griffin JA (1990). Induction of RNA-stabilized DNA conformers by transcription of an immunoglobulin switch region. *Nature* 348: 342–4.
- Reaban ME, Lebowitz J, Griffin JA (1994). Transcription induces the formation of a stable RNA-DNA hybrid in the immunoglobulin alpha switch region. *J Biol Chem* 269: 21850–7.
- Reina-San-Martin B, Chen HT, Nussenzweig A, Nussenzweig MC (2004). ATM is required for efficient recombination between immunoglobulin switch regions. *J Exp Med* 200: 1103–10.

- Richardson C, Jasin M (2000). Frequent chromosomal translocations induced by DNA double-strand breaks. *Nature* 405: 697–700.
- Richardson C, Moynahan ME, Jasin M (1998). Double-strand break repair by interchromosomal recombination: suppression of chromosomal translocations. *Genes Dev* 12: 3831–42.
- Richardson C, Moynahan ME, Jasin M (1999). Homologous recombination between heterologs during repair of a double-strand break. Suppression of translocations in normal cells. *Ann N Y Acad Sci* 886: 183–6.
- Rodriguez-Lopez R, Osorio A, Ribas G, Pollan M, Sanchez-Pulido L, de la Hoya M et al. (2004). The variant E233G of the RAD51D gene could be a low-penetrance allele in high-risk breast cancer families without BRCA1/2 mutations. *Int J Cancer* 110: 845–9.
- Rooney S, Sekiguchi J, Whitlow S, Eckersdorff M, Manis JP, Lee C et al. (2004). Artemis and p53 cooperate to suppress oncogenic N-myc amplification in progenitor B cells. *Proc Natl Acad Sci U S A* 101: 2410–5.
- Rooney S, Sekiguchi J, Zhu C, Cheng HL, Manis J, Whitlow S et al. (2002). Leaky Scid phenotype associated with defective V(D)J coding end processing in Artemis-deficient mice. *Mol Cell* 10: 1379–90.
- Sakano H, Huppi K, Heinrich G, Tonegawa S (1979). Sequences at the somatic recombination sites of immunoglobulin light-chain genes. *Nature* 280: 288–94.
- Samper E, Goytisolo FA, Slijepcevic P, van Buul PP, Blasco MA (2000). Mammalian Ku86 protein prevents telomeric fusions independently of the length of TTAGGG repeats and the G-strand overhang. *EMBO Rep* 1: 244–52.
- Schatz DG, Oettinger MA, Baltimore D (1989). The V(D)J recombination activating gene, RAG-1. *Cell* 59: 1035–48.
- Sharma S, Sommers JA, Wu L, Bohr VA, Hickson ID, Brosh RM, Jr. (2004). Stimulation of flap endonuclease-1 by the Bloom's syndrome protein. *J Biol Chem* 279: 9847–56.
- Sharples GJ (2001). The X philes: structure-specific endonucleases that resolve Holliday junctions. *Mol Microbiol* 39: 823–34.
- Shechter D, Costanzo V, Gautier J (2004). ATR and ATM regulate the timing of DNA replication origin firing. *Nat Cell Biol* 6: 648–55.
- Shinkai Y, Rathbun G, Lam KP, Oltz EM, Stewart V, Mendelsohn M et al. (1992). RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* 68: 855–67.
- Shinohara A, Ogawa T (1998). Stimulation by Rad52 of yeast Rad51-mediated recombination. *Nature* 391: 404–7.
- Sigurdsson S, Van Komen S, Bussen W, Schild D, Albala JS, Sung P (2001). Mediator function of the human Rad51B-Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange. *Genes Dev* 15: 3308–18.
- Sonoda E, Hochegger H, Saberi A, Taniguchi Y, Takeda S (2006). Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. *DNA Repair (Amst)* 5: 1021–9.
- Sonoda E, Sasaki MS, Buerstedde JM, Bezzubova O, Shinohara A, Ogawa H et al. (1998). Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. *Embo J* 17: 598–608.
- Sugimoto J, Hatakeyama T, Narducci MG, Russo G, Isobe M (1999). Identification of the TCL1/MTCP1-like 1 (TML1) gene from the region next to the TCL1 locus. *Cancer Res* 59: 2313–7.
- Sung P (1997). Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. *J Biol Chem* 272: 28194–7.
- Swanson PC (2001). The DDE motif in RAG-1 is contributed in trans to a single active site that catalyzes the nicking and transesterification steps of V(D)J recombination. *Mol Cell Biol* 21: 449–58.
- Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW (1983). The double-strand-break repair model for recombination. *Cell* 33: 25–35.

- Taccioli GE, Amatucci AG, Beamish HJ, Gell D, Xiang XH, Torres Arzayus MI et al. (1998). Targeted disruption of the catalytic subunit of the DNA-PK gene in mice confers severe combined immunodeficiency and radiosensitivity. *Immunity* 9: 355–66.
- Taccioli GE, Cheng HL, Varghese AJ, Whitmore G, Alt FW (1994). A DNA repair defect in Chinese hamster ovary cells affects V(D)J recombination similarly to the murine scid mutation. *J Biol Chem* 269: 7439–42.
- Takahashi A, Ohnishi T (2005). Does gammaH2AX foci formation depend on the presence of DNA double strand breaks? *Cancer Lett* 229: 171–9.
- Taylor AM, Byrd PJ (2005). Molecular pathology of ataxia telangiectasia. *J Clin Pathol* 58: 1009–15.
- Taylor AM, Metcalfe JA, Thick J, Mak YF (1996). Leukemia and lymphoma in ataxia telangiectasia. *Blood* 87: 423–38.
- Thacker J (2005). The RAD51 gene family, genetic instability and cancer. *Cancer Lett* 219: 125–35.
- Tian M, Alt FW (2000). Transcription-induced cleavage of immunoglobulin switch regions by nucleotide excision repair nucleases in vitro. *J Biol Chem* 275: 24163–72.
- Tischkowitz M, Dokal I (2004). Fanconi anaemia and leukaemia – clinical and molecular aspects. *Br J Haematol* 126: 176–91.
- Tremblay A, Jaslin M, Chartrand P (2000). A double-strand break in a chromosomal LINE element can be repaired by gene conversion with various endogenous LINE elements in mouse cells. *Mol Cell Biol* 20: 54–60.
- Treuner K, Helton R, Barlow C (2004). Loss of Rad52 partially rescues tumorigenesis and T-cell maturation in Atm-deficient mice. *Oncogene* 23: 4655–61.
- Tsuzuki T, Fujii Y, Sakumi K, Tominaga Y, Nakao K, Sekiguchi M et al. (1996). Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc Natl Acad Sci U S A* 93: 6236–40.
- Valerie K, Povirk LF (2003). Regulation and mechanisms of mammalian double-strand break repair. *Oncogene* 22: 5792–812.
- Villa A, Santagata S, Bozzi F, Giliani S, Frattini A, Imberti L et al. (1998). Partial V(D)J recombination activity leads to Omenn syndrome. *Cell* 93: 885–96.
- Waldmann TA, Broder S, Goldman CK, Frost K, Korsmeyer SJ, Medici MA (1983). Disorders of B cells and helper T cells in the pathogenesis of the immunoglobulin deficiency of patients with ataxia telangiectasia. *J Clin Invest* 71: 282–95.
- Walker JR, Corpina RA, Goldberg J (2001). Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* 412: 607–14.
- Wang WW, Spurdle AB, Kolachana P, Bove B, Modan B, Ebbers SM et al. (2001). A single nucleotide polymorphism in the 5' untranslated region of RAD51 and risk of cancer among BRCA1/2 mutation carriers. *Cancer Epidemiol Biomarkers Prev* 10: 955–60.
- Weinert BT, Rio DC (2007). DNA strand displacement, strand annealing and strand swapping by the Drosophila Bloom's syndrome helicase. *Nucleic Acids Res* 35: 1367–76.
- Weinstock DM, Elliott B, Jaslin M (2006a). A model of oncogenic rearrangements: differences between chromosomal translocation mechanisms and simple double-strand break repair. *Blood* 107: 777–80.
- Weinstock DM, Richardson CA, Elliott B, Jaslin M (2006b). Modeling oncogenic translocations: distinct roles for double-strand break repair pathways in translocation formation in mammalian cells. *DNA Repair (Amst)* 5: 1065–74.
- Welzel N, Le T, Marculescu R, Mitterbauer G, Chott A, Pott C et al. (2001). Templated nucleotide addition and immunoglobulin JH-gene utilization in t(11;14) junctions: implications for the mechanism of translocation and the origin of mantle cell lymphoma. *Cancer Res* 61: 1629–36.
- Woo Y, Wright SM, Maas SA, Alley TL, Caddle LB, Kamdar S et al. (2007). The nonhomologous end joining factor Artemis suppresses multi-tissue tumor formation and prevents loss of heterozygosity. *Oncogene*.

- Wu L, Hickson ID (2003). The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* 426: 870–4.
- Zha S, Alt FW, Cheng HL, Brush JW, Li G (2007). Defective DNA repair and increased genomic instability in Cernunnos-XLF-deficient murine ES cells. *Proc Natl Acad Sci U S A* 104: 4518–23.
- Zhang JG, Goldman JM, Cross NC (1995). Characterization of genomic BCR-ABL break-points in chronic myeloid leukaemia by PCR. *Br J Haematol* 90: 138–46.
- Zhu C, Mills KD, Ferguson DO, Lee C, Manis J, Fleming J et al. (2002). Unrepaired DNA breaks in p53-deficient cells lead to oncogenic gene amplification subsequent to translocations. *Cell* 109: 811–21.
- Zou L, Cortez D, Elledge SJ (2002). Regulation of ATR substrate selection by Rad17-dependent loading of Rad9 complexes onto chromatin. *Genes Dev* 16: 198–208.

# Chapter 5

## Modeling Human Leukemia Using Immune-Compromised Mice

Fumihiko Ishikawa, Yariko Saito, and Leonard D. Shultz

### Contents

5.1	Introduction . . . . .	121
5.2	Development of Immune-Compromised Mice and Humanized Mice . . . . .	122
5.3	Engraftment Studies of Hematological Malignancies . . . . .	123
5.3.1	In Vivo Models of Human AML . . . . .	123
5.3.2	In Vivo Models of Human ALL . . . . .	124
5.3.3	In Vivo Models of Human CML . . . . .	125
5.3.4	In Vivo Models of Human MM . . . . .	126
5.4	Human Leukemia Stem Cells (LSCs) . . . . .	127
5.4.1	LSCs in Human AML . . . . .	127
5.4.2	LSCs in Human ALL . . . . .	128
5.5	Summary . . . . .	129

### 5.1 Introduction

Various mutations have been found to cause leukemia, myeloid proliferative disorders, or lymphoid proliferative disorders in mice when directly introduced as transgenes or by transplantation of mouse hematopoietic cells expressing these genes through targeted vectors. These mouse models of hematological malignancies have provided important insights into leukemogenesis. However, models that facilitate studies of human leukemia cells *in vivo* are required to understand the biology of human leukemia. Studies of human leukemia cells that engraft in immunodeficient mouse models permit analyses of the mechanisms underlying human leukemic cell proliferation within the bone marrow, migration from the bone marrow into peripheral circulation, identification of specific microenvironmental niches for human leukemic cells, and the functional interactions of human leukemic cells with the elements of such niches. Such *in vivo* human leukemia models can also be used to simulate therapy

---

F. Ishikawa

Research Unit for Human Disease Models, RIKEN Research Center for Allergy and Immunology, Yokohama 230-0045, Japan  
f\_ishika@rcai.riken.jp

targeting human leukemic cells. To accomplish these goals, xenogeneic transplantation of human leukemia cells into various strains of immunodeficient mouse recipients has been attempted. This chapter reviews the history of xenotransplantation for primary human leukemia and discusses new generations of xenotransplantation models for human leukemia.

## 5.2 Development of Immune-Compromised Mice and Humanized Mice

To allow the engraftment of human normal or leukemic cells in the mouse microenvironment, immunodeficient mouse strains have been used as recipients to prevent rejection of human cells. Immunodeficient strains that support engraftment of primary human cells resulted from the discovery of the protein kinase, DNA-activated, catalytic polypeptide (*Prkdc*); severe combined immunodeficiency (*Prkdc<sup>scid</sup>*) mutation in CB17 mice [1]. However, the engraftment levels of human hematopoietic cells were unacceptably low due to the age-dependent appearance of mouse T and B cells (leakiness) and the high levels of host innate immune function. In contrast to CB17-*scid* mice, targeted mutations in either of the recombination activating genes (*Rag*) 1 and *Rag2* resulted in the development of mouse strains with improved depletion of acquired immunity without age-dependent leakiness [2, 3]. In these mice, as with CB17-*scid* mice, the high activity of residual innate immunity limited the engraftment of human cells.

These limitations in the engraftment of human cells were partially overcome by backcrossing the *scid* mutation onto the non-obese diabetic (NOD) strain, which are utilized as a model for type I diabetes mellitus. The advantage of NOD-*scid* (NOD/SCID) mice over CB17-*scid* mice is the lower NK cell activity and impaired complement activity conferred by the NOD strain background [4]. Additional defects in the innate immunity in NOD/SCID mice have led to heightened levels of human hematopoietic cell engraftment.

Further systematic introductions of mutations into NOD or NOD/SCID backgrounds resulted in the development of NOD-*Rag1<sup>null</sup>* [5], NOD-*Rag1<sup>null</sup>* *Prf1<sup>null</sup>* [6], and NOD-*scidβ2m<sup>null</sup>* mice [7] with incremental improvements in human hematopoietic cell engraftment. However, the relatively short life spans of these mouse strains and poor engraftment of the human T-lymphoid compartment continued to pose significant obstacles in development of the xenotransplantation model with a fully humanized immuno-hematopoietic system.

Targeted mutations at the interleukin-2 receptor (IL-2R) gamma-chain locus (*Il2rg*) were found to result not only in severe impairments in B- and T-cell development and function but also in complete prevention of NK cell development [8, 9]. Several strains with targeted mutations in the *Il2rg* locus were developed independently, using various genetic modifications to this locus backcrossed onto a number of diverse strain backgrounds including the

NOD/SCID [10, 11], BALB/c-*Rag2*<sup>null</sup>, and *H2<sup>d</sup>-Rag2*<sup>null</sup>. The strain developed by backcrossing a targeted mutation leading to the complete absence of the  $\gamma$ -chain into the NOD/SCID strain is the NOD/LtSz-*scid Il2rg*<sup>null</sup>, referred to as NOD/SCID/IL2r $\gamma$ <sup>null</sup> mice in this chapter. The NOD/SCID/IL2r $\gamma$ <sup>null</sup> mice, especially when used as neonatal recipients, proved to be superior in terms of the efficiency of normal human hematopoietic stem cell (HSC) engraftment compared to the NOD/SCID/ $\beta$ 2m<sup>null</sup> recipients [12]. The transplantation of purified human HSCs into the NOD/SCID/IL2r $\gamma$ <sup>null</sup> newborns resulted in long-term engraftment of normal human hematopoiesis, leading to the propagation of not only differentiated myeloid progeny but also lymphoid (T, B, NK) progeny, dendritic cells, erythrocytes, and platelets. These findings suggest that the NOD/SCID/IL2r $\gamma$ <sup>null</sup> recipients may be useful for the establishment of the *in vivo* mouse models of human leukemia.

### 5.3 Engraftment Studies of Hematological Malignancies

#### 5.3.1 *In Vivo Models of Human AML*

Acute myelogenous leukemia (AML) is the most common leukemia in adults. While there are subtypes of AML that carry favorable prognoses, overall survival in AML is quite poor, even with current treatment protocols that include combined chemotherapy and stem cell transplantation. *In vivo* animal models for human AML are crucial in understanding leukemogenesis and in developing therapeutic modalities. While immortalized human leukemic cell lines have been shown to engraft in CB17-*scid* mice, the engraftment of primary AML cells in CB17-*scid* mice has been hindered presumably due to high residual NK cell activity [13–16].

The limitation in primary AML engraftment was partially overcome by using NOD/SCID mice as recipients, due to the attenuation of NK cell activity in these mice [17]. Ailles et al. reported the successful engraftment of four different subtypes (M1, M2, M4, and M5) of primary AML cells [18]. Two other groups reported that the engraftment levels of primary AML cells correlate with disease severity in patients [19, 20]. The observation that primary AML cells from AML M3 (acute promyelocytic leukemia) patients carrying a relatively good prognosis do not engraft well in NOD/SCID mice is consistent with these findings.

Using AML-engrafted NOD/SCID mice, an *in vivo* chemotherapy model has been developed to predict or simulate sensitivity/resistance of patient-derived AML cells against various anti-cancer drugs [21]. Although the decrease in leukemic cell burden and leukemia cell apoptosis in the engrafted mice after treatment with various chemotherapeutic drugs has been reported, whether therapeutic response in the mouse model correlates with that in patients is undetermined. Transplantation of leukemic cells derived from

patients at different stages of disease, such as initial diagnosis and relapse, will provide information that may help predict sensitivity and resistance of leukemic cells in the patients. To answer these questions, however, the efficiency of AML engraftment needs to be improved, to achieve consistent levels of long-term engraftment using limited patient material available.

To achieve higher levels of AML engraftment, researchers have taken two approaches. First, induction of myeloid cell proliferation and differentiation using exogenous cytokine administration or endogenous production of cytokines by transgenes has been attempted. Second, residual innate immunity in recipient mice has been further depleted by backcrossing mutations in major histocompatibility complex (MHC) class I light chain ( $\beta 2m$ ) or common cytokine gamma chain ( $\gamma c$ ) onto the *scid*, *Rag-1<sup>null</sup>*, or *Rag2<sup>null</sup>* backgrounds. Both NOD/SCID/cytokine transgenic and NOD/SCID/ $\beta 2m^{null}$  mice supported higher levels of AML engraftment compared with NOD/SCID mice [22]. However, the improvement of AML chimerism in the recipients by exogenous cytokine administration varied among investigators. Development of innate immunity-depleted NOD/SCID mouse strains enables us to obtain more efficient engraftment of primary AML cells. When NOD/SCID/ $\beta 2m^{null}$  and NOD/SCID/ $IL2r\gamma^{null}$  recipients were compared, AML engraftment levels as well as normal HSC engraftment levels were significantly higher in the NOD/SCID/ $IL2r\gamma^{null}$  mice [23]. NOG mice [24] and *Rag2<sup>null</sup>*/ $\gamma c^{null}$  mice carrying truncated form of *IL2rg* gene also supported efficient engraftment of primary AML mononuclear cells (MNCs). The suppression of innate immunity by blocking  $IL2r\gamma$ -mediated signaling is considered essential to inhibit the rejection of primary human AML cells in xenogeneic microenvironment.

### 5.3.2 In Vivo Models of Human ALL

Acute lymphocytic leukemia (ALL) is the most common hematological malignancy in childhood. Based on ontogenetic classification, pediatric ALL is divided into T-ALL, B-precursor ALL, and mature B-ALL. B-precursor ALL accounts for 80–85% of total pediatric ALL cases. B-ALL can be further classified into subgroups based on the presence of chromosome abnormalities such as t(9;22) generating the Philadelphia (Ph) chromosome, t(12;21) generating the *TEL-AML1* fusion gene, and t(4;11) and other rearrangements involving the *MLL* gene at 11q23. Xenogeneic transplantation has been performed using subgroups of B-precursor ALL carrying the *MLL* rearrangements or the Ph+ abnormality. In the long-term observation of ALL engraftment in NOD/SCID mice, the chromosomal abnormalities or Ig gene arrangements identified in the primary ALL cells are largely retained in the ALL cells that have engrafted in NOD/SCID recipients, suggesting that these ALL engraftment models reproduce ALL disease present in the patients [25, 26]. As in human primary AML engraftment models, in vivo chemosensitivity and chemoresistance

of engrafted ALL cells to anti-cancer drugs, including vincristine, dexamethasone, methotrexate, and L-arginine, have been examined [25, 27]. In these models, both single-drug treatment and combined chemotherapy resulted in the decrease of ALL cells *in vivo* and prolonged the survival of ALL-engrafted mice. Furthermore, immunotherapeutic modalities such as induction of anti-minor antigen cytotoxic T lymphocytes (CTL) and donor lymphocyte infusion have been tested using ALL-engrafted SCID models [28, 29]. In the future, the reconstitution of immunity from normal HSCs along with leukemia derived from individual patients may allow direct and precise examination of immunotherapy against various types of leukemic cells *in vivo*.

### 5.3.3 *In Vivo Models of Human CML*

Chronic myeloid leukemia (CML) is a clonal multilineage myeloproliferative disorder caused by the constitutive activation of the ABL tyrosine kinase through the *BCR-ABL* oncogene. The *BCR-ABL* oncogene and the chimeric 210-kDa BCR-ABL protein is the product of the Ph chromosome 22q, created by the translocation of the *c-abl* proto-oncogene on human chromosome 9 to chromosome 22, almost invariably in CML. An alternative translocation breakpoint in the *BCR* gene, resulting in a 190-kDa BCR-ABL protein, also occurs in Ph-positive (Ph+) ALL. The presence of the Ph chromosome in multiple lineages, including the granulocytic, monocytic, megakaryocytic, erythroid, and B-lymphoid lineages, suggests that the malignant clone in CML originates in the HSC compartment. Recently, the presence of leukemic colony-forming, *in vitro* self-renewing granulocyte-macrophage progenitors (GMPs) were reported in patients with CML in blast crisis [30], and similar findings have been reported in mouse models of CML [31], suggesting complex multistep processes in the pathogenesis of this disorder. An animal model of human primary CML that can recapitulate these processes *in vivo* is required. Some of the first such *in vivo* models used the SCID and SCID-hu mice as recipient [32–35]. While the infusion of blast crisis CML cell lines such as K562, EM-2, BV173, and KBM-5 [34, 36, 37] into SCID recipients led to disseminated leukemia, similar attempts using primary patient samples resulted in low levels of engraftment even at very high graft doses (up to  $1.4 \times 10^8$  cells) in the case of chronic phase CML [34, 35]. Similarly, intraperitoneal or subrenal capsule injection of blast crisis CML cells into the human fetal bone implants resulted in poor dissemination of CML to the recipient BM [33, 34]. With the availability of the NOD/SCID strain, multiple groups reported improved levels of long-term engraftment of both chronic phase and blast crisis primary human CML cells [38–41]. Using this system, it was possible to phenotypically restrict CML-initiating cells into the CD34+ fraction. In one study, preselection of patient samples with high frequencies of Ph+ long-term culture-initiating cells (LTC-ICs) was used to improve engraftment efficiencies in both NOD/SCID and

NOD/SCID/ $\beta$ 2m<sup>null</sup> recipients [42]. While these models represent significant progress in the development of *in vivo* models of primary human CML, xenotransplantation systems that allow the demonstration of self-renewing long-term engrafting CML-initiating cells are required to further characterize CML pathogenesis. The clarification of the complex cellular evolution in which CML disease develops over multiple lineages would require efficient engraftment of highly purified primary CML populations. Development of such models will also allow *in vivo* examination of therapeutic responses and drug resistance, for instance in the case of imatinib-resistant CML.

### 5.3.4 *In Vivo Models of Human MM*

Multiple myeloma (MM) is an aggressive clonal B-cell malignancy originating from the plasma cell. It is characterized by BM plasmacytosis accompanied by lytic bone lesions and excess production of monoclonal immunoglobulin production detectable in serum and/or urine. With currently available treatment modalities including high-dose therapies, the prognosis is dismal, with a median survival of 4 years. The development of *in vivo* models for MM is crucial for basic understanding of MM pathogenesis as well as for preclinical evaluation of novel therapies. However, an efficient and reproducible engraftment of primary human MM cells has been difficult to achieve. Currently available *in vivo* models for MM include the injection of pre-established human MM cell lines into NOD/SCID recipients subcutaneously or intravenously [43–45] and the SCID-hu model in which human MM cell lines or primary MM cells are injected into subcutaneous human fetal bone implants in the SCID recipients [46, 47]. In one study, the intravenous injection of KMS-11 MM cell line into NOD/SCID recipients resulted in progressive BM infiltration, hind-leg paralysis due to central nervous system involvement, and the production of monoclonal kappa light chain of human type into the recipient sera [43]. Using this xenograft model, the authors tested the *in vivo* efficacy of alemtuzumab. In another study, the RPMI8226 human MM cell line was injected subcutaneously into NOD/SCID recipients, resulting in subcutaneous tumor formation [44]. This system was used to test the effects of 1-acetoxychavicol acetate, a nuclear factor kappa B (NF- $\kappa$ B) inhibitor *in vivo*. Mitsiades et al. transplanted RPMI8226/S human MM cell line stably expressing green fluorescent protein (GFP), allowing visualization of MM cell infiltration by whole-body fluorescence imaging [45]. Yaccoby et al. first reported the transplantation of primary human MM cells in the SCID-hu system, where MM cells from 12 out of 15 patients were found to engraft [47]. On the other hand, Tassone et al. used the SCID-hu system to monitor the growth and response to chemotherapy of GFP-transduced INA-6 human MM cell line [46]. While these systems represent the currently available *in vivo* models of MM, they are certainly not the optimal system to study primary human MM engraftment. The majority of studies use

cell lines, rather than primary cells, and the engraftment of primary cells requires injection of a large number ( $1 \times 10^6$ – $1 \times 10^7$ ) of cells. Successful serial transplantation demonstrating the presence of a self-renewing MM stem cell population has not been reported to date. Therefore, a more sensitive xenotransplantation system is required to establish a human primary MM model that recapitulates human MM disease *in vivo*. NOG mice have been used as recipients of KMM-1 and U-266 MM cell lines [48]. While this has resulted in engraftment of the cell lines, cell doses required were still high ( $2 \times 10^6$ – $1 \times 10^7$ ). Development of a more sensitive and efficient xenotransplantation model that allows not only the *in vivo* recapitulation of primary human MM disease but also the examination of MM stem cells by the engraftment of purified primary MM cell populations in limiting dilutions and serial transplants is needed in the future.

## 5.4 Human Leukemia Stem Cells (LSCs)

Past paradigms of leukemia development have focused on malignant clones that result in disease due to their highly proliferative nature. However, the presence of developmental hierarchy in leukemia, which parallels that of normal hematopoiesis, has been suggested by clonality studies such as those using specific chromosomal translocations found in certain types of leukemia. These studies have suggested the presence of leukemia stem cells (LSCs) analogous to normal HSCs. The development of xenotransplantation systems with significant levels of human leukemia engraftment has provided the *in vivo* assay systems required for the identification of putative LSCs prospectively and functionally.

### 5.4.1 LSCs in Human AML

Bonnet and Dick reported that CD34+CD38– AML cells, not CD34+CD38+ or CD34–AML cells, initiate leukemia after transplantation into NOD/SCID recipients [49]. These findings have been confirmed by other investigators using SCID-repopulating models [50, 51]. In some models, CD34+CD38– AML cells seem to meet all the criteria for stem cells, that is, multilineage development capacity, long-term engraftment capacity, and self-renewal capacity. Especially, transplantation into *scid*-, *Rag1<sup>null</sup>*-, and *Rag2<sup>null</sup>*-deficient strains that are also  $\gamma c^{null}$  result in efficient engraftment, allowing the examination of the self-renewal capacity of purified candidate LSCs [24]. Currently, one of the most promising approaches is the identification of antigens that can discriminate AML stem cells from normal HSCs. CD123 (IL3-Ra) and CD90 are antigens differentially expressed in AML stem cells and normal HSCs [52, 53]. Hosen et al. reported CD96 as a potential marker specifically expressed in LSCs (CD34+CD38–CD90– cells) but not in normal

HSCs (CD34+CD38–CD90+ cells) [54]. Jordan and colleagues have been studying agents that can selectively eradicate leukemic cells including LSCs (CD34+CD38–CD123+ cells) but spare normal HSCs (CD34+CD38–CD123– cells) [55, 56]. CD44, a physiological E-selectin ligand, is expressed on the surface of LSCs, and LSCs adhere to their niche via CD44-hyaluronic acid binding. Neutralizing antibody against CD44 prevented homing and engraftment of human primary LSCs [57]. Intriguingly, blocking of CD44 signal resulted in the induction of differentiation/maturation of leukemic blasts. Similar findings were confirmed using mouse leukemia model where *Bcr-abl*-transduced CD44<sup>+/−</sup> murine hematopoietic stem progenitors exhibited defective homing capacity resulting in decreased engraftment [58].

Although the microenvironment including osteoblasts, endothelial cells, or fibroblasts are of mouse origin, xenograft models using *scid* mice enabled investigators to analyze the interaction between stem cells and their niche. The pioneer work by Lapidot and colleagues clarified the role of SDF-1 and CXCR-4 in mobilization and homing of normal HSCs and leukemic cells using a *scid*-repopulating assay [59, 60]. Although human AML cells express lower and heterogenous level CXCR-4 on their surface, the blockade of signaling between these two molecules by neutralizing antibody against CXCR-4 and SDF-1 inhibited the growth of AML in NOD/SCID mice. As LSCs retain their quiescence and self-renewal capacity in the mouse microenvironment, further studies would be expected to identify other molecular mechanism underlying stem–niche interaction.

#### 5.4.2 LSCs in Human ALL

In contrast to AML stem cells, LSCs and stem cell hierarchy of pediatric B-ALL have not been clarified. Within B-precursor ALL, LSCs with distinct chromosomal abnormalities might exhibit different biology or might have different origins of leukemogenesis. LSCs need to be determined not only based on ontogenetic classification but also on genetic subtypes.

Using in vitro colony assay and fluorescence in situ hybridization (FISH) analysis, Hotfilder et al. reported that LSCs are present in CD34+CD19– fraction in Ph+ ALL and MLL-AF4 ALL [61]. FISH analyses revealed that 50–60% CD34+CD19– cells derived from ALL patient BM possess these leukemic translocations. In the MEC assay, CD34+CD19– cells generate G, M, GM, E, and mixed colonies, while CD34+CD19+ cells could not differentiate into myeloid lineage.

Castor et al. investigated the functional heterogeneity within three distinct genetic subtypes of B-ALL: p210 Ph+ ALL, p190 Ph+ ALL, and ETV6/RUNX1 ALL [62]. The frequency of translocation in CD34+CD38–CD19+ and CD34+CD38–CD19– cells is different among three different subtypes. The vast majority of CD34+CD38–CD19+ cells carry the t(12;21) translocation

generating the *ETV6/RUNX1 (TEL/AML1)* fusion gene, while CD34+CD38– cells do not. These findings suggest that the expression of CD19 determines the functional characteristics of primitive B-ALL cells.

Consistent with these observations, B-ALL patient BM CD34+CD38– cell populations have been found to contain cells with clonogenic leukemic T-cell receptor rearrangement [63]. Therefore, CD19 may serve as an antigen allowing the discrimination between LSCs and normal HSCs, allowing successful purging of LSCs from autologous grafts. At the same time, the significance of CD38 expression within CD34+CD19+ and CD34+CD19– populations in primary B-ALL must be further clarified using highly purified cells in sensitive xenotransplantation systems.

## 5.5 Summary

The development of in vivo models of human hematopoietic malignancies has occurred concomitantly with that of highly immunodeficient mouse strains that act as xenotransplantation recipients. The establishment of sensitive and reproducible xenotransplantation assays has allowed the in vivo examination of human leukemia biology and the identification and characterization of LSCs. Technical advances such as intrafacial vein injection in newborn recipients and intrafemoral injection in adults have also facilitated successful engraftment of highly purified primary human cells. These models of human hematopoietic malignancies are expected to provide new insights into leukemia biology and pathophysiology and contribute to the development of novel therapies, bridging the gap between the bench and the bedside.

## References

1. Bosma, G.C., Custer, R.P. & Bosma, M.J. A severe combined immunodeficiency mutation in the mouse. *Nature* **301**, 527–530 (1983).
2. Mombaerts, P. et al. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell* **68**, 869–877 (1992).
3. Shinkai, Y. et al. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* **68**, 855–867 (1992).
4. Shultz, L.D. et al. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. *J Immunol* **154**, 180–191 (1995).
5. Shultz, L.D. et al. NOD/LtSz-Rag1null mice: an immunodeficient and radioresistant model for engraftment of human hematolymphoid cells, HIV infection, and adoptive transfer of NOD mouse diabetogenic T cells. *J Immunol* **164**, 2496–2507 (2000).
6. Shultz, L.D. et al. NOD/LtSz-Rag1nullPfpnull mice: a new model system with increased levels of human peripheral leukocyte and hematopoietic stem-cell engraftment. *Transplantation* **76**, 1036–1042 (2003).
7. Christianson, S.W. et al. Enhanced human CD4+ T cell engraftment in beta2-microglobulin-deficient NOD-scid mice. *J Immunol* **158**, 3578–3586 (1997).

8. Cao, X. et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. *Immunity* **2**, 223–238 (1995).
9. Sugamura, K. et al. The interleukin-2 receptor gamma chain: its role in the multiple cytokine receptor complexes and T cell development in XSCID. *Annu Rev Immunol* **14**, 179–205 (1996).
10. Ito, M. et al. NOD/SCID/gamma(c)null mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* **100**, 3175–3182 (2002).
11. Shultz, L.D. et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J Immunol* **174**, 6477–6489 (2005).
12. Ishikawa, F. et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor {gamma} chain(null) mice. *Blood* **106**, 1565–1573 (2005).
13. Honma, Y., Ishii, Y., Sassa, T. & Asahi, K. Treatment of human promyelocytic leukemia in the SCID mouse model with corytenin A, an inducer of myelomonocytic differentiation of leukemia cells. *Leuk Res* **27**, 1019–1025 (2003).
14. Kiser, M. et al. Oncogene-dependent engraftment of human myeloid leukemia cells in immunosuppressed mice. *Leukemia* **15**, 814–818 (2001).
15. Pirruccello, S.J. et al. OMA-AML-1: a leukemic myeloid cell line with CD34+ progenitor and CD15+ spontaneously differentiating cell compartments. *Blood* **80**, 1026–1032 (1992).
16. Terpstra, W. et al. Conditions for engraftment of human acute myeloid leukemia (AML) in SCID mice. *Leukemia* **9**, 1573–1577 (1995).
17. Lapidot, T. et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* **367**, 645–648 (1994).
18. Ailles, L.E., Gerhard, B., Kawagoe, H. & Hogge, D.E. Growth characteristics of acute myelogenous leukemia progenitors that initiate malignant hematopoiesis in nonobese diabetic/severe combined immunodeficient mice. *Blood* **94**, 1761–1772 (1999).
19. Pearce, D.J. et al. AML engraftment in the NOD/SCID assay reflects the outcome of AML: implications for our understanding of the heterogeneity of AML. *Blood* **107**, 1166–1173 (2006).
20. Lumkul, R. et al. Human AML cells in NOD/SCID mice: engraftment potential and gene expression. *Leukemia* **16**, 1818–1826 (2002).
21. Yalcintepe, L., Frankel, A.E. & Hogge, D.E. Expression of interleukin-3 receptor subunits on defined subpopulations of acute myeloid leukemia blasts predicts the cytotoxicity of diphtheria toxin interleukin-3 fusion protein against malignant progenitors that engraft in immunodeficient mice. *Blood* **108**, 3530–3537 (2006).
22. Feuring-Buske, M. et al. Improved engraftment of human acute myeloid leukemia progenitor cells in beta 2-microglobulin-deficient NOD/SCID mice and in NOD/SCID mice transgenic for human growth factors. *Leukemia* **17**, 760–763 (2003).
23. Ishikawa, fF. et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone marrow endosteal region. *Nature Biotechnology* **25**, 1315–1321 (2007).
24. Ninomiya, M. et al. Homing, proliferation and survival sites of human leukemia cells in vivo in immunodeficient mice. *Leukemia* **21**, 136–142 (2007).
25. Liem, N.L. et al. Characterization of childhood acute lymphoblastic leukemia xenograft models for the preclinical evaluation of new therapies. *Blood* **103**, 3905–3914 (2004).
26. Nijmeijer, B.A. et al. Monitoring of engraftment and progression of acute lymphoblastic leukemia in individual NOD/SCID mice. *Experimental Hematology* **29**, 322–329 (2001).
27. Schimmel, K.J., Nijmeijer, B.A., van Schie, M.L., Falkenburg, J.H. & Guchelaar, H.J. Limited antitumor-effect associated with toxicity of the experimental cytotoxic drug cyclopentenyl cytosine in NOD/scid mice with acute lymphoblastic leukemia. *Leuk Res* (2007).
28. Nijmeijer, B.A., van Schie, M.L., Verzaal, P., Willemze, R. & Falkenburg, J.H. Responses to donor lymphocyte infusion for acute lymphoblastic leukemia may be determined by both

- qualitative and quantitative limitations of antileukemic T-cell responses as observed in an animal model for human leukemia. *Exp Hematol* **33**, 1172–1181 (2005).
- 29. Nijmeijer, B.A., Willemze, R. & Falkenburg, J.H. An animal model for human cellular immunotherapy: specific eradication of human acute lymphoblastic leukemia by cytotoxic T lymphocytes in NOD/scid mice. *Blood* **100**, 654–660 (2002).
  - 30. Jamieson, C.H. et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med* **351**, 657–667 (2004).
  - 31. Jaiswal, S. et al. Expression of BCR/ABL and BCL-2 in myeloid progenitors leads to myeloid leukemias. *Proc Natl Acad Sci U S A* **100**, 10002–10007 (2003).
  - 32. Hoyle, C.F. & Negrin, R.S. Engraftment of chronic myeloid leukemia in SCID mice. *Hematol Oncol* **16**, 87–100 (1998).
  - 33. Namikawa, R., Ueda, R. & Kyoizumi, S. Growth of human myeloid leukemias in the human marrow environment of SCID-hu mice. *Blood* **82**, 2526–2536 (1993).
  - 34. Sawyers, C.L., Gishizky, M.L., Quan, S., Golde, D.W. & Witte, O.N. Propagation of human blastic myeloid leukemias in the SCID mouse. *Blood* **79**, 2089–2098 (1992).
  - 35. Sirard, C. et al. Normal and leukemic SCID-repopulating cells (SRC) coexist in the bone marrow and peripheral blood from CML patients in chronic phase, whereas leukemic SRC are detected in blast crisis. *Blood* **87**, 1539–1548 (1996).
  - 36. Beran, M. et al. Biological properties and growth in SCID mice of a new myelogenous leukemia cell line (KBM-5) derived from chronic myelogenous leukemia cells in the blastic phase. *Cancer Res* **53**, 3603–3610 (1993).
  - 37. Skorski, T., Nieborowska-Skorska, M. & Calabretta, B. A model of Ph' positive chronic myeloid leukemia-blast crisis cell line growth in immunodeficient SCID mice. *Folia Histochem Cytobiol* **30**, 91–96 (1992).
  - 38. Dazzi, F. et al. The kinetics and extent of engraftment of chronic myelogenous leukemia cells in non-obese diabetic/severe combined immunodeficiency mice reflect the phase of the donor's disease: an in vivo model of chronic myelogenous leukemia biology. *Blood* **92**, 1390–1396 (1998).
  - 39. Lewis, I.D., McDiarmid, L.A., Samels, L.M., To, L.B. & Hughes, T.P. Establishment of a reproducible model of chronic-phase chronic myeloid leukemia in NOD/SCID mice using blood-derived mononuclear or CD34+ cells. *Blood* **91**, 630–640 (1998).
  - 40. Verstegen, M.M., Cornelissen, J.J., Terpstra, W., Wagemaker, G. & Wognum, A.W. Multi-lineage outgrowth of both malignant and normal hemopoietic progenitor cells from individual chronic myeloid leukemia patients in immunodeficient mice. *Leukemia* **13**, 618–628 (1999).
  - 41. Wang, J.C. et al. High level engraftment of NOD/SCID mice by primitive normal and leukemic hematopoietic cells from patients with chronic myeloid leukemia in chronic phase. *Blood* **91**, 2406–2414 (1998).
  - 42. Eisterer, W. et al. Different subsets of primary chronic myeloid leukemia stem cells engraft immunodeficient mice and produce a model of the human disease. *Leukemia* **19**, 435–441 (2005).
  - 43. Carlo-Stella, C. et al. CD52 antigen expressed by malignant plasma cells can be targeted by alemtuzumab in vivo in NOD/SCID mice. *Exp Hematol* **34**, 721–727 (2006).
  - 44. Ito, K. et al. 1'-acetoxychavicol acetate is a novel nuclear factor kappaB inhibitor with significant activity against multiple myeloma in vitro and in vivo. *Cancer Res* **65**, 4417–4424 (2005).
  - 45. Mitsiades, C.S. et al. Fluorescence imaging of multiple myeloma cells in a clinically relevant SCID/NOD in vivo model: biologic and clinical implications. *Cancer Res* **63**, 6689–6696 (2003).
  - 46. Tassone, P. et al. A clinically relevant SCID-hu in vivo model of human multiple myeloma. *Blood* **106**, 713–716 (2005).
  - 47. Yaccoby, S., Barlogie, B. & Epstein, J. Primary myeloma cells growing in SCID-hu mice: a model for studying the biology and treatment of myeloma and its manifestations. *Blood* **92**, 2908–2913 (1998).

48. Dewan, M.Z. et al. Prompt tumor formation and maintenance of constitutive NF-kappaB activity of multiple myeloma cells in NOD/SCID/gammacnull mice. *Cancer Sci* **95**, 564–568 (2004).
49. Bonnet, D. & Dick, J.E. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* **3**, 730–737 (1997).
50. Feuring-Buske, M. & Hogge, D.E. Hoechst 33342 efflux identifies a subpopulation of cytogenetically normal CD34(+)CD38(−) progenitor cells from patients with acute myeloid leukemia. *Blood* **97**, 3882–3889 (2001).
51. Sperr, W.R. et al. Human leukaemic stem cells: a novel target of therapy. *European journal of clinical investigation* **34 Suppl 2**, 31–40 (2004).
52. Buccisano, F. et al. CD90/Thy-1 is preferentially expressed on blast cells of high risk acute myeloid leukaemias. *Br J Haematol* **125**, 203–212 (2004).
53. Jordan, C.T. et al. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. *Leukemia* **14**, 1777–1784 (2000).
54. Hosen, N. et al. CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. *Proc Natl Acad Sci U S A* **104**, 11008–11013 (2007).
55. Guzman, M.L. et al. The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. *Blood* **105**, 4163–4169 (2005).
56. Guzman, M.L. et al. Preferential induction of apoptosis for primary human leukemic stem cells. *Proc Natl Acad Sci U S A* **99**, 16220–16225 (2002).
57. Jin, L., Hope, K.J., Zhai, Q., Smadja-Joffe, F. & Dick, J.E. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nat Med* **12**, 1167–1174 (2006).
58. Krause, D.S., Lazarides, K., von Andrian, U.H. & Van Etten, R.A. Requirement for CD44 in homing and engraftment of BCR-ABL-expressing leukemic stem cells. *Nat Med* **12**, 1175–1180 (2006).
59. Dar, A., Kollet, O. & Lapidot, T. Mutual, reciprocal SDF-1/CXCR4 interactions between hematopoietic and bone marrow stromal cells regulate human stem cell migration and development in NOD/SCID chimeric mice. *Exp Hematol* **34**, 967–975 (2006).
60. Tavor, S. et al. CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice. *Cancer Res* **64**, 2817–2824 (2004).
61. Hotfilder, M. et al. Leukemic stem cells in childhood high-risk ALL/t(9;22) and t(4;11) are present in primitive lymphoid-restricted CD34+CD19- cells. *Cancer Res* **65**, 1442–1449 (2005).
62. Castor, A. et al. Distinct patterns of hematopoietic stem cell involvement in acute lymphoblastic leukemia. *Nat Med* **11**, 630–637 (2005).
63. George, A.A. et al. Detection of leukemic cells in the CD34(+)CD38(−) bone marrow progenitor population in children with acute lymphoblastic leukemia. *Blood* **97**, 3925–3930 (2001).

# **Chapter 6**

## **Dietary Restriction: A Model System**

### **Probing the Cell Fate Decision**

### **Between Cancer and Senescence**

**Robin P. Ertl and David E. Harrison**

## **Contents**

6.1	Introduction . . . . .	133
6.2	Current Paradigm . . . . .	135
6.3	Cancer, Senescence, and Evolution . . . . .	136
6.4	The Role of p16 in Mice . . . . .	138
6.5	The Role of p53 in Mice . . . . .	140
6.6	Does Dietary Restriction Break the Paradigm? . . . . .	142
6.7	Model Systems to Examine Effects of DR . . . . .	142
6.8	Competitive Repopulation . . . . .	143
6.9	DR in BALB Mice . . . . .	144
6.10	DR in B6 Mice . . . . .	148
6.11	The need to Examine Multiple Strains . . . . .	149
6.12	Problems with Comparisons Between Strains . . . . .	150
6.13	Proposed Model . . . . .	151
6.14	Future Research . . . . .	152
6.15	Conclusion . . . . .	153

## **6.1 Introduction**

A major aspect of cancer is unregulated cell proliferation. Yet, in general as organisms age, their cells lose the ability to proliferate, which leads to cellular senescence (Hayflick 1965; Smith and Pereira-Smith 1996). It is important to remember that the ability of stem cells to repopulate a tissue *in vivo* requires more than just proliferation. It requires a coordinated pattern of homing, engraftment, self-renewal, differentiation, and proliferation. In this chapter, all of these necessary functions are referred to collectively as repopulating ability (RA). The loss of RA can cause severe clinical problems with age, such as anemia (Robinson 2003; Guralnik et al. 2004; Penninx et al. 2004). There are treatments that can increase RA and delay senescence. Unfortunately, most of

---

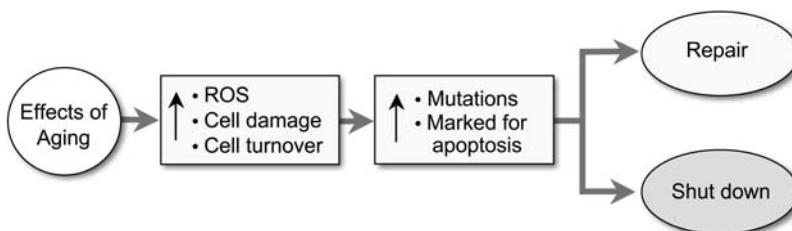
D.E. Harrison  
The Jackson Laboratory, Bar Harbor, ME 04609, USA  
david.harrison@jax.org

these treatments also increase the risk of cancer (Campisi 2003; Pardal et al. 2005; Beausejour and Campisi 2006). The maximal potential lifespan of an organism, therefore, seems to depend on balancing the need to maintain the RA versus the potential risk that cells will transform.

The exact mechanisms of aging are largely unknown. However, we do know that the balance of these two cell fates—cell senescence versus cancer—depends, in part, on the type of cellular damage present. General cellular damage can be a marker for cellular turnover or apoptosis, and accumulated genetic damage may lead to cellular senescence or cancer. To avoid the lineage of genetically damaged cells from having systemic consequences, the organism can either repair the damage or minimize the effect by shutting down the cell (Fig. 6.1). DNA repair is a major field and a topic for other reviews. The focus of this chapter is the cellular response when DNA repair is insufficient and damaged cells must try to minimize deleterious effects by balancing risks.

The central role of stem cells in cancer has come under increasing scrutiny. Krivtsov et al. (2006) showed that it is possible to generate cancerous cells from definitive hematopoietic cells by a single transposition. In general, though, more than a single mutation is needed to transform a cell line (Pardal, Clarke and Morrison 2003; Pardal et al. 2005; Krivtsov et al. 2006; Giordano et al. 2007). It is unlikely that all the requisite mutations will occur within the lifespan of a single cell. Thus, to acquire the several mutations necessary for transformation into a carcinoma, mutations must occur in cells that have a self-renewal capacity, such as, stem cells.

Tumor suppressors may be the mechanism by which this cell fate decision is regulated. Recent studies have shown that in hematopoietic stem cells (HSCs) (Janzen et al. 2006), pancreatic islets (Krishnamurthy et al. 2006), and neural stem cells (Molofsky et al. 2005, 2006), a decrease in the tumor suppressor p16<sup>INK4a</sup> results in an increase in stem cell/precursor cell RA. Conversely, as p16<sup>INK4a</sup> increases with age, there is a decrease in RA. The tumor suppressor p53 also exhibits a similar inverse relationship between a tissue's RA and gene



**Fig. 6.1 Cell fate decision imposed by damage.** Effects of aging result from lifelong exposure to damaging conditions such as reactive oxygen species (ROS) leading to mutations and apoptosis. This damage forces a cell fate decision: to avoid cancer, cells must either repair the damage or, if the damage is irreparable, shut down

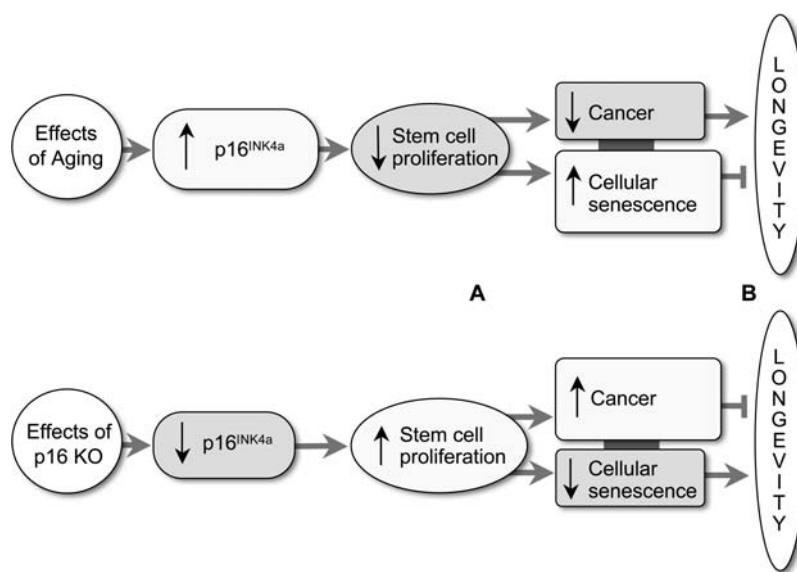
expression (Tyner et al. 2002; TeKippe, Harrison and Chen 2003; Maier et al. 2004; Dumble et al. 2007; Gatz et al. 2007).

Recent studies of p16<sup>INK4a</sup> expression in stem cells indicate that its highest levels occur in the most primitive precursors and that expression of tumor suppressors increases with age (Molofsky et al. 2005, 2006; Janzen et al. 2006; Krishnamurthy et al. 2006). This is consistent with the hypothesis that most cancerous cell lines are derived from stem cells acquiring multiple mutations and that induction of tumor suppressors with age is necessary to obviate the effects of accumulated, spontaneous mutations. The lack of induction of p16<sup>INK4a</sup> in less primitive progenitors is potentially explained by the fact that they exist in the body for a shorter period of time, acquire fewer mutations, and are ultimately replaced by new lineages derived from differentiation of the most primitive precursors.

Mice are an ideal organism in which to study the relationships between senescence and cancer. With age, they exhibit an increased incidence of spontaneous cancers, a general decrease in RA, and altered stem cell functions. The fact that they have a short lifespan—aging 30 times faster than humans—and are relatively small makes longitudinal studies of mammalian aging practical in a modest space. In addition, the full genomic sequence is known for several strains of mice, many antibodies and other reagents are available, cell surface markers to identify specific cell types are known, and many assays and techniques have been worked out in detail (Information available through The Jackson Laboratory public website, [www.jax.org](http://www.jax.org)). In this chapter, we will examine stem cell aging in several mouse models and discuss possible mechanisms regulating the balance between cancer and senescence.

## 6.2 Current Paradigm

The recent studies that utilized p16<sup>INK4a</sup> knockouts (KOs) caused Beausejour and Campisi (2006), Janzen et al. (2006), Krishnamurthy et al. (2006), and Molofsky et al. (2006) to propose a model governing the cell fate decision which, in part, determines the maximal lifespan of an organism; their similar models are summarized in Fig. 6.2. These models relate the regulation of stem cell proliferative ability by p16<sup>INK4a</sup> to the intricate balance between cancer risk and cellular senescence. This bimodal fate can be envisioned as walking down a path that runs along a mountain ridge. To each side there is a steep chasm, and as we go along, the path narrows. To reach our maximal lifespan, we try to balance the two fates: If we lose our balance and go to the right, we fall off the path and die due to lack of proliferation leading to senescence. If we lose our balance in the other direction, while we increase proliferation, we fall off the path and die from cancer. Thus, to reach the maximal lifespan, we need to allow just enough proliferation to avoid senescence, yet the least proliferation possible to avoid cancer. The longer we live, though, the narrower the path becomes



**Fig. 6.2 Current paradigm.** The recent articles of Beausejour and Campisi (2006), Janzen et al. (2006), Krishnamurthy et al. (2006), and Molofsky et al. (2006) have proposed a model governing the cell fate decision that balances the consequences of cancer risk and cellular senescence as a function of cell proliferation (**A**) regulated by P16. This balance, in part, determines the longevity (**B**)

and the more difficult it is to avoid either senescence or cancer due to the accumulation of spontaneous mutations.

### 6.3 Cancer, Senescence, and Evolution

For any theoretical relationship between cancer and senescence to be more than a laboratory artifact created by random chance in a particular species, the relationship must have evolved as a result of positive selective pressure or pleiotropy via an evolutionary stable strategy. Aranda-Anzaldo and Dent (2007) suggested that cancer and senescence are not viable targets of selective pressures because, through evolutionary history, these events usually occur post-reproduction. They cite, as an example, feral mice with 90% mortality by 40 weeks of age, yet with little or no evidence of cancer at that age. That does not mean that the feral mice cannot get cancer; however, it occurs so late in life that it does not exert enough selective pressure to overcome genetic drift. Aranda-Anzaldo and Dent (2007) suggest that tumor suppressors such as p53 and p16<sup>INK4a</sup> in the Campisi model (Fig. 6.2) are primarily required during development to eliminate cells that have mutated, failed to replicate properly, or are not required for later stages of development. Their conclusions depend in

part on two factors: (1) the occurrence of cancer in juvenile or reproducing animals was rare in evolutionary history and (2) prior to the industrial revolution, there were no cancer-causing environmental factors that required tumor suppressors for the survival of young adults.

In fact, these questions are far from settled. The data indicating that cancer is rare in juvenile and reproducing animals are based on organisms that have already evolved intricate systems of DNA repair, tumor suppression, and apoptosis. The deletion of only one of these pathways (e.g., p53) dramatically reduces lifespan, with cancers that develop by 6 months of age or less, an age at which it would significantly decrease reproductive fitness.

While the biological burden of cancer-causing mutagens is commonly associated with discharge of pollutants starting with the industrial revolution, in fact, the greatest burden is from the environmental factors and the food we consume (Ames and Gold 1998), with the most potent mutagens occurring naturally. These mutagens are thought to have evolved by what is referred to as plant–animal warfare (Gonzalez and Nebert 1990). Plants, to expand their habitat, developed natural defense mechanisms to limit predation. Animals, to continue to occupy that niche, then evolved detoxification pathways to these compounds, thus causing plants to generate ever more potent mutagens, in a cycle of adaptation and evolution that continues.

Compared to many animals, mammals mature and reproduce slowly. Through evolutionary history, maturing and reproducing mammals were faced with the normal background mutation rate and the consequences of naturally occurring mutagens. These could have provided the selective pressure required to develop the complex pathways of tumor suppression and DNA repair needed to maintain genomic stability and postpone cancers until after the necessary reproductive period.

The mutation rate and the incidence of cancer are, therefore, reasonable targets upon which evolutionary forces can act. In fact, the mutation rate is thought to be highly regulated: a certain number of mutations must occur, prior to reproductive fitness, to provide natural selection with the needed genetic diversity in subsequent generations. However, excessive mutations will have deleterious, if not lethal, effects.

Research into the age when senescence occurs, and whether senescence is a valid target of evolutionary selective pressure, has similarly been considered in species that have already undergone significant evolutionary selection to determine their lifespans. The selective pressure that determined the age at which cellular senescence occurs may have been the pressure to ensure fitness during reproduction—that is to say senescence is caused by exhaustion or decline of mechanisms evolved to be present until the age-specific force of selection approaches zero. In the mouse, this age would be near the end of the female reproductive lifespan. The feral mouse, for example, must avoid predators during its reproductive period, so diseases of aging, such as muscular atrophy, are not expressed until reproductive fitness declines. In the case of cellular senescence, a number of cellular characteristics may be functionally linked. The forces of

evolution may exert pressure so that all processes in the linked pathways are functional until an age just prior to the end of reproductive fitness. Such linkages may explain, for instance, why HSCs can be transplanted and repopulate the marrow far more than would be needed in several mouse lifetimes.

Some post-mitotic organisms do not appear to develop cancer, having neither the opportunity, with no adult cell turnover, nor the time, given their short lifespan. Yet, they express p53. This indicates, as Aranda-Anzaldo and Dent (2007) suggest, that p53 arose due to developmental requirements. However, in mammals, cancer and senescence are in an equilibrium determined by evolutionarily selective pressures. P53 may have been altered through evolutionary history to regulate the proliferative ability in response to mutations throughout the entire lifecycle, just as p53 regulates proliferative ability and apoptosis during development of post-mitotic organisms. As organisms evolved, the need for continuous cell turnover throughout their life cycles, there arose the need to mitigate the vulnerabilities to cancers caused by cell turnover. If, in fact, there was no natural selection on the balance between cancers and senescence, then this relationship should vary with the strain examined as a function of genetic drift. The rates of both cancer and senescence, however, correlate closely in many species, suggesting evolution directly by natural selection or indirectly by pleiotropy, not random drift.

Cancer and senescence are both linked by the proliferative ability upon which both depend. As we propose below, the cell fate decision may not be black and white, but rather a gray scale of possibilities where the balance between cancer and senescence exists throughout an organism's lifespan: As the organism ages, there are fewer options, forcing a cell fate decision, similar to the metaphor given earlier where the path along the precipice narrows the further you progress down the path. In this model, it is useful to think of both cancer and senescence as outcomes of processes continually altered over the lifetime of the organism—that cellular senescence is the exhaustion of a lineage of proliferating cells and that cancer results from the accumulation of mutations which short-circuit the normal mechanisms halting proliferation. The accumulation of mutations leading to cancer may occur throughout the lifespan of the organism. The need to have sufficient proliferation to avoid senescence, but at the same time defend against the increased mutations associated with that proliferation, may be the selective pressure responsible for the development of cancer defense systems. The breakdown of these systems, post-reproduction, leads to diseases of aging.

## 6.4 The Role of p16 in Mice

The current data from  $p16^{INK4a}$  studies in mice are consistent with the model proposed in Fig. 6.2 for regulating the balance between cancer and senescence. Janzen et al. (2006), Krishnamurthy et al. (2006), and Molofsky et al. (2006)

found that, with age, p16<sup>INK4a</sup> levels increased in precursor/stem cell subpopulations to a greater extent than in differentiated cells. This increase correlated with a decrease in RA while RA increased in p16<sup>INK4a</sup> KO mice. The decrease in p16<sup>INK4a</sup> and increase in RA in KO mice was accompanied by an increase in cancer incidence. These results suggest that the increase in p16<sup>INK4a</sup> with age is a preprogrammed response decreasing proliferative ability to mitigate the increase in cancer risk that occurs with age.

Janzen et al. (2006) examined these trends in the primitive precursors of hematopoiesis in C57BL/6 (B6) mice, long-term HSCs (LT-HSCs). Using the BrdU assay, they observed a decrease in the turnover of LT-HSCs with age commensurate with the increase in p16<sup>INK4a</sup>. In B6 KO p16<sup>INK4a</sup> mice, where there is a decrease in p16<sup>INK4a</sup> levels, they found a significant increase in HSC turnover. This measure of cell proliferation is a composite of both differentiation into definitive cell types and self-renewal. The increase in Hes-1, a downstream effector of Notch signaling associated with self-renewal, combined with the increase in the number of LT-HSCs present in the KO mice, indicates that part of the observed increase in proliferation is because of the increase in self-renewal. These data are consistent with the concept that increased p16<sup>INK4a</sup> expression is a preprogrammed response to age post-reproduction. However, this concept contradicts the idea that such responses cannot evolve in the life cycle during a period with little or no selective pressure. Is the increase in p16<sup>INK4a</sup> then a response to other changes with age that occur earlier in the lifecycle?

In an especially rigorous measure of RA, transplanting bone marrow (BM) serially into successive recipients causes a tremendous stress on precursor cells due to increased demand for proliferation and self-renewal. A single serial transplantation causes a decline in HSC functional abilities in B6 mice that is far greater than a lifetime of normal function (Harrison, Astle and Stone 1989; Harrison et al. 1993; Chen, Astle and Harrison 2000; Yuan et al. 2005). After three successive serial transplants, Janzen et al. (2006) found an increase in the p16<sup>INK4a</sup> expression along with a decrease in the number of leukocytes. In the p16<sup>INK4a</sup> KO mice under the same serial transplant conditions, leukocyte numbers were increased compared to similarly aged controls (Janzen et al. 2006). These data support the contention that p16<sup>INK4a</sup> is, in fact, suppressing both the proliferation and the risk of cancer in these serially transplanted HSCs. This tumor suppressor is not the entire answer, though, as the number of leukocytes also decreases after serial transplantation in p16<sup>INK4a</sup> KO mice, albeit to a lesser extent, suggesting that p53 and/or other factors are involved in this cell fate decision.

This increase in RA, caused by the decrease in p16<sup>INK4a</sup> in the KO mice, is thought to be a contributing factor to the decrease in apoptosis and the subsequent increase in survival after serial transplantation. Two fundamental questions remain: (1) What causes the increase in p16<sup>INK4a</sup> levels? The observed increase in p16<sup>INK4a</sup> levels may be a consequence of normal aging; however, if this increase occurs only at an age with little or no evolutionary forces at work,

how did this increase evolve? It also may be a response to the partial exhaustion of proliferative ability or a response to the mutation rate and the risk of cancer. Are these processes active during earlier stages of the life history and thus under evolutionary selective pressures? The observed increase in p16<sup>INK4a</sup> after serial transplantation may be a response to the mutation rate rather than the aging of the cell lineage. (2) If these processes are active during other parts of our life cycle, do we spend our entire life balancing this cell fate decision or can this decision be delayed in order to lengthen lifespan?

Thus far, all studies that examined p16<sup>INK4a</sup> in precursors/stem cells in a variety of organs suggest that aspects of RA are regulated by p16<sup>INK4a</sup>. However, while the reported measures of B6 HSC RA (discussed above) are consistent with this theory, not all measures of HSC RA decrease at times when p16<sup>INK4a</sup> levels are known to increase. Unlike other strains, in B6 mice, the RA of total marrow goes up with age (Chen, Astle and Harrison 2000) while RA per HSC remains fairly constant (Rossi et al. 2005; Ertl et al. 2008). This occurs at an age when Janzen et al. reported that p16<sup>INK4a</sup> levels were increasing in B6 mice (Janzen et al. 2006). We do know that there is an increase in lifespan in B6 mice compared to most other strains. Thus, the increase in RA of total marrow with age might be the result of a compensatory mechanism. Combined, these data suggest that the regulation of this pathway must be more complex than shown in Fig. 6.2.

## 6.5 The Role of p53 in Mice

The tumor suppressor p53 is thought to cause apoptosis, leading to a decreased RA with age at the tissue level, thus bringing about organ or organismal senescence. Similar to p16<sup>INK4a</sup>, p53 concentration showed an inverse relationship to RA at the organ level. Reduced p53 activity in p53<sup>-/-</sup> mutants was associated with twice as many LT-HSCs that gave rise to 37% more 9-day spleen colony forming units (CFU-S), along with an increase in the number of proliferating HSCs and hematopoietic progenitors (TeKippe, Harrison and Chen 2003; Dumble et al. 2007; Gatzka et al. 2007; Table 6.1). The RA of transplanted BM from p53-deficient mice is two- to four-fold greater than for controls, an advantage that increased with the time after transplant. This was in part due to the two- to three-fold increase in the number of donor LT-HSCs present in the recipient BM (TeKippe, Harrison and Chen 2003). Importantly, decreasing the p53 expression to increase HSC function has a severe cost—only half of transplant recipients survive after 4 months, and they are all dead by 8 months, time points at which HSC recipients usually survive. In the genetic mutant p53<sup>+/-m</sup>, which has greater in vivo concentrations of p53 than wild-type mice, there was reduced marrow cellularity and atrophy (Dumble et al. 2007). At these higher levels of p53, the numbers of HSCs in old mice were reduced when compared to wild-type controls. When these p53<sup>+/-m</sup> HSCs were transplanted into lethally

**Table 6.1** Relationships among p53 dosage, longevity, cancer, and hematopoietic stem cell (HSC) function

p53 genotype	Donor marrow composition*			Recipient marrow composition†		Effects of genotype on donor marrow function			Aged HSC reconstitution (%)‡
	BMC $10^6$	CFU-S, day 9	LT-HSC‡	Percent survival	LT-HSC‡	Median longevity (weeks)§	Cancer incidence (%)△	Aged HSC no. (%)**	
p53 -/-	231	20.2	0.010%	44%	0.018%	18	100	ND	ND
p53 +/-	ND	ND	ND	ND	ND	52	90	0.22	0.070
p53 +/+	296	14.7	0.004%	92%	0.006%	118	45	0.15	0.029
p53 +/m	ND	ND	ND	ND	ND	96	6	0.08	0.019

CFU-S, colony forming units; LT-HSCs, long-term HSCs.

The first six columns are data from TeKippe, Harrison and Chen (2003). Five-month data represents result of a competitive repopulation study. The next five columns are from Dumble et al. (2007).

\* 4–6 males at 2–5 months.

† 5 months post-transplantation.

‡ Lin<sup>-</sup>, Sca<sup>+</sup>, cKit<sup>+</sup>, CD34<sup>-</sup>.

§ Age in weeks at which 50% of a p53 cohort died.

△ Percentage of mice in a p53 cohort that develop cancer in a lifespan.

\*\* Percentage of HSCs (Lin<sup>-</sup>, Sca<sup>+</sup>, cKit<sup>+</sup>, Flk-2<sup>-</sup>) in total marrow cells from aged (18 to 20-months old) mice.

† Percentage of proliferating HSCs in total marrow cells.

‡‡ Percentage of hematopoietic system reconstituted by 500 aged HSCs from donors of a given p53 genotype 12 weeks after transplantation.

irradiated recipients, they exhibited reduced homing and engraftment (Dumble et al. 2007).

As mentioned in the previous section, the p16<sup>INK4a</sup> data indicate that it is not the sole regulator of the balance between cancer and senescence. The inverse correlation between p53 and apoptosis, another regulator of cell proliferation, suggests that there may be several critical regulators of this cell fate decision.

## 6.6 Does Dietary Restriction Break the Paradigm?

Insights into the regulation of cell proliferation are found in mice treated with lifelong diet restriction (DR). DR is a treatment that reduces food intake to about 70% of the normal diet. Even at these reduced levels, the NIH-31 (4% fat) diet provides all the essential nutrients without the need for dietary supplements. With lifelong DR, there is a significant increase in lifespan concurrent with a reduction in the incidence of cancer (Bronson and Lipman 1991; Blackwell et al. 1995; Turturro et al. 2002) and many other markers of aging (Miller and Harrison 1985; Effros et al. 1991; Masoro 1993; Luan et al. 1995; Chen, Astle and Harrison 1998, 2003). For the paradigm in Fig. 6.2 to hold, the risk of cancer and cellular senescence must be inversely correlated as the organism balances the risks to achieve maximal fitness. The question then is whether the resulting increase in lifespan due to the decrease in cancer is concomitant with a substantial decrease in cell proliferative abilities. At least with HSCs, this is not the case. As detailed below, there is no increase in senescence shown as a reduction of HSC RA with DR. In fact, both the risk of cancer and cell senescence are simultaneously reduced (Ertl et al. 2008). However, DR may not necessarily break the paradigm but instead delay this cell fate decision.

## 6.7 Model Systems to Examine Effects of DR

If we are to examine the underlying mechanism regulating this cell fate decision, we need a model that distinguishes the epigenetic health changes caused by under-eating from the phenotypes of aging that are genetically regulated. As noted above, recent studies of the role of tumor suppressors p53 and p16<sup>INK4a</sup>, combined with the necessary role of self-renewal needed to transform cell lines, make adult stem cells ideal candidates for study. The role of these primitive cells is to replace tissue after it becomes defective. Of the adult stem cells, for which phenotypes can be directly measured, HSCs are by far the best characterized. The lineages of cells produced from differentiation of HSCs are defined, and quantitative assays of HSC function *in vivo* have been worked out in detail (Harrison, Astle and Stone 1989; Van Zant et al. 1990; Harrison and Zhong 1992; Harrison et al. 1993; Morrison et al. 1996; Chen, Astle and Harrison 2000;

Sudo et al. 2000; Liang, Van Zant and Szilvassy 2005; Sharma et al. 2005; Yuan et al. 2005; Min, Montecino-Rodriguez and Dorshkind 2006). In addition, well-defined cell surface markers can identify and be used to separate both the primitive precursors and the definitive cell types (Spangrude, Heimfeld and Weissman 1988; Spangrude and Brooks 1993; Morrison and Weissman 1994; Goodell et al. 1996; Christensen and Weissman 2001; Ishida, Zeng and Ogawa 2002; Bryder, Rossi and Weissman 2006; Lin and Goodell 2006; Pearce et al. 2007; Rossi, Bryder and Weissman 2007).

Mice are ideal models in which to investigate the effects of lifelong DR and the accumulation of spontaneous mutations that lead to cancer (The Jackson Laboratory 1997). They age rapidly (30 times faster than humans), and the use of inbred strains allows the investigation of such phenomena without the confounding effects of genetic variability (Flurkey, Currer and Harrison 2007). Thus, studying HSCs in DR inbred mice is an ideal platform from which to probe the paradigm in Fig. 6.2 and determine how cells make a fate decision amongst normal function, cancer, and senescence.

Both BALB/cByJ (BALB) and B6 mice exhibit the same overt response to DR. Upon autopsy, old DR mice, compared to similarly aged ad libitum (AL) controls, exhibit lower incidences of most cancers, less brittle bones, less heart calcification, and a younger morphology of internal organs, all culminating in a longer lifespan (Miller and Harrison 1985; Bronson and Lipman 1991; Effros et al. 1991; Masoro 1993; Blackwell et al. 1995; Luan et al. 1995; Chen, Astle and Harrison 1998, 2003; Turturro et al. 2002). These two strains of mice, though, differ dramatically in the effect of DR on the RA of LT-HSCs, as discussed in detail below (Ertl et al. 2008).

## 6.8 Competitive Repopulation

The crux of the proposed paradigm in Fig. 6.2 depends on the relationship between cancer and proliferation. A highly sensitive assay that measures both proliferation and differentiation of HSCs is the competitive repopulation assay (CRA), given in detail elsewhere (Harrison, Astle and Stone 1989; Harrison and Zhong 1992; Harrison et al. 1993; Chen, Astle and Harrison 2000, 2003; Sharma et al. 2005; Yuan et al. 2005; Ertl et al. 2008). The CRA is an ex vivo assay that takes advantage of the standard BM transplant. The marrow to be tested (donor) is mixed with an aliquot of a standard pool of marrow obtained from genetically identifiable young mice (competitor). The mixture is injected into lethally irradiated recipient mice, where all mice—donor, competitor and recipients—are strain matched. The result is that HSCs from both the competitor and the donor repopulate the recipient. RA of each donor in an experiment are expressed in repopulating units (RUs) relative to the marrow of the standard competitor pool.

The standard dosages of irradiation used in such experiments (usually about 1000 mGy) kill or disable the host HSCs and their descendent precursor cells so they are replaced by donor and competitor cells over the next few weeks (Harrison and Zhong 1992) and for the rest of their lives, usually about 1 year. Initially after transplantation, host cells are replaced by descendants of the multipotent progenitors (MPPs) in the transplanted marrow. During the first month post-transplantation, the MPP cells and their descendants are replaced by cells differentiated from the short-term HSCs (ST-HSCs). By 4 months post-transplantation, the ST-HSCs, MPPs, and definitive cells present are due to the action of only LT-HSCs, because the other, more differentiated, cells have been exhausted.

Comparing the contributions of the donor and competitor to the definitive cells present in peripheral blood 4–6 months post-transplantation gives the RA of a given donor's LT-HSCs. The quantitative advantage of using a competitor pool as a standard to which all donors can be compared is obvious. In addition, this combination of donor and competitor cells minimizes the stress to the mouse. It assures rescue from lethal irradiation, regardless of the RA of the donor cells, because the competitor cells alone are sufficient to maintain recipient health (Harrison, Astle and Stone 1989; Harrison et al. 1993; Chen, Astle and Harrison 2000).

While the CRA has been well utilized in examining HSCs, this technique can be, in theory, extended to the measurement of RA of any transplantable adult stem cell line. A wide variety of genetic markers are available to distinguish cells descended from donor and competitor stem cells. For non-HSCs, a major difficulty is removing endogenous stem cells from the recipient while avoiding harm that might prevent growth of donor stem cells. Another difficulty is separating cells based on their stage of differentiation—from adult stem cells to definitive somatic cells.

## 6.9 DR in BALB Mice

The BALB strain of mice is an important model system because, unlike B6 mice, their BM shows a significant loss in RA with age (Miller and Harrison 1985; Chen, Astle and Harrison 2000; Ertl et al. 2008). Lifelong DR of BALB mice prevents the age-related loss in the RA, producing levels greater than found in young controls (Miller and Harrison 1985; Ertl et al. 2008). When the RA is expressed per HSC, there is a 20- to 50-fold loss with age, which is also greatly alleviated by DR (Ertl et al. 2008). Thus, by altering the function per stem cell and the number of stem cells present, DR delays the effects of aging and maintains the overall RA of BALB BM.

*HSC markers.* The combined concentration of LT- and ST-HSCs can be determined by flow cytometry of cells expressing the cell surface marker, cKit, that also have a high drug efflux capacity defined by the double negative, linear segment of the Hoechst-staining profile. The linear segment is defined as the

side population (SP) (Goodell et al. 1996, 1997; Goodell 1999; Lin and Goodell 2006; Pearce et al. 2007). While this segment contains multiple cell types, the tip is known to be enriched for LT-HSCs (Goodell et al. 1996; Lin and Goodell 2006). This method of determination (SP+Kit) shows a slight increase in the number of HSCs present in BALB mice with lifelong DR compared to young mice and a RA per stem cell that is equivalent to the young. Combined, these account for the slight increase with age in overall RA of the BM from mice on DR (SP+Kit criterion, Table 6.2).

The flow criterion historically used to enrich subpopulations for LT-HSCs in B6 mice (Spangrude, Heimfeld and Weissman 1988; Spangrude and Brooks 1993; Morrison and Weissman 1994; Christensen and Weissman 2001; Ishida, Zeng and Ogawa 2002; Bryder, Rossi and Weissman 2006; Rossi, Bryder and Weissman 2007) uses the markers lineage<sup>-</sup>, Sca<sup>+</sup>, cKit<sup>+</sup>, CD34<sup>-</sup>, and CD135<sup>-</sup> (mKSL). The expression of Sca, however, is lower in BALB marrow than in B6 marrow. While the expression is lower, initial data suggest that cells expressing these cell surface markers are still proportional to the total subpopulation of LT-HSCs (Spangrude and Brooks 1993; Ertl et al. 2008). Using this criterion in BALB mice, we see a nearly eight-fold increase in LT-HSCs after lifelong DR, compared to young controls. This is concomitant with a five-fold decrease in the RA per LT-HSC; however, even with this loss, the RA is 10-fold greater than similarly aged

**Table 6.2** Hematopoietic stem cell (HSC) frequency and function in BALB mice utilizing different flow criteria

	Young AL	Old AL	Old DR
<i>mKSL criterion</i>			
LT-HSC/10 <sup>6</sup> viable BMC	99 ± 19	1847 ± 341 ***	752 ± 145 ***
RU/10 <sup>6</sup> viable BMC	10 ± 2	6 ± 3	15 ± 4
RU/10 <sup>3</sup> LT-HSC	107 ± 29	2 ± 1 **	21 ± 2 *
<i>SP + Kit criterion</i>			
HSC/10 <sup>6</sup> viable BMC	261 ± 48	199 ± 68	336 ± 56
RU/10 <sup>6</sup> viable BMC	11 ± 3	0.3 ± 0.1 **	17 ± 7
RU/10 <sup>3</sup> HSC	47 ± 21	2 ± 1 **	54 ± 26

AL, ad libitum; DR, diet restriction; LT-HSC, long-term HSC.

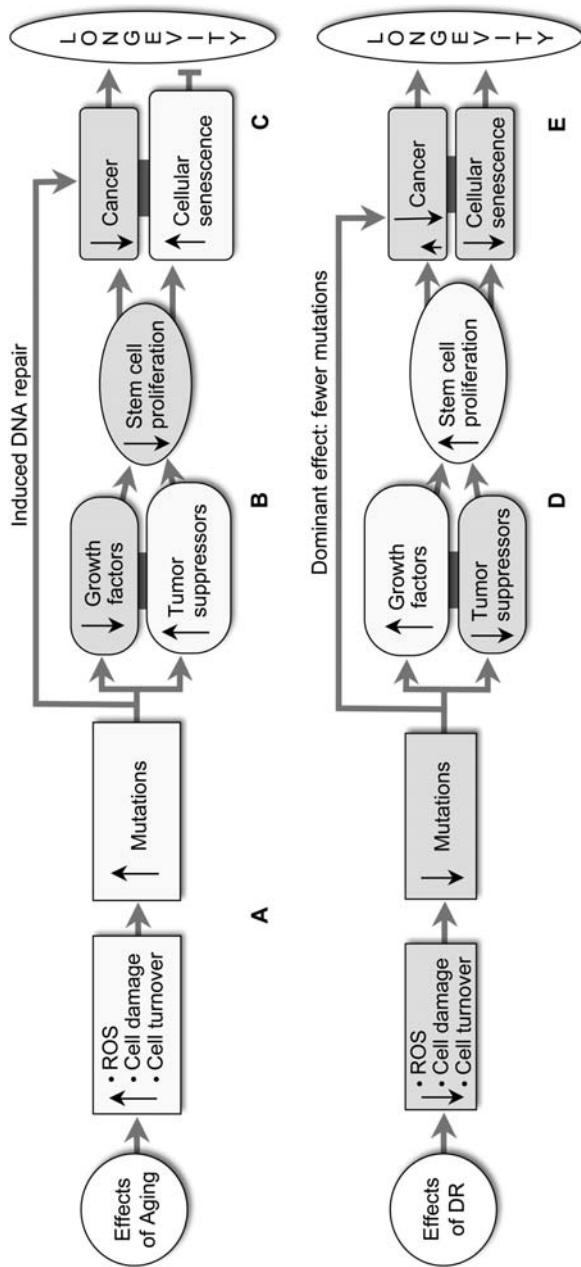
For each flow criterion, the frequency of HSCs and RA [in repopulating units (RUs)] were determined in separate groups of BALB BMC donors (young = 2–7 months; old = 2–25 months). The mKSL flow criterion (Min, Montecino-Rodriguez and Dorshkind 2006) used lineage<sup>-</sup>, Sca-1<sup>+</sup>, c-kit<sup>+</sup>, CD34<sup>-</sup>, flk2<sup>-</sup>, while the SP + Kit criterion used the Hoechst effluxing, double negative side population (Goodell et al. 1996, 1997; Goodell 1999; Lin and Goodell 2006), plus cKit<sup>+</sup>. Because HSCs were defined per viable BMC and RA (RU) per total BMCs, the ratio is a lower limit. However, flow cytometry viabilities were about the same in both experiments, so relative ratios are correct. Within any given determination and criterion, measurements are compared to young AL controls where \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. (This research was originally published in *Blood*. Ertl, R. P., Chen, J., Astle, C. M., Duffy, T. M. and Harrison, D. E. Effects of dietary restriction on hematopoietic stem-cell aging are genetically regulated. *Blood*. 2008; 111:1709–1716. © the American Society of Hematology)

AL controls. Thus, this flow criterion (mKSL criterion, Table 6.2) likewise supports the previous finding that lifelong DR prevents most of the loss of RA per stem cell with age (Ertl et al. 2008). This, combined with the increase in the number of stem cells present in the marrow, explains the slight increase in the overall RA of the BM with DR compared to young controls.

The results from the two different flow criteria are not directly comparable because they enrich different subpopulations, and in our studies, were done with different cohorts of mice. There are similarities in the overall trends. A notable exception is the trend in AL-fed aged mice, which varied greatly in total RA and number of HSCs, likely due to variations in health status of aged mice (Table 6.2). The robustness of the decline with age is seen with both flow criteria when RA is expressed per stem cell: marrow from old AL-fed donors compared to young AL-fed donors showed enormous losses of RA per LT-HSC (53-fold loss with mKSL criterion, 23-fold loss with SP+Kit criterion; Table 6.2). Also robust is the effect of DR on aged marrow, with both criteria the RA per HSC was greatly improved (10-fold improvement with mKSL criterion, 27-fold improvement with SP+Kit criterion; Table 6.2).

These studies suggest that, while DR reduces the risk of cancer and increases lifespan compared to AL controls, in BALB mice it also reduces or eliminates the loss of RA per HSC with age (Ertl et al. 2008). Because both cancer and senescence are decreased at the same time, these data appear to contradict the paradigm proposed by Campisi (Fig. 6.2). However, all of the results observed in both DR and tumor suppressor studies can be explained if DR is, in fact, *delaying* the cell fate decision between cancer and senescence, as proposed in Fig. 6.3 and discussed in detail latter in this chapter.

*Clonal stability.* One of the measures of the stem cell exhaustion in BM is whether HSCs have a stable production of all the lineages of definitive hematopoiesis. Young mice exhibit this characteristic, referred to as clonal stability, producing the different types of hematopoietic cells in the same ratios over extended periods of time (Harrison, Astle and Stone 1989; Harrison and Zhong 1992; Harrison et al. 1993). Experimentally, clonal stability is determined by setting up a CRA in which the numbers of genetically distinguishable donor and competitor cells are under limiting conditions— $1\text{--}4 \times 10^5$  cells of each per mouse. With the average number of engrafting LT-HSCs present in mouse BM at around one per  $10^5$  cells, this means that, given normal random distribution, very few LT-HSCs are present. Under these conditions, once the transplanted LT-HSCs are responsible for the entire production of definitive hematopoietic cells (after 4 months), the percent of donor-derived cells from the different lineages should be correlated. In addition, the ratios should be correlated over time, showing they are derived proportionally over time from the same few LT-HSCs (Harrison, Astle and Stone 1989; Harrison and Zhong 1992; Harrison et al. 1993). With this assay, young BALB mice exhibit clonal stability between 5 and 8 months, with a high correlation coefficient ( $r = 0.97$ ), explaining 90% of the variance (Table 6.3). With age, HSCs exhibit a loss of clonal stability, giving an insignificant correlation coefficient



**Fig. 6.3 Proposed model.** The modification of Fig. 6.2 considers both the accumulation of age-related damage (**A**, see Fig. 6.1) and the subsequent response: initiating repair and/or preventing the spread of deleterious mutations by inhibiting proliferation (**B**). Alterations in the equilibrium between cancer and cellular senescence (**C**), in part, determine the longevity. Like other factors that decrease mutations, diet restriction (DR) increases stem cell proliferation (**D**), thus decreasing cellular senescence. However, the decrease in mutations also decreases cancer (**E**), thus explaining the increase in stem cell function and longevity and the decrease in cancer observed with DR

**Table 6.3** Effects of age and diet restriction (DR) on hematopoietic stem cell (HSC) pluripotentiality and clonal stability in BALB mice

	Young AL	Old AL	Old DR
HSC pluripotentiality (correlation coefficients between percentages donor erythrocytes and lymphocytes)			
L : E at 1.5 months	0.31 (NS)	0.003 (NS)	0.55 ( $P < 0.05$ )
L : E at 5 months	0.90 ( $P < 0.01$ )	0.91 ( $P < 0.01$ )	0.96 ( $P < 0.01$ )
L : E at 8 months	0.91 ( $P < 0.01$ )	0.89 ( $P < 0.01$ )	0.92 ( $P < 0.01$ )
HSC clonal stability (correlation coefficients between average donor percentages at two time points)			
1.5:5 months	0.29 (NS)	0.24 (NS)	0.35 (NS)
1.5:8 months	0.33 (NS)	0.43 (NS)	0.20 (NS)
5:8 months	0.97 ( $P < 0.01$ )	0.48 (NS)	0.95 ( $P < 0.01$ )

AL, ad libitum.

To estimate HSC pluripotentiality, data were analyzed for correlations between percentages of donor lymphocytes (L) and erythrocytes (E) within each recipient at each of the three time points for each donor group (young = 3 months; old = 25 months). To estimate clonal stability during reconstitution, correlation coefficients were also calculated on average donor contributions between each set of two time points. NS means not significant ( $P > 0.05$ ). (This table was published in *Exp Hematol*, 31, Chen, J., Astle, C. M., and Harrison, D. E., Hematopoietic senescence is postponed and hematopoietic stem cell function is enhanced by dietary restriction, 1097–1103, Copyright Elsevier (2003)).

( $r = 0.48$ ), explaining only 23% of the variance. This demonstrates that BALB HSCs are slowly exhausted with age, leading toward senescence. DR prevents the loss of clonal stability with age, giving high correlation coefficients over time, approaching those of the young ( $r = 0.95$ ), again explaining nearly 90% of the variance. This independent measure verifies that DR can both delay senescence and reduce the risk of cancer (Miller and Harrison 1985).

## 6.10 DR in B6 Mice

While DR in B6 mice is also known to increase lifespan and decrease the risk of cancer (Bronson and Lipman 1991; Blackwell et al. 1995; Turturro et al. 2002), alterations in HSC function are vastly different from those seen in BALB mice. In B6 mice, the overall RA of BM increases three-fold with age (Harrison, Astle and Stone 1989; Chen, Astle and Harrison 2000). The increase with age results from a three-fold increase in the number of LT-HSCs present in BM (Ertl et al. 2008). Thus, with age, the RA per HSC remains fairly constant while the overall function of BM actually increases. This is not to say that B6 mice do not age, because they do exhibit many of the overt signs of aging, including a decrease in HSC homing and engraftment, alterations in the ability to produce different hematopoietic lineages, and earlier stem cell exhaustion after serial transplantation (Chen, Astle and Harrison 2000; Sudo et al. 2000; Liang, Van Zant and

Szilvassy 2005; Rossi et al. 2005; Min, Montecino-Rodriguez and Dorshkind 2006; Rossi, Bryder and Weissman 2007). However, the effects are far less severe than in BALB mice. The increase in overall marrow RA with a constant RA per LT-HSC is unaltered by lifelong DR (Ertl et al. 2008).

Overall, data on HSC aging in AL- and DR-treated B6 and BALB mice suggest that Fig. 6.2 needs to be modified, as the original model proposed by Campisi (Campisi 2003; Beausejour and Campisi 2006) does not explain all of the observed results. While the data from B6 mice do not contradict the concept that DR delays the cell fate decision between cancer and senescence, in this strain, DR is not the causative agent increasing the marrow RA. The lack of effect of DR is likely due to the elevated levels of repopulating function present in old B6 mice, which eliminate the need for further enhancement.

## 6.11 The need to Examine Multiple Strains

The greatest advantage of using mice as research models, besides the shorter lifespan, is the fact that inbred mice are genetically identical (The Jackson Laboratory 1997; Flurkey, Currer and Harrison 2007). Being genetically identical permits us to tease apart effects of specific genes. Also, the lack of genetic variance within a given strain reduces the biological variability caused by multiple alleles and recombination. The reduced biological variability gives us greater sensitivity with which to examine the contribution of different factors, each of which may only have a small effect.

The combination of alleles at different loci within a single inbred strain may not, however, give a good indication of *in vivo* conditions in wild mice on a segregating background, such as found in the human population. The process of inbreeding mice has removed the interactions created by heterozygosity and hybrid vigor. Examining different strains, though, allows us to examine the interactions of different allelic combinations. Thus, to understand the organismal relationships in complex systems involving multiple loci and pathways, such as cancer or aging, the treatments or models should be tested in multiple strains (Flurkey, Currer and Harrison 2007). This assures us that observed results are not anomalies associated with specific alleles or specific combinations of alleles whose homozygous occurrence may be selected against in a wild segregating population and thus, rarely found in nature.

Our DR studies in BALB and B6 mice illustrate the value of examining multiple strains. If the effect of cancer versus senescence had been examined in only the standard B6 mouse, we could have constructed a simpler model and missed the importance of the DR effect on retarding or preventing the dramatic changes in HSC number and RA caused by aging of BALB mice. DR effects on hematopoiesis in BALB and B6 mice have been consistently and significantly

different. However, in all animals examined, DR has decreased the overt signs of aging, decreased the incidence of cancer, and increased lifespan.

## 6.12 Problems with Comparisons Between Strains

While, in principle, the various techniques successful in one strain of mice should be directly applicable to all strains of mice, in practice, minor obstacles need to be worked out. As stated above, the comparison of HSC RA between BALB and B6 mice is hampered by the strain-dependent expression of Sca-1. This cell surface marker is used to differentiate between progenitors and definitive cells. In B6 mice, 99% of the ability to repopulate marrow comes from cells expressing Sca. However, in BALB mice, only 25% of the cells responsible for repopulating the marrow express Sca (Spangrude and Brooks 1993). While expression is lower in BALB, all the cells that do express Sca-1 have the same RA as cells isolated from B6 marrow. Thus, the concentration of LT-HSCs determined by the mKSL criterion in BALB still has utility. Although the actual number is underestimated, it should be proportional to the pool of LT-HSCs as a whole and thus give a valid comparison amongst the young, old, and old DR BALB mice.

The altered expression of cell surface markers between strains of mice calls into question the exact composition of HSC subpopulations and prevents direct comparisons. Validating trends using multiple flow criteria is one approach to overcome this problem. While each flow criterion identifies different subpopulations, a robust trend will be observable regardless of the criterion used. This approach, however, may miss minor but important trends. As an alternative, Morrison's group recently developed the Slam marker system, which appears to give valid HSC numbers in a wider variety of strains and conditions than previous marker systems (Kiel et al. 2005; Yilmaz, Kiel and Morrison 2006). This flow criterion of lineage-, CD150<sup>+</sup>, and CD48<sup>-</sup> identifies a subpopulation highly enriched for both ST<sup>-</sup> and LT-HSC. When transplanted into lethally irradiated recipients, they are capable of repopulating the BM. This subpopulation can be further gated for CD34 to separate ST-HSCs (CD34<sup>+</sup>) from LT-HSC (CD34<sup>-</sup>), as detailed elsewhere (Rossi, Bryder and Weissman 2007). Our laboratory has recently tried this set of cell surface markers on both BALB and B6 mice. Our preliminary data indicate a significant, but not complete, overlap between the different flow criteria, consistent with the observations of Morrison's group.

With the aid of a new flow cytometer with a greater number of colors, we will verify degrees of overlap between the different flow criteria in marrow cells from different strains of mice at different ages. Transplanting a limited number of cells into lethally irradiated recipients, with a genetically identifiable standard competitor, will assess the RA of HSCs sorted by the different flow criteria.

These experiments should markedly improve our ability to compare hematopoietic precursor concentrations among mouse strains.

## 6.13 Proposed Model

The current model in Fig. 6.2 does not explain how DR can decrease the incidence of both cancer and senescence (Ertl et al. 2008). We therefore propose a modification of this model that considers both the accumulation of age-related damage (Fig. 6.3A) and the subsequent response (Fig. 6.3B–E). In this new model, aging and DR are modifiers of cellular functions that alter the accumulation rate and the overall number of mutations. Other modifiers may also have these effects; however, we focus on aging and DR because they have been well studied. In our model, as organisms age, they accumulate damage (Fig. 6.3A) from a variety of sources, such as reactive oxygen species (ROS). This damage will directly or indirectly increase cell turnover and the incidence of mutations. The amount and rate of damage can, however, be altered by DR. The ability of DR to decrease tumor incidence along with other age-related damage is well documented (Bronson and Lipman 1991; Blackwell et al. 1995; Turturro et al. 2002).

As shown in Fig. 6.3b,c, cells respond to damage by initiating repair or by preventing the spread of deleterious mutations by inhibiting proliferation or both. Tumor suppressors have been shown to play an essential role in regulating proliferation (Tyner et al. 2002; TeKippe, Harrison and Chen 2003; Maier et al. 2004; Dumble et al. 2007; Gatzka et al. 2007). It is, however, equally likely that the cytokines, such as growth factors, could be down regulated in order to inhibit stem cell proliferation. Thus, an increase in age-related damage will induce DNA repair along with decreasing stem cell proliferation by increasing tumor suppressors or by decreasing growth factors. This will alter the equilibrium between cancer and cellular senescence. The induced DNA repair and decrease in stem cell proliferation will decrease the risk of cancer. The decrease in proliferation, though, has the negative consequence of increasing cellular senescence. It is this balance between cancer and senescence that, in part, determines the maximal lifespan obtainable.

As shown in Fig. 6.3d,e, a decrease from the normal level of spontaneous mutations that occur with age would lead to lower levels of tumor suppressor expression and possibly an increase in growth factors. The net result is a relative stimulation of stem cell RA compared to controls, thus decreasing cellular senescence. The previous model (Fig. 6.2) suggested that the cellular senescence and cancer were in a tight equilibrium. Here, we suggest that while they are still in an equilibrium, which is a key element in determining lifespan, they can be altered independently. In this model, DR decreases damage (including mutations) and thus decreases the need for tumor suppression. This removes inhibition of stem cell proliferation and decreases cellular senescence. The increase in

proliferation will increase the risk of cancer, but the overall reduction of mutations is the predominant effect. Thus, it is possible for DR to both decrease cellular senescence and cancer at the same time, as found in BALB *in vivo*. This model (Fig. 6.3) predicts that DR diminishes genomic damage in BALB but not B6 HSCs, a unique and testable prediction.

## 6.14 Future Research

While the linkage between tumor suppressors and RA has been established, many parts of our model are speculative. However, there are a number of specific testable predictions that will determine whether the model has value. The Campisi model (Fig. 6.2) suggests that p16<sup>INK4a</sup> expression increases with age. Our model (Fig. 6.3) predicts that the p16<sup>INK4a</sup> expression will correlate with the mutation rate. This correlation could explain the previous age relationship of p16<sup>INK4a</sup>, if under the same conditions, the numbers of mutations also increased with age. Independent measures of tumor suppressor expression and spontaneous mutation rates with age in different strains will test whether our model is valid.

It also remains to be established whether the mechanism by which DR retards aging is by a reduction of cellular damage leading to a decrease in the mutation rate. Furthermore, it is important to distinguish whether this is a transitory effect that requires a lifetime of treatment to significantly alter stem cell aging or a set-point mechanism that improves stem cell function within a few months. Initial data suggest that lifelong DR is required (Chen, Astle and Harrison 2003). It is known that ROS damage goes up with age and that ROS will directly cause mutations when it comes into contact with DNA (Wiesner, Zsurka and Kunz 2006; Dröge and Schipper 2007; Mallette and Ferbeyre 2007; Muller et al. 2007). However, *in vivo* the vast majority of naturally occurring oxygen radicals are found close to the mitochondria and the smooth endoplasmic reticulum. Oxygen radicals, by definition, are charged and very reactive and thus will travel only angstroms before donating the extra electron to another molecule. Thus, it is possible for ROS to directly damage mitochondrial DNA but less apt to directly damage genomic DNA leading to cancer. To date, researchers have searched without success for a carrier allowing a reactive species to cross the nuclear envelope and provide a direct mechanism of ROS-induced mutations. However, ROS can damage the cell in many other ways that mark the cell for turnover. This rapid turnover of cells under high-stress conditions may cause the increased mutation rate observed with elevated ROS *in vivo*. DR retards much of the damage and degradation associated with aging. If our model is valid, DR should decrease ROS and cellular turnover, resulting in a decrease in the mutations observed.

If DR decreases mutation rates, this should have two consequences: (1) It should decrease the need to shut down cell proliferation to obviate the risk of cancer and thus increase stem cell RA. (2) It should simultaneously

decrease the risk of cancer. While the latter is not surprising, it is important because most mechanisms that enhance a cell's RA also increase the incidence of cancer.

## 6.15 Conclusion

To achieve a maximal healthy lifespan, a balance must exist between increasing stem cell senescence in order to decrease cancer versus decreasing stem cell senescence at the risk of increasing cancer. Tumor suppressors such as p53 and p16<sup>INK4a</sup> may play a major role in regulating this cell fate decision as we age.

While the need to make this decision is inevitable, it can be delayed. DR demonstrates that the two cellular fates are not inexplicably linked in an inverse relationship. In fact, with DR treatment, a decrease in HSC senescence combines with a simultaneous decrease in the risk of cancer to increase lifespan. The ability of DR in aged BALB mice to improve HSC RA without increasing the risk of cancer may result from a decreased mutation rate intrinsic to the HSC.

Establishing the effects of DR on tumor suppressors, ROS and mutation rates are the first steps in verifying or disproving our proposed model. Details of the underlying mechanism are still largely unknown. Is the maximal potential lifespan determined by epigenetic conditions, is it determined by inherited genes, or is it determined by a combination? Do hormones, which regulate other pathways, such as the insulin/IGF-1 pathway, drive the effect or is it driven by a stress response to DNA damage or lipid peroxidation? If the response depends on damage, is it linked to the type and amount of damage?

Understanding the various mechanisms that regulate aging, in particular how stem cell senescence decisions are made and how they can be delayed, has important health implications. In the clinic, it might be possible for DR mimics to delay stem cell senescence without increasing cancer. A better understanding of ROS may make it possible to reduce the effective oxygen radical burden. Elucidating stem cell senescence pathways in different strains of inbred mice may help us explain the large differences in cancer susceptibility and suggest new avenues for preventative therapy.

## References

- Ames, B. N., and Gold, L. S. 1998. The causes and prevention of cancer: the role of environment. *Biotherapy*. 11:205–220.
- Aranda-Anzaldo, A., and Dent, M. A. R. 2007. Reassessing the role of p53 in cancer and ageing from an evolutionary perspective. *Mech Ageing Dev.* 128:293–302.
- Beausejour, C. M., and Campisi, J. 2006. Ageing: balancing regeneration and cancer. *Nature*. 443:404–405.
- Blackwell, B. N., Bucci, T. J., Hart, R. W., et al. 1995. A. Longevity, body weight, and neoplasia in ad libitum-fed and diet-restricted C57BL6 mice fed NIH-31 open formula diet. *Toxicol Pathol.* 23:570–582.

- Bronson, R. T., and Lipman, R. D. 1991. Reduction in rate of occurrence of age related lesions in dietary restricted laboratory mice. *Growth Dev Aging.* 55:169–184.
- Bryder, D., Rossi, D. J., and Weissman, I. L. 2006. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *Am J Pathol.* 169:338–346.
- Campisi, J. Cancer and ageing: rival demons? 2003. *Nat Rev Cancer.* 3:339–349.
- Chen, J., Astle, C. M., and Harrison, D. E. 1998. Delayed immune aging in diet-restricted B6CBAT6F1 mice is associated with preservation of naive T cells. *J Gerontol A Biol Sci Med Sci.* 53:B330–B337.
- Chen, J., Astle, C. M., and Harrison, D. E. 2000. Genetic regulation of primitive hematopoietic stem cell senescence. *Exp Hematol.* 28:442–450.
- Chen, J., Astle, C. M., and Harrison, D. E. 2003. Hematopoietic senescence is postponed and hematopoietic stem cell function is enhanced by dietary restriction. *Exp Hematol.* 31:1097–1103.
- Christensen, J. L., and Weissman, I. L. 2001. Flk-2 is a marker in hematopoietic stem cell differentiation: A simple method to isolate long-term stem cells. *Proc Natl Acad Sci USA.* 98:14541–14546.
- Dröge, W., and Schipper, H. M. 2007. Oxidative stress and aberrant signaling in aging and cognitive decline. *Aging Cell.* 6:361–370.
- Dumble, M., Moore, L., Chambers, S. M., et al. 2007. The impact of altered p53 dosage on hematopoietic stem cell dynamics during aging. *Blood.* 109:1736–1742.
- Effros, R. B., Walford, R. L., Weindruch, R., et al. 1991. Influences of dietary restriction on immunity to influenza in aged mice. *J Gerontol.* 46:B142–B147.
- Ertl, R. P., Chen, J., Astle, C. M., et al. (2008). Effects of dietary restriction on hematopoietic stem cell aging are genetically regulated. *Blood.* 111:1709–1716.
- Flurkey, K., Currer, J. M., and Harrison, D. E. 2007. The Mouse in Aging Research. In: *The Mouse in Biomedical Research*, 2nd Edition, Vol III, Normative Biology, Husbandry, and Models. Fox J. G. et al., (eds). American College of Laboratory Animal Medicine (Elsevier), Burlington, MA. pp. 637–672.
- Gatza, C., Moore, L., Dumble, M., et al. 2007. Tumor suppressor dosage regulates stem cell dynamics during aging. *Cell Cycle.* 6:52–55.
- Giordano, A., Fucito, A., Romano, G., et al. 2007. Carcinogenesis and environment: the cancer stem cell hypothesis and implications for the development of novel therapeutics and diagnostics. *Front Biosci.* 12:3475–3482.
- Gonzalez, F. J., and Nebert, D. W. 1990. Evolution of the P450 gene superfamily: animal-plant ‘warfare’, molecular drive and human genetic differences in drug oxidation. *Trends Genet.* 6:182–186.
- Goodell, M. A. 1999. Introduction: Focus on hematology. CD34(+) or CD34(–): does it really matter? *Blood.* 94:2545–2547.
- Goodell, M. A., Brose, K., Paradis, G., et al. C. 1996. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med.* 183:1797–1806.
- Goodell, M. A., Rosenzweig, M., Kim, H., et al. 1997. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nat Med.* 3:1337–1345.
- Guralnik, J. M., Eisenstaedt, R. S., Ferrucci, L., et al. 2004. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood.* 104:2263–2268.
- Harrison, D. E., Astle, C. M., and Stone, M. 1989. Numbers and functions of transplantable primitive immunohematopoietic stem cells. Effects of age. *J Immunol.* 142:3833–3840.
- Harrison, D. E., and Zhong, R. K. 1992. The same exhaustible multilineage precursor produces both myeloid and lymphoid cells as early as 3–4 weeks after marrow transplantation. *Proc Nat Acad Sci USA.* 89:10134–10138.
- Harrison, D. E., Jordan, C. T., Zhong, R. K., et al. 1993. Primitive hematopoietic stem cells: direct assay of most productive populations by competitive repopulation with simple binomial, correlation and covariance calculations. *Exp Hematol.* 21:206–219.

- Hayflick, L. 1965. The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res.* 37:614–636.
- Ishida, A., Zeng, H., and Ogawa, M. 2002. Expression of lineage markers by CD34+ hematopoietic stem cells of adult mice. *Exp Hematol.* 30:361–365.
- Janzen, V., Forkert, R., Fleming, H. E., et al. 2006. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. *Nature.* 443:421–426.
- Kiel, M. J., Yilmaz, O. H., Iwashita, T., et al. 2005. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *2005;121:1109–1121.*
- Krishnamurthy, J., Ramsey, M. R., Ligon, K. L., et al. 2006. p16INK4a induces an age-dependent decline in islet regenerative potential. *Nature.* 443:453–457.
- Krivtsov, A. V., Twomey, D., Feng, Z., et al. 2006. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature.* 442:818–822.
- Liang, Y., Van Zant, G., and Szilvassy, S. J. 2005. Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. *Blood.* 106:1479–1487.
- Lin, K. K., and Goodell, M. A. 2006. Purification of hematopoietic stem cells using the side population. *Methods Enzymol.* 420:255–264.
- Luan, X., Zhao, W., Chandrasekar, B., et al. 1995. G. Calorie restriction modulates lymphocyte subset phenotype and increases apoptosis in MRL/lpr mice. *Immunol Lett.* 47:181–186.
- Maier, B., Gluba, W., Bernier, B., et al. 2004. Modulation of mammalian life span by the short isoform of p53. *Genes Dev.* 18:306–319.
- Mallette, F.A., and Ferbeyre, G. 2007. The DNA damage signaling pathway connects oncogenic stress to cellular senescence. *Cell Cycle.* 6:1831–1836.
- Masoro, E. J. 1993. Dietary restriction and aging. *J Am Geriatr Soc.* 41:994–999.
- Miller, R. A., and Harrison, D. E. 1985. Delayed reduction in T cell precursor frequencies accompanies diet-induced lifespan extension. *J Immunol.* 134:1426–1429.
- Min, H., Montecino-Rodriguez, E., and Dorshkind, K. 2006. Effects of aging on the common lymphoid progenitor to pro-B cell transition. *J Immunol.* 176:1007–1012.
- Molofsky, A. V., He, S., Bydon, M., et al. 2005. Bmi-1 promotes neural stem cell self-renewal and neural development but not mouse growth and survival by repressing the p16Ink4a and p19Arf senescence pathways. *Genes Dev.* 19:1432–1437.
- Molofsky, A. V., Slutsky, S. G., Joseph, N. M., et al. 2006. Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing. *Nature.* 443:448–452.
- Morrison, S. J., and Weissman, I. L. 1994. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity.* 1:661–673.
- Morrison, S. J., Wandycz, A. M., Akashi, K., et al. 1996. The aging of hematopoietic stem cells. *Nat Med.* 2:1011–1016.
- Muller, F. L., Lustgarten, M. S., Jang, Y., et al. 2007. Trends in oxidative aging theories. *Free Radic Biol Med.* 43:477–503.
- Pardal, R., Clarke, M. F., and Morrison, S. J. 2003. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer.* 3:895–902.
- Pardal, R., Molofsky, A. V., He, S., et al. 2005. Stem cell self-renewal and cancer cell proliferation are regulated by common networks that balance the activation of proto-oncogenes and tumor suppressors. *Cold Spring Harb Symp Quant Biol.* 70:177–185.
- Pearce, D. J., Anjos-Afonso, F., Ridler, C. M., et al. 2007. Age dependent increase in SP distribution within Hematopoiesis: implications for our understanding of the mechanism of aging. *Stem Cells.* 25:828–835.
- Penninx, B.W., Pahor, M., Cesari, M., et al. 2004. Anemia is associated with disability and decreased physical performance and muscle strength in the elderly. *J Am Geriatr Soc.* 52:719–724.
- Robinson, B. 2003. Cost of anemia in the elderly. *J Am Geriatr Soc.* 51:S14–S17.
- Rossi, D. J., Bryder, D., Zahn, J. M., et al. 2005. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc Nat Acad Sci USA.* 102:9194–9199.

- Rossi, D. J., Bryder, D., and Weissman, I. L. 2007. Hematopoietic stem cell aging: Mechanism and consequence. *Exp Gerontol.* 42:385–390.
- Sharma, Y., Flurkey, K., Astle, C. M., et al. 2005. Mice severely deficient in growth hormone have normal hematopoiesis. *Exp Hematol.* 33:776–783.
- Smith, J. R., and Pereira-Smith, O.M. 1996. Replicative senescence: implications for in vivo aging and tumor suppression. *Science.* 273:63–67.
- Spangrude, G.J., and Brooks, D. M. 1993. Mouse strain variability in the expression of the hematopoietic stem cell antigen Ly-6A/E by bone marrow cells. *Blood.* 82:3327–3332.
- Spangrude, G. J., Heimfeld, S., and Weissman, I. L. 1988. Purification and characterization of mouse hematopoietic stem cells. *Science.* 241:58–62. [Erratum in *Science.* 1989;244:1030].
- The Staff of The Jackson Laboratory. 1997. *Handbook on Genetically Standardized JAX® Mice.* Bar Harbor, ME: The Jackson Laboratory.
- Sudo, K., Ema, H., Morita, Y., et al. 2000. Age-associated characteristics of murine hematopoietic stem cells. *J Exp Med.* 192:1273–1280.
- TeKippe, M., Harrison, D. E., and Chen, J. 2003. Expansion of hematopoietic stem cell phenotype and activity in *Tsp53*-null mice. *Exp Hematol.* 31:521–527.
- Turturro, A., Duffy, P., Hass, B., et al. 2002. Survival characteristics and age-adjusted disease incidences in C57BL/6 mice fed a commonly used cereal-based diet modulated by dietary restriction. *J Gerontol A Biol Sci Med Sci.* 57:B379–B389.
- Tyner, S. D., Venkatachalam, S., Choi, J., et al. 2002. p53 mutant mice that display early ageing-associated phenotypes. *Nature.* 415:45–53.
- Van Zant, G., Holland, B. P., Eldridge, P. W., et al. 1990. Genotype-restricted growth and aging patterns in hematopoietic stem cell populations of allophenic mice. *J Exp Med.* 171:1547–1565.
- Wiesner, R. J., Zsurka, G., and Kunz, W. S. 2006. Mitochondrial DNA damage and the aging process: facts and imaginations. *Free Radic Res.* 40:1284–1294.
- Yilmaz, O. H., Kiel, M. J., and Morrison, S. J. 2006. SLAM family markers are conserved among hematopoietic stem cells from old and reconstituted mice and markedly increase their purity. *Blood.* 107:924–930.
- Yuan, R., Astle, C. M., Chen, J., et al. 2005. Genetic regulation of hematopoietic stem cell exhaustion during development and growth. *Exp Hematol.* 33:243–250.

# **Chapter 7**

## **Modeling Human Philadelphia Chromosome-Positive Leukemia in Mice**

**Shaoguang Li**

### **Contents**

7.1	Introduction . . . . .	157
7.2	Molecular Mechanisms of Ph <sup>+</sup> Leukemia . . . . .	158
7.3	BCR–ABL Signaling . . . . .	159
7.4	Mouse Models of Ph <sup>+</sup> Leukemia . . . . .	161
7.5	Translational Research Using Ph <sup>+</sup> Leukemia Mouse Models . . . . .	164
7.6	Future Directions . . . . .	170

**Abstract** The BCR-ABL oncogene transforms cells through sustained activation of signal transduction pathways in the cells. Identification of signaling pathways that play critical roles in leukemogenesis is the key to developing effective therapies against these targets. The success of this approach relies on establishment and use of physiological disease models to determine and evaluate potential therapeutic targets. Mouse models provide a powerful tool for studying signaling pathways in leukemic cells and for developing new therapeutic strategies for treating leukemia patients.

### **7.1 Introduction**

Human Philadelphia chromosome-positive (Ph<sup>+</sup>) leukemias induced by the *BCR-ABL* oncogene are among the most common hematologic malignancies and include chronic myeloid leukemia (CML) and B-cell acute lymphoblastic leukemia (B-ALL). CML has a triphasic clinical course: a chronic phase, in which BCR-ABL-expressing pluripotent stem cells massively expand but undergo normal differentiation to form mature neutrophils; an accelerated phase, in which neutrophil differentiation becomes progressively impaired

---

S. Li

Division of Hematology/Oncology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA  
Shaoguang.Li@umassmed.edu

and the cells become less sensitive to myelosuppressive medications; and blast crisis, a condition resembling acute leukemia in which myeloid or lymphoid blasts fail to differentiate. The transition from chronic phase to blast crisis results from additional genetic alterations, and this process is not well understood. CML and Ph<sup>+</sup> B-ALL have a common stem cell origin [1] and often co-exist in a patient [2, 3]. This suggests that these two diseases are closely related and may represent two different stages or forms of the same disease. Curative therapy for CML requires management of both CML (chronic phase and myeloid/lymphoid blast crisis) and Ph<sup>+</sup> B-ALL.

The Abl tyrosine kinase inhibitor imatinib mesylate (Gleevec, ST1571) has become the most effective drug for leukemia therapy and has been shown to induce a complete hematologic response in the majority of chronic phase CML patients [4]. However, imatinib was unable to abrogate BCR-ABL-expressing leukemic cells [5] and induced cellular and clinical drug resistance [6–12], suggesting that use of imatinib as a single agent may not prevent eventual disease progression to terminal blast crisis or cure CML. Moreover, imatinib is much less effective in treating CML blast crisis patients [13, 14]. Recently, a newly developed Abl kinase inhibitor (termed dasatinib or BMS-354825 and produced by Bristol-Myers Squibb) has been shown to have an inhibitory effect on almost all imatinib-resistant BCR-ABL mutants [15], offering some hope for overcoming imatinib resistance. However, the BCR-ABL-T315I mutant, which frequently appears in patients resistant to imatinib therapy, is still resistant to dasatinib [15]. Two other novel Abl kinase inhibitors, termed AP23464 and AMN107 (nilotinib), have also been effective against several frequently observed imatinib-resistant BCR-ABL mutants but ineffective against the BCR-ABL-T315I mutant [16, 17]. While clinical trials are ongoing to determine the long-term effectiveness of these drugs in treating imatinib-resistant Ph<sup>+</sup> leukemia patients, it has been shown that imatinib does not completely eradicate CML stem cells [18]. It is critical to develop anti-stem cell therapies that are synergistic with available treatment strategies. The success of this approach requires understanding the signaling pathways utilized by BCR-ABL to induce Ph<sup>+</sup> leukemias and active in leukemic stem cells. Investigation of signaling pathways active in leukemic stem cells is critical to developing curative therapeutic strategies for Ph<sup>+</sup> leukemia.

## 7.2 Molecular Mechanisms of Ph<sup>+</sup> Leukemia

The molecular basis of Philadelphia chromosome is the *BCR-ABL* oncogene. The *BCR* gene, on chromosome 22, breaks at either exon 1, exon 12/13, or exon 19 and fuses to the *c-ABL* gene on chromosome 9 to form, respectively, three types of *BCR-ABL*: P190, P210, or P230. In humans, each of the three forms of the *BCR-ABL* oncogene is associated with a distinct type of leukemia [19]. The P190 form is most often present in B-ALL but only rarely in CML. However, P190-induced B-ALL may proceed from a transient chronic phase CML

[20, 21]. P210 is the predominate form in CML and in some acute lymphoid and myeloid leukemias in CML blast crisis. P230 was recently found in a very mild form of CML [22]. Lymphoid blast crisis of CML and Ph<sup>+</sup> B-ALL are pathologically similar and account for 20% of adults and 5% of children with acute lymphoblastic leukemia. Among those patients, 50% of adults and 20% of children carry P210BCR-ABL and the rest carry P190BCR-ABL [13, 23]. In addition, Ph<sup>+</sup> B-ALL is pathologically similar to CML diagnosed in lymphoid blast crisis (which is derived from chronic phase CML) [24], effective therapies for acute lymphoid leukemia are still not available.

CML transition from chronic phase to blast crisis is a devastating process in Ph<sup>+</sup> leukemia. Although the mechanism underlying the disease progression remains unclear, additional genetic changes are believed to play a role in this process. Mutations of tumor suppressor genes, including the retinoblastoma gene (Rb), p16, and p53, have been found to be associated with CML blast crisis patients [25–27]. However, it is still not known how BCR-ABL-expressing cells acquire these additional genetic lesions. A plausible mechanism is an increase in genetic instability caused by BCR-ABL. Several studies have shown that BCR-ABL deregulates the functions of DNA repair-related genes. For example, BCR-ABL down-regulates expression of the DNA repair enzyme DNA-PKcs [28]. P210BCR-ABL may interact with the xeroderma pigmentosum group B protein, which could lead to the impairment of DNA repair function [29]. Expression of two other genes related to genetic stability, *BRCA-1* and *RAD51*, is also deregulated by BCR-ABL [30, 31]. BCR-ABL can also cause over-expression and increased activity of the error-prone polymerase  $\beta$ , leading to an increased mutagenesis [30]. A recent study showed that BCR-ABL associates with rad 3-related protein (ATR), which is involved in DNA repair, and inhibits the activation of ATR following DNA damage, leading to alteration of cellular responses to DNA damage [32]. Although BCR-ABL is a primary growth stimulator for leukemic cells [33], it is generally accepted that the concomitant effect of BCR-ABL on cell survival and DNA double strand break repair may lead to the acquisition of secondary genetic abnormalities contributing to CML disease progression [34]. The above examples providing evidence that BCR-ABL can cause disruption of DNA repair mechanisms indicate that this effect of BCR-ABL may play a major role in progression of chronic phase CML.

### 7.3 BCR-ABL Signaling

BCR-ABL has been shown to activate multiple signaling molecules/pathways, including Ras, MAPK, STAT, JNK/SAPK, PI-3 kinase, NF- $\kappa$ B, and c-MYC [35], as well as cytokine production [36, 37]. Studies also link BCR-ABL to apoptotic pathways [38–49] and to activation of Src family kinases in cultured cells [50–52] and in mice [53]. It is not known what signaling pathways are active in leukemic stem cells that are insensitive to almost all anti-leukemia drugs.

Importantly, the role of a particular signaling pathway in BCR–ABL leukemogenesis could be cell-content dependent.

*Different signaling pathways are required for proliferation of myeloid or lymphoid leukemic cells.* As described above, P190 and P210 forms of BCR–ABL are associated with distinct leukemia: B-ALL for P190 and CML for P210 although P210 also induces acute lymphoid leukemia in CML blast crisis. A simple explanation for the induction of different type leukemia by P190 and P210 is that this could be caused by structural differences between these two forms of the *BCR-ABL* oncogene. Different forms of *BCR-ABL* (P190, P210, and P230) contain the same portion of the *c-ABL* gene but different lengths of the *BCR* gene. BCR in BCR–ABL comprises three functional domains: a coiled-coil domain, serine-rich sequences, and the C-terminal domain. P190 contains the first two domains of BCR, and P210 and P230 also contain some and the majority of the C-terminal domain of BCR, respectively. The C-terminal domain that is lacking in the P190 BCR protein comprises the *dbl*-like domain (present in both P210 and P230) and the *GAP<sup>rac</sup>* domain (present only in P230). Both *dbl*-like domain and *GAP<sup>rac</sup>* domain are homologous to regulators of small, Ras-related GTP-binding proteins. Despite the structural differences in P190 and P210BCR–ABL proteins, there is little evidence showing that these two kinases may stimulate different signaling pathways in cells, which lead to induction of different type of leukemia. In fact, P190 can induce CML in mice, as efficiently as P210 can [54], suggesting that distinct leukemia induced by P190 and P210 does not reflect the structural differences between the two kinases. An alternative explanation for induction of either CML or B-ALL is that different cell types (myeloid or lymphoid) require different signaling networks for malignant transformation. This cell-content-dependent signaling in leukemia induction is supported by the finding that three Src kinases (Lyn, Hck, and Fgr) are required for proliferation of leukemic cells in B-ALL but not CML induced by BCR–ABL [53] although these Src kinases are activated by BCR–ABL in both myeloid and lymphoid leukemic cells. This suggests that these three Src kinases are potential targets for B-ALL but not for chronic phase CML although this study does not exclude a role for other Src family kinases in the induction of CML by BCR–ABL. These results indicate that BCR–ABL utilizes different signaling network to induce B-ALL or CML, suggesting different therapeutic strategies for treating these two diseases. This idea about different signaling network used by BCR–ABL to induce B-ALL or CML is supported by the observation that the BCR–ABL SH2 domain is required for efficient induction of CML-like disease but not of B-ALL [55]. The cell type-specific signaling may represent a common mechanism in cancer development, and identification of unique signaling network involved in the induction of each type of cancer is critical to developing effective therapies.

*Src kinases in BCR–ABL signaling.* Src kinases activated by BCR–ABL are key signaling molecules in B-ALL development [53]. It is important to identify signaling pathways downstream of Src kinases in  $\text{Ph}^+$  leukemia. Hck has been shown to phosphorylate and activate STAT5 in BCR–ABL-expressing myeloid

cells [56]. However, a cell culture study using a mutant form of BCR–ABL that is resistant to the BCR–ABL kinase inhibitor imatinib shows that inhibition of Src kinases with CGP76030 does not affect activation of STAT5, and instead, activation of Akt is impaired by this drug [57]. A study using SHP-1 (SH2-containing tyrosine phosphatase-1)-deficient mice shows that SHP-1 interacts with Lyn in myeloid [58] and lymphoid [59] cells; BCR–ABL forms complex with SHP-1 [60]. Several lines of evidence also implicate relationship between cytokines and activation of Src kinases. Fyn, Hck, and Lyn are activated following IL-3 stimulation, and growth of progenitor cells induced by stem cell factor (SCF) is reduced in the absence of Lyn that is activated by SCF [61]. On the other hand, BCR–ABL can render cells independent of cytokines such as IL-3 [37, 62, 63]. These results indicate that the signaling pathways stimulated by BCR–ABL overlap at least partially with those activated by these cytokines.

Src kinases directly interact with BCR–ABL [50, 51] through its SH3, SH2, and SH1 domains and through the distal portion of the C-terminal tail, which is required for transformation of the myeloid leukemia cell line to IL-3 independence by BCR–ABL [52]. Src kinases are also involved in BCR–ABL function indirectly through other signaling molecules or pathways. BCR–ABL forms a stable complex with protein tyrosine phosphatases [60, 64], which could be regulated by Src kinases [58, 65]. Src kinases are also functionally associated with Btk (Bruton's tyrosine kinase). Btk activation has been shown to induce cell transformation [66], and Lyn phosphorylates and activates Btk [66, 67].

## 7.4 Mouse Models of Ph<sup>+</sup> Leukemia

*Transgenic model.* Since more than two decades ago, attempts have been made to generate transgenic mice expressing different forms (P190, P210, or P230) of BCR–ABL oncogene. In these different strains of transgenic mice, variable types of promoters are used to drive BCR–ABL transgenes. These promoters include Eμ [68], MPSV–LTR [68], metallothionein [69–71], BCR [72, 73], tec [74], and MSCV–LTR [75]. The BCR–ABL transgenic models show evidence that BCR–ABL is important in the initiation of leukemia and provide useful tools for studying molecular mechanisms by which the BCR–ABL oncogene induces the disease. However, there are two general problems related to these transgenic models. First, the P210 BCR–ABL transgene driven by the BCR promoter causes embryonic lethality [73]. Second, in the mice that develop leukemia, the disease type is mainly lymphoid (B-ALL or T-ALL) but not myeloid [68–71] although some degree of myeloproliferative disorder develops when the promoter of the *tec* gene is used [74]. In addition, disease latency in these mice is relatively long, making it difficult to use these models for developing therapeutic strategies.

*Inducible tet-off model.* Although the BCR–ABL transgenic models show the requirement of BCR–ABL in the initiation of leukemia, they do not indicate whether expression of BCR–ABL is necessary to maintain leukemia phenotype. The first animal model to address this concern is the inducible tet-off system in transgenic mice [33], in which the induction of BCR–ABL expression is induced by withdrawal of tetracycline from drinking water of the mice. Upon tetracycline withdrawal, all mice develop lethal B-ALL. Importantly, the leukemic phenotype is reversibly dependent on the continuous expression of BCR–ABL, even after multiple rounds of induction and reversion of BCR–ABL expression. These results demonstrate that BCR–ABL is required for both induction and maintenance of leukemia. Because the MMTV–LTR promoter is used in this transgenic strain to control expression of tTA (tetracycline transactivator), BCR–ABL is turned on only in B220<sup>+</sup> B-lymphoid cells but not in hematopoietic stem cells (HSCs), explaining the lack of the induction of CML-like disease in these mice. Subsequently, the inducible tet-off BCR–ABL transgenic mouse is generated using human CD34 regulatory element [76]. In this model, the *cre* responder gene is expressed in HSCs, common myeloid progenitor cells, and megakaryocytic/erythroid progenitor cells. However, the mice did not display an increase in granulopoiesis, a characteristic of CML, although megakaryocytic lineage is affected in the mice. A better model that mimics human CML is developed using the murine stem cell leukemia (*SCL*) gene 3'-enhancer [77]. In these SCLtTA/BCR–ABL mice, BCR–ABL causes neutrophilia, leukocytosis, and organ invasion by myeloid cells, closely resembling CML-like disease although white blood cell counts in the mice are relatively lower compared to human CML. It is surprising that CML in these mice are not transplantable in NOD/SCID recipient mice, and this needs to be explained mechanistically in the future, as human CML is viewed as a stem cell disease. In addition, the SCLtTA/BCR–ABL mice are of mixed-strain background, resulting in variable life span ranging from 4 weeks to 17 weeks. Although this model is very useful in studying molecular mechanisms of BCR–ABL leukemogenesis, variable survival and mixed genetic background of the mice may not favor drug-testing experiments.

*Xenotransplantation model.* CML is a stem cell disorder. When leukemic cells from bone marrow and peripheral blood of CML patients are intravenously injected into irradiated severe combined immunodeficient (SCID) mice, the mice are repopulated with neoplastic Ph<sup>+</sup> CD34<sup>+</sup> cells [78, 79]. This model provides a powerful tool for studying disease mechanisms of Ph<sup>+</sup> leukemia. However, mice with engrafted human CML cells do not develop lethal leukemia although leukemic cells proliferate in the mice. This could be caused by many reasons. First, the latency of leukemia is longer than the lifespan of immune-deficient recipient mice. Second, residual immunity of recipient mice may reduce engraftment of human CML cells. Third, it is possible that technically the number of leukemic stem cells in intravenously injected human CML cells is not high enough to allow development of leukemia in recipient mice. Recently, a better NOD/SCID strain with less immune resistance and longer lifespan has

been developed [80], and this NOD/SCID model will significantly improve engraftment of human CML cells.

*Retroviral transduction/transplantation model.* The initial efforts in establishing a BCR–ABL retroviral mouse model were taken about two decades ago [81], but at that time the system was hampered by low efficiency of disease induction. For example, in the originally described mouse retroviral bone marrow transduction/transplantation system, only about 25% of the recipients of BCR–ABL-transduced bone marrow cells developed CML-like leukemia [82–84], and some mice did not develop any disease, probably due to low viral titer and inefficient viral transduction. To overcome these deficiencies, during last 10 years efforts have been made to significantly improve the existing systems to develop an efficient and accurate mouse model of human CML and B-ALL. These improvements include modifications of the retroviral vector backbone, use of a transient retroviral packaging system, and change of viral transduction conditions [54, 85, 86]. After retrovirally expressing the *BCR–ABL* oncogene in bone marrow cells, which are derived from donor BALB/c mice pretreated with 5-fluorouracil (5-FU), 100% of syngeneic recipients develop CML-like leukemia within 4–6 weeks. The same CML-like disease can also be induced in C57BL/6 [53] and other inbred strains (unpublished results). The control retroviral construct does not induce leukemia in mice. Mouse CML-like disease is characterized by leukocytosis with greatly elevated numbers of maturing neutrophils, organ infiltration, and splenomegaly (Table 7.1). The target cells for BCR–ABL are primitive multipotential HSCs [54], precisely mimicking most of the pathological characteristics of human CML. Myeloid cells transduced by the *BCR–ABL* oncogene express BCR–ABL protein [54].

In addition to inducing human CML, BCR–ABL is also associated with a subset of human B-ALL as described above. To induce B-ALL, effort has been made to express BCR–ABL in the progenitor cells that give rise to the lymphoid cell lineage. The target cells for Abelson virus-induced pre-B leukemia, which is phenotypically similar to BCR–ABL-induced B-ALL, are fairly abundant in normal bone marrow from BALB/c mice. Hence, bone marrow cells from

**Table 7.1** Pathological characteristics of leukemias induced by BCR–ABL

	CML-like disease		B-ALL	
	BALB/c	C57BL/6	BALB/c	C57BL/6
WEC ( $\times 10^3/\mu\text{l}$ )	295±59	105±38	68±21	45±17
% of neutrophils in PB	>60	>50	<2	<1
% of B-lymphocyte in PB	<7	<9	>90	>80
Spleen wt. (g)	0.91±0.11	0.46±0.09	0.43±0.07	0.29±0.04
Liver enlargement	↑↑↑↑	↑↑↑	↑↑	↑
Lung hemorrhage	+	+	—	—
Pleural effusion	—	—	+	+

B-ALL, B-cell acute lymphoblastic leukemia; CML, chronic myeloid leukemia.

Note that normal white blood cell count should be less than  $10 \times 10^3/\mu\text{l}$ .

non-5-FU-treated donor mice were used to induce B-ALL in recipient mice. In doing so, the recipient mice develop B-ALL, and Southern blot analysis of these mice shows that the BCR-ABL provirus exists only in the malignant B cells [54, 55]. The leukemic cells express the B-cell markers CD19, B220 (CD45R), BP1, CD24, and CD43 and show rearrangement of immunoglobulin heavy chain genes, suggesting that these leukemic cells are pro- or pre-B cells and are phenotypically similar to human Ph<sup>+</sup> B-ALL. Diseased mice developed lymphadenopathy, moderate splenomegaly (Table 7.1), and bone marrow involvement with leukemic cells. In addition, these mice developed hemorrhagic pleural effusion (Table 7.1), which was a major cause of death. Furthermore, B-leukemic cells also cause the same leukemia in secondary recipients and can grow in culture. This efficient retroviral transduction/transplantation system provides a powerful tool for elucidating the common and distinct signaling pathways contributing to the diseases.

## 7.5 Translational Research Using Ph<sup>+</sup> Leukemia Mouse Models

The retroviral transduction/transplantation model of BCR-ABL-induced leukemia has been successfully used in studying BCR-ABL domain functions and *BCR-ABL* cooperative genes [87]. More importantly, the model has been used widely in studying BCR-ABL-activated downstream signaling pathways and developing new therapeutic strategies as described below.

*Identification of crucial signaling pathways.* Because the retroviral transduction/transplantation model produces efficient and accurate leukemia induced by BCR-ABL, this model provides a powerful system in exploring molecular mechanisms and testing therapeutic strategies. One of earliest examples of utilizing this model to genetically determine contribution of a particular signaling pathway in leukemogenesis is to test the role of interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in the development of BCR-ABL-induced CML. In this study, mice deficient for IL-3 and GM-CSF were used to investigate whether induction of CML by BCR-ABL is affected in the absence of these two cytokine genes. The results show that IL-3 and GM-CSF are not required for induction of CML by BCR-ABL [88] although some studies suggest a role of these two genes in BCR-ABL transformation. Other examples for using this model in exploring signaling pathways activated by BCR-ABL include studies of STAT5, Ras, Grb-2, JNK, Cbl, ICSBP [87], and Src family kinases [53].

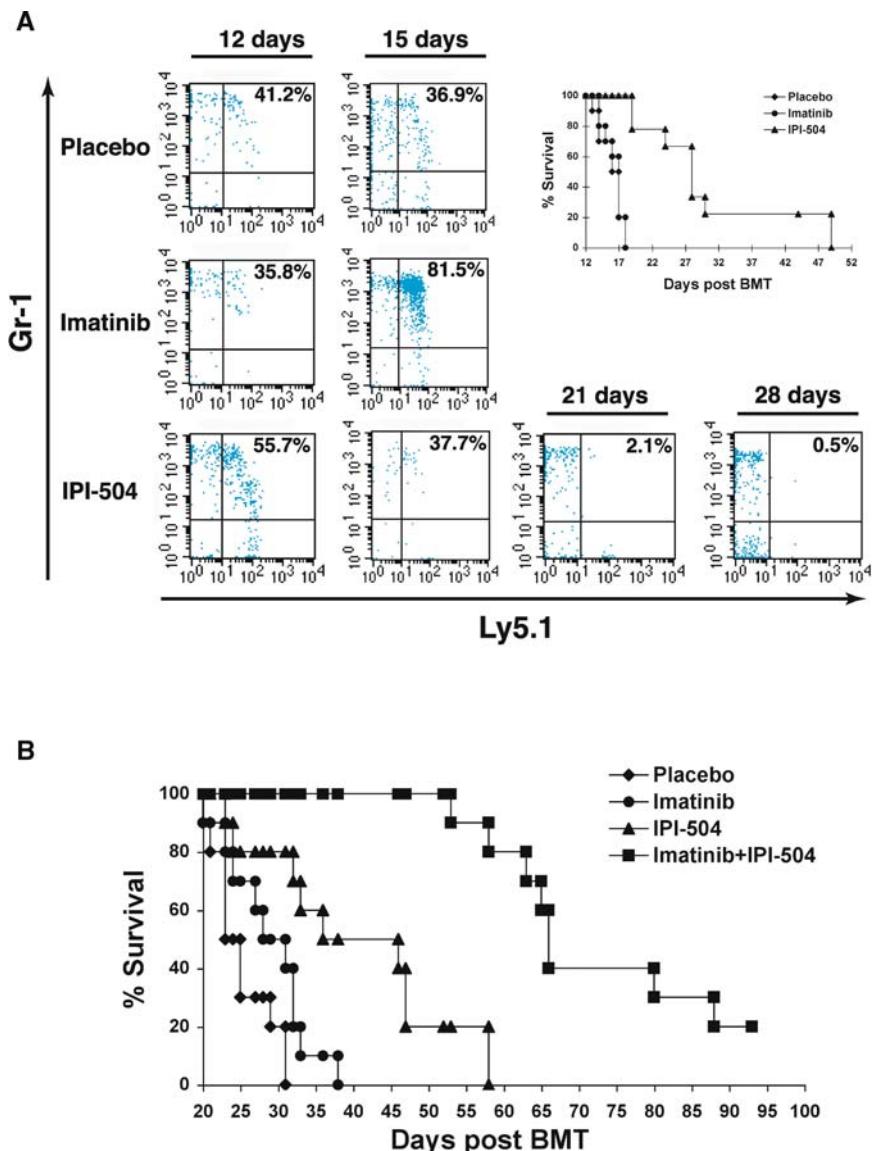
*Testing of new therapeutic targets and strategies.* For the reasons described above, retroviral transduction/transplantation mouse model of Ph<sup>+</sup> leukemia provides an excellent system for identifying novel therapeutic targets and evaluating efficacy of therapeutic agents. Effectiveness of imatinib in treating CML or B-ALL induced by BCR-ABL has been tested in this leukemia mouse model. The results show that imatinib significantly prolongs survival of CML

[53, 89] or ALL [53] mice but does not cure the diseases, suggesting that imatinib cannot eradicate leukemic stem cells. Another good example of testing therapeutic drugs using the leukemia model is to test heat shock protein 90 (Hsp90) as a therapeutic target for the treatment of Ph<sup>+</sup> leukemia [90]. Treatment with an Hsp90 inhibitor (termed IPI-504) results in BCR–ABL protein degradation, decreased numbers of CML stem cells, and prolonged survival of CML mice bearing wild-type or mutant BCR–ABL T315I. Hsp90 inhibition more potently suppresses BCR–ABL–T315I-expressing leukemia clones relative to the wild-type clones in mice (Fig. 7.1). Combination treatment with IPI-504 and imatinib is more effective than either treatment alone in prolonging survival of mice simultaneously bearing both wild-type and T315I leukemic cells (Fig. 7.2). Together, these results provide a rationale for use of an Hsp90 inhibitor as a first-line treatment in CML by inhibiting leukemia stem cells and preventing the emergence of imatinib-resistant clones in patients. Although current therapeutic drugs such as BCR–ABL kinase inhibitors and Hsp90 inhibitors are effective in treating leukemia mice and patients, none of available drugs is curative for Ph<sup>+</sup> leukemia, likely due to the inability of these drugs to completely kill leukemic stem cells. Study of biology of leukemic stem cells and identification of effective targets in these cells will provide better therapeutic strategies for Ph<sup>+</sup> leukemia.

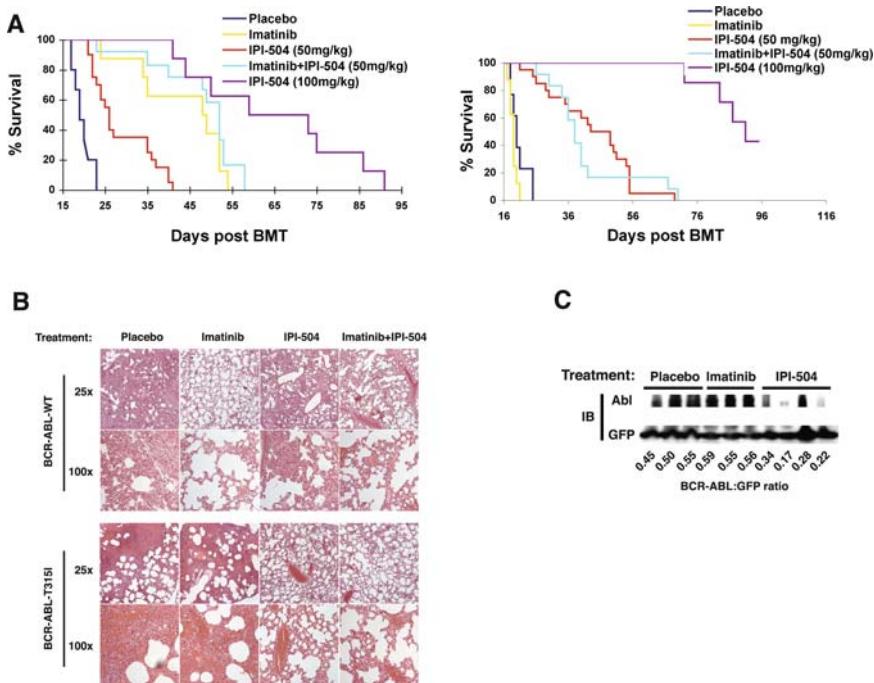
*Identification of leukemic stem cells.* Study of biology of leukemic stem cells requires having a reliable in vivo model system, and the development of anti-stem cells therapy depends on the understanding of differences between normal and leukemic stem cells. An important characteristic of stem cells is the ability for self-renewal, and only long- and short-term self-renewing HSCs have this capability [91] although other cell lineages in the hematopoietic system may have an effect on HSCs. However, in abnormal situation, some hematopoietic progenitors that do not normally self-renew can aberrantly acquire self-renewing capacity during leukemogenesis to become leukemic stem cells. For example, granulocyte–macrophage progenitors have been found to acquire stem cell property in human CML myeloid blast crisis through activation of β-catenin [92], which is also involved in self-renewal of normal HSCs [93, 94]. It is still an open question whether cancer stem cells exist in all types of tumors; however, it is convincing that leukemic stem cells and normal HSCs share mechanisms for regulation of self-renewal. For example, the cells capable of initiating human

---

**Fig. 7.1** represented BCR–ABL–WT-expressing myeloid cells. Percentages of BCR–ABL–T315I-expressing myeloid cells in peripheral blood of IPI-504-treated CML mice were further analyzed at days 21 and 28 post-BMT. The FACS results for one representative mouse from each treatment group were shown. IPI-504 but not imatinib significantly prolonged survival of the CML mice. **(B)** Simultaneous inhibition of Hsp90 and BCR–ABL kinase activity with IPI-504 and imatinib significantly prolongs survival of CML mice carrying both T315-expressing and WT–BCR–ABL leukemia cells. BALB/c mice were used to induce CML, and each treatment group had 10 mice. Adapted from [90]. (See color insert)



**Fig. 7.1 (continued) Inhibition of Hsp90 by IPI-504 preferentially reduces growth of myeloid leukemic cells harboring the BCR-ABL-T315I mutant.** (A) Bone marrow cells from C57BL/6-Ly5.2 mice were transduced by BCR-ABL-WT, and bone marrow cells from C57BL/6-Ly5.1 mice were transduced by BCR-ABL-T315I. The transduced cells were 1:1 mixed, and  $0.5 \times 10^6$  mixed cells were injected into each recipient mouse (C57BL/6-Ly5.2). The mice were treated with placebo ( $n = 10$ ), imatinib (100 mg/kg, twice a day) ( $n = 10$ ), and IPI-504 (50 mg/kg, once every 2 days) ( $n = 10$ ), respectively, beginning at 8 days post BMT. At days 12 and 15 post BMT, GFP<sup>+</sup> cells viable cells in peripheral blood of the mice were analyzed for Gr-1<sup>+</sup>Ly5.1<sup>+</sup> cells that represented BCR-ABL-T315I-expressing myeloid cells. Gr-1<sup>+</sup>Ly5.1<sup>-</sup> cells



**Fig. 7.2 Hsp90 is a therapeutic target for CML induced by either BCR-ABL-WT or BCR-ABL-T315I.** (A) Treatment with the Hsp90 inhibitor IPI-504 prolonged the survival of CML mice. Mice with BCR-ABL-WT (left panel)- or BCR-ABL-T315I (right panel)-induced CML were treated with placebo ( $n = 15$  for BCR-ABL-WT;  $n = 13$  for BCR-ABL-T315I), imatinib (100 mg/kg, twice a day by gavage) ( $n = 8$  for both BCR-ABL-WT and BCR-ABL-T315I), IPI-504 (50 mg/kg, once every 2 days by gavage) ( $n = 20$  for both BCR-ABL-WT and BCR-ABL-T315I), IPI-504 (100 mg/kg, once every 2 days by gavage) ( $n = 8$  for both BCR-ABL-WT;  $n = 7$  for BCR-ABL-T315I), and imatinib+IPI-504 ( $n = 12$  for both BCR-ABL-WT and BCR-ABL-T315I), respectively, beginning at day 8 post-transplantation. The IPI-504-treated mice with BCR-ABL-T315I-induced CML lived longer than those with BCR-ABL-WT-induced CML (comparing between left and right panels) (B) Photomicrographs of hematoxylin- and eosin-stained lung sections from drug-treated mice at day 14 post-transplantation. (C) Western blot analysis of spleen cell lysates for degradation of BCR-ABL in IPI-504-treated CML mice. IB, immunoblot. Adapted from [90]. (See color insert)

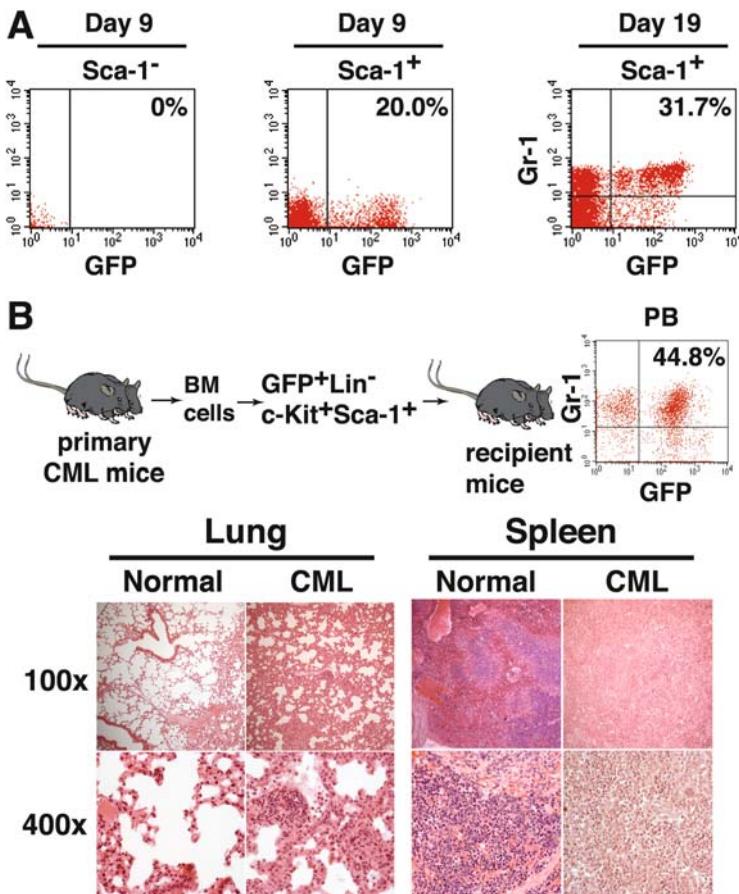
AML in NOD/SCID mice are exclusively CD34<sup>+</sup>CD38<sup>-</sup> cells [95], which is characteristic of normal HSCs. The existence of similar biological characteristics between normal and leukemic stem cells is supported by the studies using *Bim-1*-deficient mice; *Bim-1* is required for maintenance of self-renewal of normal HSCs [96, 97] and stem cells for AML, as *Bim1*<sup>-/-</sup> bone marrow cells from AML mice are incapable of re-producing the disease in secondary recipients [96]. However, failure to repopulate malignant diseases to secondary recipients does not exclude the possibility that the transferred cancer stem

cells with self-renewal capability did not engraft due to complex mechanisms related to the donor–recipient interactions, because the interaction between stem cells and their specific bone marrow microenvironment is critical for regulating the balance between self-renewal and differentiation of HSCs [98]. To better understand physiopathology of human hematologic malignancies, it is important to fully understand how leukemic stem cells communicate with bone marrow microenvironment.

Mouse CML stem cells have been recently identified and characterized using leukemia models (Fig. 7.3). C57BL/6 bone marrow cells transduced with BCR–ABL retrovirus were first sorted into Sca-1<sup>−</sup> or Sca-1<sup>+</sup> population, and only BCR–ABL-transduced Sca-1<sup>+</sup> cells transferred lethal CML to secondary B6 recipient mice [18], suggesting that early bone marrow progenitors contain CML stem cells. Because normal and leukemic stem cells share some common properties, HSCs (Lin<sup>−</sup>c-kit<sup>+</sup>Sca-1<sup>+</sup>) are likely to be a candidate population. This idea is proven to be true, as BCR–ABL-expressing HSCs (GFP<sup>+</sup>Lin<sup>−</sup>c-Kit<sup>+</sup>Sca-1<sup>+</sup>) isolated from bone marrow cells of primary CML mice induce CML in recipient mice. Thus, BCR–ABL expressing HSCs function as CML stem cells. In the future, it is necessary to test whether other cell lineages serve as CML stem cells.

Because chronic phase CML can progress into lymphoid blast crisis and Ph<sup>+</sup> B-ALL can co-exist with CML [99], it is possible that Ph<sup>+</sup> B-ALL and CML develop from a common stem cell. This assumption is supported by the observation that an anti-serum that recognizes B-ALL cells also detects cells from CML patients [24]. Furthermore, lymphoid and myeloid leukemias induced by BCR–ABL originate from the same progenitor cells in mice [54]. However, P190-induced Ph<sup>+</sup> B-ALL is rarely present in CML [100], suggesting a possibility that early lymphoid progenitors serve as the stem cells for Ph<sup>+</sup> B-ALL. This hypothesis is supported by identification of B-ALL stem cells using mice with BCR–ABL-induced lymphoid leukemia. In this study, dasatinib remarkably prolonged survival of B-ALL mice, but a small percentage of leukemic cells (<1%) remained in peripheral blood of these treated mice. These residual cells cause disease relapse after the treatment is stopped and are identified as B220<sup>+</sup>/CD43<sup>+</sup> pro-B cells [18]. These progenitor leukemic cells have acquired self-renewal capacity and function as B-ALL stem cells.

*Sensitivity of leukemic stem cells to kinase inhibitors.* A critical question to ask is whether leukemic stem cells are sensitive to inhibition by kinase inhibitors, based on the fact that a complete and sustained molecular remission (undetectable levels of BCR–ABL transcripts) is difficult to attain in CML patients after a complete cytogenetic remission achieved through imatinib treatment [101–104]. These studies suggest that imatinib and probably other Abl kinase inhibitors can efficiently kill highly proliferating leukemic cells but are insufficient to eradicate leukemic stem cells. This idea is supported by the finding that neither imatinib nor dasatinib can completely eradicate leukemic stem cells in CML and B-ALL mice [18]. Consistent with the results from this study in mice,



**Fig. 7.3 Identification of bone marrow cell populations that function as CML stem cells.** (A) BCR-ABL-transduced BM cells from B6 mice were sorted by Sca-1 MACS columns (Miltenyi Biotec), followed by transferring Sca-1<sup>-</sup> or Sca-1<sup>+</sup> population into B6 mice ( $1 \times 10^5$  cells/mouse; 4 mice per cell population group) to induce CML. GFP<sup>+</sup> myeloid cells (Gr-1<sup>+</sup>) in peripheral blood (PB) of the mice were examined at days 9 and 19 after the induction of leukemia. All mice receiving Sca-1<sup>+</sup> population died of CML by day 42. (B) BCR-ABL-expressing HSCs function as CML stem cells. BM cells from CML mice in B6 background were sorted by FACS for BCR-ABL-expressing HSCs (GFP<sup>+</sup>Lin<sup>-</sup>c-kit<sup>+</sup>Sca-1<sup>+</sup>), followed by transferring into lethally irradiated B6 mice ( $2 \times 10^4$  cells/mouse). GFP<sup>+</sup> myeloid cells (Gr-1<sup>+</sup>) were detected in peripheral blood. In contrast to the normal control mice, CML mice showed complete infiltration of the lungs with myeloid leukemic cells and complete disruption of follicular architecture of the spleen by infiltrating leukemic cells. Adapted from [18]. (See color insert)

the majority of B-ALL patients treated with imatinib have a marked but unsustained hematologic response [13, 14, 105, 106].

*Src kinases as therapeutic targets for Ph<sup>+</sup>B-ALL.* Although allogeneic bone marrow transplantation (BMT) is the only established curative therapy for Ph<sup>+</sup> leukemia, this option is available to less than 15–20% of patients due to age

restrictions and a lack of suitable donors. Imatinib was introduced to clinical trial for CML therapy more than a half decade ago [107, 108] and has been shown to induce a complete hematologic response in all interferon-resistant chronic phase CML patients [4]. Imatinib is, however, much less effective in treating patients with CML blast crisis and Ph<sup>+</sup> B-ALL [13, 14], and this type of insensitivity of leukemia to imatinib may not be associated with the development of imatinib-resistant BCR-ABL kinase domain mutations. To overcome this type of imatinib resistance, targeting BCR-ABL downstream signaling pathways that play an essential role in BCR-ABL leukemogenesis is likely a legitimate strategy. The success of this approach requires identification of key downstream signaling pathways utilized by BCR-ABL to induce leukemia. A good example that supports this idea is to identify Src kinases as key BCR-ABL downstream signaling molecules and to show that inhibition of Src kinases by the Src kinase inhibitor CGP76030 overcomes imatinib resistance to inhibit proliferation and induce apoptosis of pre-B-lymphoid cells that express BCR-ABL-T315I mutant [53]. These results are also supported by induction of apoptosis of leukemic cells from CML lymphoid blast crisis patients using anti-Lyn short interfering RNA [109].

## 7.6 Future Directions

Available mouse CML and B-ALL models have served as an excellent assay system in understanding the molecular basis of Ph<sup>+</sup> leukemia and in testing new therapeutic strategies. However, significant improvements are needed to allow answering some critical questions. First, Ph<sup>+</sup> leukemia is believed to be initiated from a stem cell that acquires Philadelphia chromosome. The mechanisms by which this stem cell accumulates other genetic mutations and subsequently develops into a fatal leukemia are largely unknown. The development of a model that allows studying the initial events of Ph<sup>+</sup> leukemia will be helpful. Second, most challenging issue in therapy of Ph<sup>+</sup> leukemia deals with leukemic stem cells. Although imatinib and dasatinib are effective in treating patients with Ph<sup>+</sup> leukemia and significantly prolong survival of leukemia mice, they are incapable of eliminating quiescent leukemic stem cells in patients [110] and in mice [18]. Study of biology of leukemic stem cells is key to developing anti-stem cell therapy for curing the disease and modeling Ph<sup>+</sup> leukemia using stem cells will provide a better model system for this area of research. Third, in human CML patients, the ineffectiveness of kinase inhibitors to completely eradicate leukemic cells can be caused by BCR-ABL mutations and could also be caused by the pre-existing BCR-ABL kinase domain mutations [111]. Investigation of developing mechanisms by which BCR-ABL mutations are initiated is critical to establishing curative therapy of Ph<sup>+</sup> leukemia. On the other hand, combination therapy using different kinase inhibitors to inhibit imatinib- or dasatinib-resistant leukemic cells is necessary [112-117]. It is also worth testing whether

this strategy would help to inhibit leukemic stem cells. The development of a model system that focuses on elucidating imatinib-resistant mechanisms and developing novel therapies will be essential for curing Ph<sup>+</sup> leukemia.

## References

1. Cobaleda C, Gutierrez-Cianca N, Perez-Losada J, Flores T, Garcia-Sanz R, Gonzalez M, Sanchez-Garcia I. A primitive hematopoietic cell is the target for the leukemic transformation in human philadelphia-positive acute lymphoblastic leukemia. *Blood*. 2000; 95: 1007–13.
2. van Rhee F, Hochhaus A, Lin F, Melo JV, Goldman JM, Cross NC. p190 BCR-ABL mRNA is expressed at low levels in p210-positive chronic myeloid and acute lymphoblastic leukemias. *Blood*. 1996; 87: 5213–7.
3. Roumier C, Daudignon A, Soenen V, Dupriez B, Wetterwald M, Lai JL, Cosson A, Fenaux P, Preudhomme C. p190 bcr-abl rearrangement: a secondary cytogenetic event in some chronic myeloid disorders? *Haematologica*. 1999; 84: 1075–80.
4. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*. 2001; 344: 1031–7.
5. Marley SB, Deininger MW, Davidson RJ, Goldman JM, Gordon MY. The tyrosine kinase inhibitor ST1571, like interferon-alpha, preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors from patients with chronic myeloid leukemia. *Exp Hematol*. 2000; 28: 551–7.
6. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science*. 2001; 293: 876–80.
7. Weisberg E, Griffin JD. Mechanism of resistance to the ABL tyrosine kinase inhibitor ST1571 in BCR/ABL-transformed hematopoietic cell lines. *Blood*. 2000; 95: 3498–505.
8. le Coutre P, Tassi E, Varella-Garcia M, Barni R, Mologni L, Cabrita G, Marchesi E, Supino R, Gambacorti-Passerini C. Induction of resistance to the Abelson inhibitor ST1571 in human leukemic cells through gene amplification. *Blood*. 2000; 95: 1758–66.
9. Mahon FX, Deininger MW, Schultheis B, Chabrol J, Reiffers J, Goldman JM, Melo JV. Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor ST1571: diverse mechanisms of resistance. *Blood*. 2000; 96: 1070–9.
10. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, Sawyers CL. Multiple BCR-ABL kinase domain mutants confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (ST1571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell*. 2002; 2: 117–25.
11. Branford S, Rudzki Z, Walsh S, Grigg A, Arthur C, Taylor K, Herrmann R, Lynch KP, Hughes TP. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (ST1571) resistance. *Blood*. 2002; 99: 3472–5.
12. von Bubnoff N, Schneller F, Peschel C, Duyster J. BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to ST1571: a prospective study. *Lancet*. 2002; 359: 487–91.
13. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast

- crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med.* 2001; 344: 1038–42.
- 14. Talpaz M, Sawyers CL, Kantarjain H, Resta D, Fernandes Rees S, Ford J, Bruker BJ. Activity of an ABL specific tyrosine kinase inhibitor in patients with BCR/ABL positive acute leukemias, including chronic myelogenous leukemia in blast crisis. *Oncologist.* 2000; 5: 282–3 (Abstr.).
  - 15. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science.* 2004; 305: 399–401.
  - 16. O'Hare T, Pollock R, Stoffregen EP, Keats JA, Abdullah OM, Moseson EM, Rivera VM, Tang H, Metcalf CA, 3rd, Bohacek RS, Wang Y, Sundaramoorthi R, Shakespeare WC, Dalgarno D, Clackson T, Sawyer TK, Deininger MW, Druker BJ. Inhibition of wild-type and mutant Bcr-Abl by AP23464, a potent ATP-based oncogenic protein kinase inhibitor: implications for CML. *Blood.* 2004; 104: 2532–9.
  - 17. Weisberg E, Manley PW, Breitenstein W, Bruggen J, Cowan-Jacob SW, Ray A, Huntly B, Fabbro D, Fendrich G, Hall-Meyers E, Kung AL, Mestan J, Daley GQ, Callahan L, Catley L, Cavazza C, Mohammed A, Neuberg D, Wright RD, Gilliland DG, Griffin JD. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell.* 2005; 7: 129–41.
  - 18. Hu Y, Swerdlow S, Duffy TM, Weinmann R, Lee FY, Li S. Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph<sup>+</sup> leukemia in mice. *Proc Natl Acad Sci USA.* 2006; 103: 16870–75.
  - 19. Advani AS, Pendergast AM. Bcr-Abl variants: biological and clinical aspects. *Leuk Res.* 2002; 26: 713–20.
  - 20. Van Etten RA. Malignant transformation by abl and BCR/ABL. *Cancer Treat Res.* 1992; 63: 167–92.
  - 21. Van Etten RA. The molecular pathogenesis of the Philadelphia-positive leukemias: implications for diagnosis and therapy. *Cancer Treat Res.* 1993; 64: 295–325.
  - 22. Pane F, Frigeri F, Sindona M, Luciano L, Ferrara F, Cimino R, Meloni G, Saglio G, Salvatore F, Rotoli B. Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). *Blood.* 1996; 88: 2410–4.
  - 23. Sawyers CL. Chronic myeloid leukemia. *N Engl J Med.* 1999; 340: 1330–40.
  - 24. Janossy G, Roberts M, Greaves MF. Target cell in chronic myeloid leukaemia and its relationship to acute lymphoid leukaemia. *Lancet.* 1976; 2: 1058–61.
  - 25. Towatari M, Adachi K, Kato H, Saito H. Absence of the human retinoblastoma gene product in the megakaryoblastic crisis of chronic myelogenous leukemia. *Blood.* 1991; 78: 2178–81.
  - 26. Sill H, Goldman JM, Cross NC. Homozygous deletions of the p16 tumor-suppressor gene are associated with lymphoid transformation of chronic myeloid leukemia. *Blood.* 1995; 85: 2013–6.
  - 27. Feinstein E, Cimino G, Gale RP, Alimena G, Berthier R, Kishi K, Goldman J, Zaccaria A, Berrebi A, Canaani E. p53 in chronic myelogenous leukemia in acute phase. *Proc Natl Acad Sci USA.* 1991; 88: 6293–7.
  - 28. Deutsch E, Dugray A, AbdulKarim B, Marangoni E, Maggiorella L, Vaganay S, M'Kacher R, Rasy SD, Eschwege F, Vainchenker W, Turhan AG, Bourhis J. BCR-ABL down-regulates the DNA repair protein DNA-PKcs. *Blood.* 2001; 97: 2084–90.
  - 29. Takeda N, Shibuya M, Maru Y. The BCR-ABL oncprotein potentially interacts with the xeroderma pigmentosum group B protein. *Proc Natl Acad Sci USA.* 1999; 96: 203–7.
  - 30. Canitrot Y, Lautier D, Laurent G, Frechet M, Ahmed A, Turhan AG, Salles B, Cazaux C, Hoffmann JS. Mutator phenotype of BCR-ABL transfected Ba/F3 cell lines and its association with enhanced expression of DNA polymerase beta. *Oncogene.* 1999; 18: 2676–80.
  - 31. Slupianek A, Schmutte C, Tomblaine G, Nieborowska-Skorska M, Hoser G, Nowicki MO, Pierce AJ, Fishel R, Skorski T. BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. *Mol Cell.* 2001; 8: 795–806.

32. Dierov J, Dierova R, Carroll M. BCR/ABL translocates to the nucleus and disrupts an ATR-dependent intra-S phase checkpoint. *Cancer Cell*. 2004; 5: 275–85.
33. Huettnner CS, Zhang P, Van Etten RA, Tenen DG. Reversibility of acute B-cell leukaemia induced by BCR-ABL1. *Nat Genet*. 2000; 24: 57–60.
34. Calabretta B, Perrotti D. The biology of CML blast crisis. *Blood*. 2004; 103: 4010–22.
35. Sawyers CL. Signal transduction pathways involved in BCR-ABL transformation. *Baillieres Clin Haematol*. 1997; 10: 223–31.
36. Anderson SM, Mladenovic J. The BCR-ABL oncogene requires both kinase activity and src-homology 2 domain to induce cytokine secretion. *Blood*. 1996; 87: 238–44.
37. Hariharan IK, Adams JM, Cory S. bcr-abl oncogene renders myeloid cell line factor independent: potential autocrine mechanism in chronic myeloid leukemia. *Oncogene Res*. 1988; 3: 387–99.
38. Skorski T, Nieborowska-Skorska M, Wlodarski P, Perrotti D, Martinez R, Wasik MA, Calabretta B. Blastic transformation of p53-deficient bone marrow cells by p210bcr/abl tyrosine kinase. *Proc Natl Acad Sci USA*. 1996; 93: 13137–42.
39. Honda H, Hirai H. Model mice for BCR/ABL-positive leukemias. *Blood Cells Mol Dis*. 2001; 27: 265–78.
40. Neshat MS, Raitano AB, Wang HG, Reed JC, Sawyers CL. The survival function of the Bcr-Abl oncogene is mediated by Bad-dependent and -independent pathways: roles for phosphatidylinositol 3-kinase and Raf. *Mol Cell Biol*. 2000; 20: 1179–86.
41. Majewski M, Nieborowska-Skorska M, Salomoni P, Slupianek A, Reiss K, Trotta R, Calabretta B, Skorski T. Activation of mitochondrial Raf-1 is involved in the antiapoptotic effects of Akt. *Cancer Res*. 1999; 59: 2815–9.
42. Sanchez-Garcia I, Martin-Zanca D. Regulation of Bcl-2 gene expression by BCR-ABL is mediated by Ras. *J Mol Biol*. 1997; 267: 225–8.
43. Dubrez L, Eymen B, Sordet O, Droin N, Turhan AG, Solary E. BCR-ABL delays apoptosis upstream of procaspase-3 activation. *Blood*. 1998; 91: 2415–22.
44. Amarante-Mendes GP, Naekyung Kim C, Liu L, Huang Y, Perkins CL, Green DR, Bhalla K. Bcr-Abl exerts its antiapoptotic effect against diverse apoptotic stimuli through blockage of mitochondrial release of cytochrome C and activation of caspase-3. *Blood*. 1998; 91: 1700–5.
45. McGahon AJ, Nishioka WK, Martin SJ, Mahboubi A, Cotter TG, Green DR. Regulation of the Fas apoptotic cell death pathway by Ab1. *J Biol Chem*. 1995; 270: 22625–31.
46. Skorski T, Bellacosa A, Nieborowska-Skorska M, Majewski M, Martinez R, Choi JK, Trotta R, Wlodarski P, Perrotti D, Chan TO, Wasik MA, Tsichlis PN, Calabretta B. Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3 k/Akt-dependent pathway. *Embo J*. 1997; 16: 6151–61.
47. Jonuleit T, van der Kuip H, Miethling C, Michels H, Hallek M, Duyster J, Aulitzky WE. Bcr-Abl kinase down-regulates cyclin-dependent kinase inhibitor p27 in human and murine cell lines. *Blood*. 2000; 96: 1933–9.
48. Parada Y, Banerji L, Glassford J, Lea NC, Collado M, Rivas C, Lewis JL, Gordon MY, Thomas NS, Lam EW. BCR-ABL and interleukin 3 promote haematopoietic cell proliferation and survival through modulation of cyclin D2 and p27Kip1 expression. *J Biol Chem*. 2001; 276: 23572–80.
49. Goetz AW, van der Kuip H, Maya R, Oren M, Aulitzky WE. Requirement for Mdm2 in the survival effects of Bcr-Abl and interleukin 3 in hematopoietic cells. *Cancer Res*. 2001; 61: 7635–41.
50. Danhauser-Riedl S, Warmuth M, Druker BJ, Emmerich B, Hallek M. Activation of Src kinases p53/56 lyn and p59hck by p210bcr/abl in myeloid cells. *Cancer Res*. 1996; 56: 3589–96.
51. Warmuth M, Bergmann M, Priess A, Hauslmann K, Emmerich B, Hallek M. The Src family kinase Hck interacts with Bcr-Abl by a kinase-independent mechanism and phosphorylates the Grb2-binding site of Bcr. *J Biol Chem*. 1997; 272: 33260–70.

52. Lionberger JM, Wilson MB, Smithgall TE. Transformation of myeloid leukemia cells to cytokine independence by Bcr-Abl is suppressed by kinase-defective Hck. *J Biol Chem.* 2000; 275: 18581–5.
53. Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, Hallek M, Van Etten RA, Li S. Requirement of Src kinases Lyn, Hck and Fgr for BCR-ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. *Nat Genet.* 2004; 36: 453–61.
54. Li S, Ilaria RL, Jr., Million RP, Daley GQ, Van Etten RA. The P190, P210, and p230 forms of the *BCR/ABL* oncogene induce a similar chronic myeloid leukemia-like syndrome in mice but have different lymphoid leukemogenic activity. *J Exp Med.* 1999; 189: 1399–412.
55. Roumiantsev S, de Aos IE, Varticovski L, Ilaria RL, Van Etten RA. The src homology 2 domain of Bcr/Abl is required for efficient induction of chronic myeloid leukemia-like disease in mice but not for lymphoid leukemogenesis or activation of phosphatidylinositol 3-kinase. *Blood.* 2001; 97: 4–13.
56. Klejman A, Schreiner SJ, Nieborowska-Skorska M, Slupianek A, Wilson M, Smithgall TE, Skorski T. The Src family kinase Hck couples BCR/ABL to STAT5 activation in myeloid leukemia cells. *Embo J.* 2002; 21: 5766–74.
57. Warmuth M, Simon N, Mitina O, Mathes R, Fabbro D, Manley PW, Buchdunger E, Forster K, Moarefi I, Hallek M. Dual-specific Src and Abl kinase inhibitors, PP1 and CGP76030, inhibit growth and survival of cells expressing imatinib mesylate-resistant Bcr-Abl kinases. *Blood.* 2003; 101: 664–72.
58. Daigle I, Yousefi S, Colonna M, Green DR, Simon HU. Death receptors bind SHP-1 and block cytokine-induced anti-apoptotic signaling in neutrophils. *Nat Med.* 2002; 8: 61–7.
59. Yang W, McKenna SD, Jiao H, Tabrizi M, Lynes MA, Shultz LD, Yi T. SHP-1 deficiency in B-lineage cells is associated with heightened lyn protein expression and increased lyn kinase activity. *Exp Hematol.* 1998; 26: 1126–32.
60. Tauchi, Feng GS, Shen R, Song HY, Donner D, Pawson T, Broxmeyer HE. SH2-containing phosphotyrosine phosphatase Syp is a target of p210bcr-abl tyrosine kinase. *J Biol Chem.* 1994; 269: 15381–7.
61. Anderson SM, Jorgensen B. Activation of src-related tyrosine kinases by IL-3. *J Immunol.* 1995; 155: 1660–70.
62. Daley GQ, Baltimore D. Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl protein. *Proc Natl Acad Sci USA.* 1988; 85: 9312–6.
63. Kabarowski JH, Allen PB, Wiedemann LM. A temperature sensitive p210 BCR-ABL mutant defines the primary consequences of BCR-ABL tyrosine kinase expression in growth factor dependent cells. *Embo J.* 1994; 13: 5887–95.
64. Bruecher-Encke B, Griffin JD, Neel BG, Lorenz U. Role of the tyrosine phosphatase SHP-1 in K562 cell differentiation. *Leukemia.* 2001; 15: 1424–32.
65. Gardai S, Whitlock BB, Helgason C, Ambruso D, Fadok V, Bratton D, Henson PM. Activation of SHIP by NADPH oxidase-stimulated Lyn leads to enhanced apoptosis in neutrophils. *J Biol Chem.* 2002; 277: 5236–46.
66. Park H, Wahl MI, Afar DE, Turck CW, Rawlings DJ, Tam C, Scharenberg AM, Kinet JP, Witte ON. Regulation of Btk function by a major autophosphorylation site within the SH3 domain. *Immunity.* 1996; 4: 515–25.
67. Rawlings DJ, Scharenberg AM, Park H, Wahl MI, Lin S, Kato RM, Fluckiger AC, Witte ON, Kinet JP. Activation of BTK by a phosphorylation mechanism initiated by SRC family kinases. *Science.* 1996; 271: 822–5.
68. Hariharan IK, Harris AW, Crawford M, Abud H, Webb E, Cory S, Adams JM. A bcr-abl oncogene induces lymphomas in transgenic mice. *Mol Cell Biol.* 1989; 9: 2798–805.
69. Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, Groffen J. Acute leukaemia in bcr/abl transgenic mice. *Nature.* 1990; 344: 251–3.

70. Honda H, Fujii T, Takatoku M, Mano H, Witte ON, Yazaki Y, Hirai H. Expression of p210bcr/abl by metallothionein promoter induced T-cell leukemia in transgenic mice. *Blood*. 1995; 85: 2853–61.
71. Voncken JW, Kaartinen V, Pattengale PK, Germeraad WT, Groffen J, Heisterkamp N. BCR/ABL P210 and P190 cause distinct leukemia in transgenic mice. *Blood*. 1995; 86: 4603–11.
72. Castellanos A, Pintado B, Weruaga E, Arevalo R, Lopez A, Orfao A, Sanchez-Garcia I. A BCR-ABL(p190) fusion gene made by homologous recombination causes B-cell acute lymphoblastic leukemias in chimeric mice with independence of the endogenous bcr product. *Blood*. 1997; 90: 2168–74.
73. Heisterkamp N, Jenster G, Kioussis D, Pattengale PK, Groffen J. Human bcr-abl gene has a lethal effect on embryogenesis. *Transgenic Res*. 1991; 1: 45–53.
74. Honda H, Oda H, Suzuki T, Takahashi T, Witte ON, Ozawa K, Ishikawa T, Yazaki Y, Hirai H. Development of acute lymphoblastic leukemia and myeloproliferative disorder in transgenic mice expressing p210bcr/abl: a novel transgenic model for human Ph1-positive leukemias. *Blood*. 1998; 91: 2067–75.
75. Inokuchi K, Dan K, Takatori M, Takahashi H, Uchida N, Inami M, Miyake K, Honda H, Hirai H, Shimada T. Myeloproliferative disease in transgenic mice expressing P230 Bcr/Ab1: longer disease latency, thrombocytosis, and mild leukocytosis. *Blood*. 2003; 102: 320–3.
76. Huettner CS, Koschmieder S, Iwasaki H, Iwasaki-Arai J, Radomska HS, Akashi K, Tenen DG. Inducible expression of BCR/ABL using human CD34 regulatory elements results in a megakaryocytic myeloproliferative syndrome. *Blood*. 2003; 102: 3363–70.
77. Koschmieder S, Gottgens B, Zhang P, Iwasaki-Arai J, Akashi K, Kutok JL, Dayaram T, Geary K, Green AR, Tenen DG, Huettner CS. Inducible chronic phase of myeloid leukemia with expansion of hematopoietic stem cells in a transgenic model of BCR-ABL leukemogenesis. *Blood*. 2005; 105: 324–34.
78. Sirard C, Lapidot T, Vormoor J, Cashman JD, Doedens M, Murdoch B, Jamal N, Messner H, Addy L, Minden M, Laraya P, Keating A, Eaves A, Lansdorp PM, Eaves CJ, Dick JE. Normal and leukemic SCID-repopulating cells (SRC) coexist in the bone marrow and peripheral blood from CML patients in chronic phase, whereas leukemic SRC are detected in blast crisis. *Blood*. 1996; 87: 1539–48.
79. Wang JC, Lapidot T, Cashman JD, Doedens M, Addy L, Sutherland DR, Nayar R, Laraya P, Minden M, Keating A, Eaves AC, Eaves CJ, Dick JE. High level engraftment of NOD/SCID mice by primitive normal and leukemic hematopoietic cells from patients with chronic myeloid leukemia in chronic phase. *Blood*. 1998; 91: 2406–14.
80. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nat Rev Immunol*. 2007; 7: 118–30.
81. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science*. 1990; 247: 824–30.
82. Elefanty AG, Hariharan IK, Cory S. bcr-abl, the hallmark of chronic myeloid leukaemia in man, induces multiple haemopoietic neoplasms in mice. *Embo J*. 1990; 9: 1069–78.
83. Kelliher MA, McLaughlin J, Witte ON, Rosenberg N. Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. *Proc Natl Acad Sci USA*. 1990; 87: 6649–53.
84. Pear WS, Miller JP, Xu L, Pui JC, Soffer B, Quackenbush RC, Pendergast AM, Bronson R, Aster JC, Scott ML, Baltimore D. Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. *Blood*. 1998; 92: 3780–92.
85. Zhang X, Ren R. Bcr-Ab1 efficiently induces a myeloproliferative disease and production of excess interleukin-3 and granulocyte-macrophage colony-stimulating factor in mice: a novel model for chronic myelogenous leukemia. *Blood*. 1998; 92: 3829–40.

86. Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukemia. *Nat Rev Cancer.* 2005; 5: 172–83.
87. Li S, Gillessen S, Tomasson MH, Dranoff G, Gilliland DG, Van Etten RA. Interleukin 3 and granulocyte-macrophage colony-stimulating factor are not required for induction of chronic myeloid leukemia-like myeloproliferative disease in mice by *BCR/ABL*. *Blood.* 2001; 97: 1442–50.
88. Wolff NC, Ilaria RL, Jr. Establishment of a murine model for therapy-treated chronic myelogenous leukemia using the tyrosine kinase inhibitor ST1571. *Blood.* 2001; 98: 2808–16.
89. Peng C, Brain J, Hu Y, Goodrich A, Kong L, Grayzel D, Park R, Read M, Li S. Inhibition of heat shock protein 90 prolongs survival of mice with BCR-ABL-T315I-induced leukemia and suppresses leukemic stem cells. *Blood.* 2007; 110: 678–85.
90. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature.* 2001; 414: 105–11.
91. Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, Gotlib J, Li K, Manz MG, Keating A, Sawyers CL, Weissman IL. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med.* 2004; 351: 657–67.
92. Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, Hintz L, Nusse R, Weissman IL. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature.* 2003; 423: 409–14.
93. Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, Yates JR, 3rd, Nusse R. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature.* 2003; 423: 448–52.
94. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med.* 1997; 3: 730–7.
95. Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature.* 2003; 423: 255–60.
96. Park IK, Qian D, Kiel M, Becker MW, Pihlaja M, Weissman IL, Morrison SJ, Clarke MF. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature.* 2003; 423: 302–5.
97. Wilson A, Murphy MJ, Oskarsson T, Kaloulis K, Bettess MD, Oser GM, Pasche AC, Knabenhans C, Macdonald HR, Trumpp A. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. *Genes Dev.* 2004; 18: 2747–63.
98. Deininger M. Src kinases in Ph+ lymphoblastic leukemia. *Nat Genet.* 2004; 36: 440–1.
99. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. *Blood.* 2000; 96: 3343–56.
100. Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML, Gathmann I, Bolton AE, van Hoornissen IC, Goldman JM, Radich JP. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med.* 2003; 349: 1423–32.
101. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med.* 2003; 348: 994–1004.
102. Lin F, Drummond M, O'Brien S, Cervantes F, Goldman J, Kaeda J. Molecular monitoring in chronic myeloid leukemia patients who achieve complete cytogenetic remission on imatinib. *Blood.* 2003; 102: 1143.
103. Drummond MW, Lush CJ, Vickers MA, Reid FM, Kaeda J, Holyoake TL. Imatinib mesylate-induced molecular remission of Philadelphia chromosome-positive myelodysplastic syndrome. *Leukemia.* 2003; 17: 463–5.

104. Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller CB, Ottmann OG, Schiffer Ca, Talpaz M, Guilhot F, Deininger MW, Fischer T, O'Brien SG, Stone RM, Gambacorti-Passerini C, Russell NH, Reiffers JJ, Shea TC, Chapuis B, Coutre S, Tura S, Morra E, Larson RA, Saven A, Peschel C, Gratwohl A, Mandelli F, Ben-Am M, Gathmann I, Capdeville R, Paquette RL, Druker B. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood*. 2002; 99: 3530–9.
105. Kantarjian HM, Cortes J, O'Brien S, Giles FJ, Albitar M, Rios MB, Shan J, Faderl S, Garcia-Manero G, Thomas DA, Resta D, Talpaz M. Imatinib mesylate (ST1571) therapy for Philadelphia chromosome-positive chronic myelogenous leukemia in blast phase. *Blood*. 2002; 99: 3547–53.
106. Druker BJ, Lydon NB. Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. *J Clin Invest*. 2000; 105: 3–7.
107. Lydon NB, Druker BJ. Lessons learned from the development of imatinib. *Leuk Res*. 2004; 28 Suppl 1: S29–S38.
108. Ptaszniak A, Nakata Y, Kalota A, Emerson SG, Gewirtz AM. Short interfering RNA (siRNA) targeting the Lyn kinase induces apoptosis in primary, and drug-resistant, BCR-ABL1(+) leukemia cells. *Nat Med*. 2004; 10: 1187–9.
109. Elrick LJ, Jorgensen HG, Mountford JC, Holyoake TL. Punish the parent not the progeny. *Blood*. 2005; 105: 1862–6.
110. Pfeifer H, Wassmann B, Pavlova A, Wunderle L, Oldenburg J, Binckebanck A, Lange T, Hochhaus A, Wystub S, Bruck P, Hoelzer D, Ottmann OG. Kinase domain mutations of BCR-ABL frequently precede imatinib-based therapy and give rise to relapse in patients with de novo Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). *Blood*. 2007; 110: 727–34.
111. Harrington EA, Bebbington D, Moore J, Rasmussen RK, Ajose-Adeogun AO, Nakayama T, Graham JA, Demur C, Hercend T, Diu-Hercend A, Su M, Golec JM, Miller KM. VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth in vivo. *Nat Med*. 2004; 10: 262–7.
112. Doggett SA. Dawn of Aurora kinase inhibitors as anticancer drugs. *Expert Opin Investig Drugs*. 2004; 13: 1199–201.
113. Carter TA, Wodicka LM, Shah NP, Velasco AM, Fabian MA, Treiber DK, Milanov ZV, Atteridge CE, Biggs WH, 3rd, Edeen PT, Floyd M, Ford JM, Grotfeld RM, Herrgard S, Insko DE, Mehta SA, Patel HK, Pao W, Sawyers CL, Varmus H, Zarrinkar PP, Lockhart DJ. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc Natl Acad Sci U S A*. 2005; 102: 11011–6.
114. Young MA, Shah NP, Chao LH, Seeliger M, Milanov ZV, Biggs WH, 3rd, Treiber DK, Patel HK, Zarrinkar PP, Lockhart DJ, Sawyers CL, Kuriyan J. Structure of the kinase domain of an imatinib-resistant Abl mutant in complex with the Aurora kinase inhibitor VX-680. *Cancer Res*. 2006; 66: 1007–14.
115. Giles FJ, Cortes J, Jones D, Bergstrom D, Kantarjian H, Freedman SJ. MK-0457, a novel kinase inhibitor, is active in patients with chronic myeloid leukemia or acute lymphocytic leukemia with the T315I BCR-ABL mutation. *Blood*. 2007; 109: 500–2.
116. Cheetham GM, Charlton PA, Golec JM, Pollard JR. Structural basis for potent inhibition of the Aurora kinases and a T315I multi-drug resistant mutant form of Abl kinase by VX-680. *Cancer Lett*. 2007; 251: 323–9.

# **Chapter 8**

## **Mouse Models of Human Mature B-Cell and Plasma Cell Neoplasms**

**Siegfried Janz, Herbert C. Morse III, and Michael A. Teitel**

### **Contents**

8.1	Introduction . . . . .	180
8.2	Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma . . . . .	183
8.2.1	CLL/Small Lymphocytic Lymphoma in Humans . . . . .	183
8.2.2	Mouse Models of CLL/SLL . . . . .	184
8.2.2.1	Spontaneous Small B-Cell Lymphoma/Leukemia in Mice . . . . .	184
8.2.2.2	Models of CLL Based on Studies of New Zealand Mice . . . . .	184
8.2.2.3	The TCL1 TG Mouse Model of CLL . . . . .	187
8.2.2.4	The BLC2/TRAF Model of CLL—the NF $\kappa$ B Connection . . . . .	188
8.2.2.5	APRIL and CLL? . . . . .	189
8.2.2.6	Conclusions Regarding Mouse Models of CLL . . . . .	190
8.3	Marginal Zone Lymphomas . . . . .	191
8.3.1	MZL in Humans . . . . .	191
8.3.2	Mouse Models of MZL . . . . .	192
8.3.2.1	Spontaneous MZL in Mice . . . . .	192
8.3.2.2	Mouse Model of MALT . . . . .	192
8.3.2.3	Mouse Models of Splenic MZL . . . . .	192
8.3.2.4	Conclusions Regarding Mouse Models of MZL . . . . .	193
8.4	Mantle Cell Lymphoma . . . . .	193
8.4.1	MCL in Humans . . . . .	193
8.4.2	Mouse Models of MCL . . . . .	194
8.4.2.1	MCL in Genetically Engineered Mice . . . . .	194
8.5	Follicular B-Cell Lymphoma . . . . .	194
8.5.1	FBL in Humans . . . . .	194
8.5.2	Mouse Models of FBL . . . . .	198
8.5.2.1	Spontaneous FBL in Mice . . . . .	198
8.5.2.2	FBL in Genetically Engineered Mice . . . . .	198
8.5.2.3	Conclusions Regarding Mouse Models of FBL . . . . .	199
8.6	Diffuse Large B-Cell Lymphoma . . . . .	200
8.6.1	DLBCL in Humans . . . . .	200
8.6.2	Mouse Models of DLBCL . . . . .	200
8.6.2.1	Spontaneous DLBCL in Mice . . . . .	200

---

S. Janz

Department of Pathology, Carver College of Medicine, University of Iowa,  
500 Newton Road, 1046C ML, Iowa City, IA 52242, USA  
Siegfried-janz@uiowa.edu

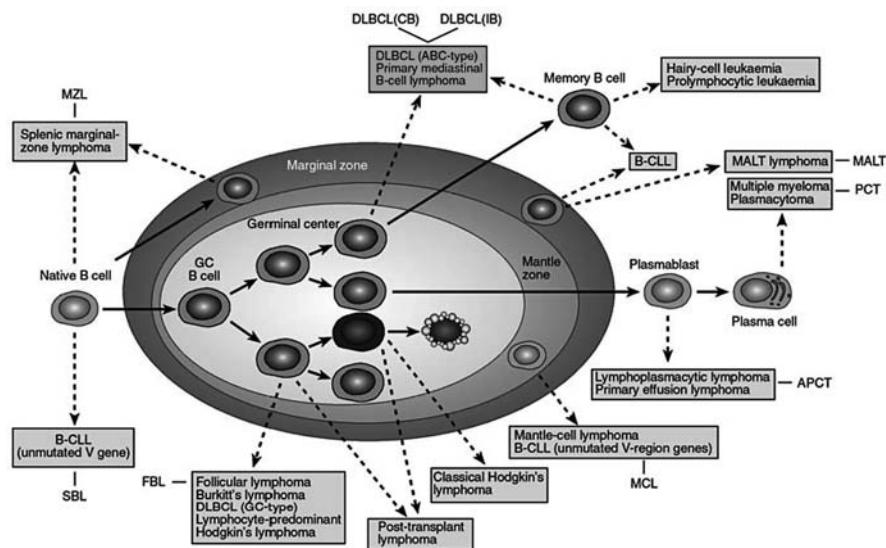
8.6.2.2	Spontaneous Variants of DLBCL in Mice . . . . .	200
8.6.2.3	DLBCL in Genetically Engineered Mice . . . . .	202
8.6.2.4	Conclusions Regarding Mouse Models of DLBCL . . . . .	203
8.7	Diffuse High-Grade Blastic B-cell Lymphoma/Leukemia (DBLL) in Mice . . . . .	203
8.7.1	DBLL in Genetically Engineered Mice . . . . .	204
8.8	Plasma Cell Neoplasms . . . . .	204
8.8.1	Human PCN Including Multiple Myeloma . . . . .	204
8.8.2	Established and Newly Emerging Mouse Models of Human PCN . . . . .	208
8.8.3	Xenograft Models of Human Myeloma in Mice . . . . .	215
8.8.4	Conclusions Regarding Mouse Models of Human PCN . . . . .	217

## 8.1 Introduction

Developing mouse models that accurately reflect features of human B-cell lineage neoplasms has been a daunting but increasingly rewarding task. Studies of spontaneous tumors or those induced by chemicals, irradiation, or retroviruses performed before the 1980s provided remarkable insights into mechanisms and genetics of lymphomagenesis. With the advent of genetic engineering, it became possible to rapidly develop and explore new models and to enhance the value of established systems. Here, we will review past and present accomplishments in modeling mature human B-cell lymphomas and plasmas cell neoplasms (PCN) in mice, examine their strengths and limitations, and discuss obstacles that must be addressed in future work. These systems have accelerated our ability to understand the development of complex disease *in vivo* and to develop novel therapeutic approaches to diseases, many of which are almost uniformly lethal.

The cellular origins of human mature B cell and PCN defined by the consensus WHO classification (Jaffe, Harris et al. 2001a) are presented diagrammatically in Fig. 8.1. The lymphomas reflect various features of normal pregerminal center (pre-GC) and post-GC cells including anatomic location, expression patterns of differentiation markers, and mutational status of immunoglobulin gene variable region (IgV) sequences. Recently, further distinctions have been made based on the results of microarray gene expression profiling of normal B cells and lymphomas.

Most types of human B-cell lymphoma are derived from GC or post-GC B cells. A number of these are recognized as having close parallels among mouse B-lineage tumors classified according to the Bethesda proposals (Morse, Anver et al. 2002) including follicular B-cell lymphoma (FBL) and diffuse large B-cell lymphomas (DLBCL) as well as plasmacytomas (PCT) (Fig. 8.1). Human lymphomas of pre-GC origin, including a subset of chronic lymphocytic leukemia (CLL), termed small B-cell lymphoma (SBL) in mice and splenic marginal zone lymphoma (SMZL), also have parallels among spontaneous and induced tumors of mice. To date, there are no reports of mouse neoplasms with significant similarities to human Hodgkin, Burkitt, primary effusion or post-transplant lymphomas or to hairy cell or prolymphocytic leukemias. There are reports, however, of mouse models of marginal zone lymphomas (MZL) of



**Fig. 8.1 Cellular origins of human and mouse mature B-cell lymphomas and plasma cell neoplasms.** After undergoing early maturation in the bone marrow, naïve transitional B cells populate peripheral lymphoid tissues where they are recruited into the follicular and marginal zone B-cell subsets. Following interaction with antigen and helper T cells, follicular B cells establish germinal centers. There they undergo rapid clonal expansion and somatic hypermutation in the dark zone and move to the light zone where they undergo class switch recombination and positive selection on antigen-bearing follicular dendritic cells. Selected cells exit the germinal center to form memory B cells or to become plasma cells. Reciprocal chromosomal translocations involving *IgH* or *IgL* gene and a proto-oncogene, characteristic of most non-Hodgkin lymphomas (NHL), are thought to be generated as unfortunate by-products of the several mechanisms involved in *Ig* gene diversification that involves the generation of double-stranded DNA breaks. Some, such as *IgH*-*BCL2* in follicular lymphoma, appear to occur as a consequence of aberrant  $V_H DJ_H$  recombination. Others, located adjacent to rearranged, somatically mutated  $V_H DJ_H$  genes, such as *IgH*-*BCL6* in diffuse large B-cell lymphoma, most likely occur as a consequence of somatic hypermutation. A third group features breakpoint in *IgH* switch regions and appears to reflect aberrant class switch recombination, as in a subset of Burkitt lymphoma. The classes of human lymphomas and the mouse neoplasms most closely related when known are indicated. [Modified from Kuppers (2005).]

mucosa-associated lymphoid tissue (MALT) and mantle cell lymphomas (MCL) as well as lymphoplasmacytic lymphoma, termed anaplastic PCT (APCT) in mice. Systems that help define, explore, and extend these parallels are the subject of this chapter.

An ongoing challenge for developing accurate mouse models of human NHL and PCN is the need to reproduce recurrent somatic mutations in the appropriate B-cell targets. Deciding which B cell to target—pre-GC, GC, or MZ B cell or extrafollicular plasmablast, for example—will be difficult as long as the precise nature and differentiation stage of the human tumor precursors remains unknown. Additional challenges for transgenic (TG) mouse models of NHL

and PCN relate to the recapitulation of tumor progression pathways, mechanisms of stromal interactions, and responses to drugs used in human patients. Although mouse models of NHL and PCN might well be expected to phenocopy the human tumors, engineering the signaling pathways and responses is much more difficult than inducing rapid onset neoplasms such as those using retroviral transfer of classic oncogenes, which have remarkable value in their own right (Hu, Swerdlow et al. 2006).

A variety of TG techniques are now available to address the challenges mentioned above. It is possible to introduce into the mouse germ line gain-of-function mutations such as constitutively expressed oncogenes under control of B cell or plasma cell-specific enhancers and promoters. Conversely, normal mouse germ line genes can be replaced with loss-of-function alleles, which may take the form of classic null alleles ("knock-out" mice) or, of increasing importance, weak-efficiency alleles that retain some gene activity and thus more closely mimic alleles segregating in the human population. A more sophisticated method involves inducible transgenes, which provide for the temporal and spatial control of gene expression or gene attenuation, thereby circumventing potential developmental problems including embryonic toxicity and lethality that may be posed by constitutively expressed TG. Adenoviruses (Strair, Sheay et al. 2002) and retroviruses (Mikkelsen and Berns 2003) offer additional means for spatial and temporal gene regulation. A lesser known but very attractive example of the latter strategy takes advantage of cell type-specific retroviral binding to specific receptors of the subgroup A avian leucosis virus, affording introduction of several cancer genes into mouse target cells (Du, Podsypanina et al. 2006). Lentivirus-mediated transgenesis (Aronoff and Petersen 2006) represents yet another alternative to conventional technologies for generating the panel of TG mouse strains that may be required to accurately model human plasma cell tumors.

Mouse models of NHL and PCN should not only phenocopy their human counterparts at the histopathologic and molecular level but also exhibit desirable features including high tumor penetrance, short latencies, and predictable stages of tumor progression. Additional expectations, such as means for monitoring the tumor burden in live animals in a repeatable and reproducible manner, need also be considered.

Barriers to using TG mouse models of human mature B-cell lymphomas and PCN more widely are several and include the following:

1. *Problems related to establishment and maintenance of mouse colony.* Differences in genetic background of mice require costly and time-consuming backcrosses of individual TG from donor strains to desired recipient strains. In addition, differences in the microbial floras of mouse colonies frequently results in quarantine and monitoring of microbiological features and health status frequently resulting in rederivation of strains under specific pathogen-free (SPF) conditions in the recipient institution.
2. *Logistical problems.* There are limited financial and space resources for scaling up mouse breeding, husbandry, and genotyping to perform statistically robust

time course analyses of tumor development and drug testing. In addition, there are intellectual property issues that seriously affect time, cost, and mechanisms to negotiate and acquire mouse strains. The complexities of multiparty agreements and differing priorities of academic and commercial outfits lend an additional layer of difficulties. Finally, we are faced with problems related to data acquisition and analysis that include, as one example, a paucity of methods for high-throughput biomedical imaging and high-throughput microscopy.

## 8.2 Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

### 8.2.1 *CLL/Small Lymphocytic Lymphoma in Humans*

CLL and SLL are clonal, accumulative diseases of monomorphic, small, round, slowly proliferating CD5<sup>+</sup> B cells in peripheral blood, bone marrow, and lymph nodes admixed with smaller numbers of prolymphocytes and para-immunoblasts. Lymph nodes exhibit a pseudofollicular pattern. The term small lymphocytic lymphoma (SLL) is reserved for non-leukemic cases with similar tissue involvement and cellular phenotype. SLL is not considered to be the precursor to CLL. Rather, tissue involvement is thought to be almost always secondary to an established leukemia. Transformation to clonally related high-grade lymphoma, usually DLBCL, termed Richter syndrome, occurs in about 5% of cases (Jaffe, Harris et al. 2001). Interestingly, CLL may be preceded by a condition, tentatively designated benign monoclonal lymphocytosis (Victor Hoffbrand and Hamblin 2007), that may bear a similar relationship to CLL as monoclonal gammopathy of undetermined significance (MGUS) does to multiple myeloma (MM) (Kyle, Therneau et al. 2002).

Abnormal karyotypes are found by FISH in ~80% of cases with deletions of 13q14 (50%) and 12q trisomy (20%) being among the most common; translocation t(14q32) occurs in 5% of cases (Dohner, Stilgenbauer et al. 2000). The 13q14 deletion encompasses the micro-RNA genes, miR-15a, and miR-16-1 (miR15/16) (Calin, Sevignani et al. 2004). Familial aggregations of CLL are not uncommon. CLL/SLL is usually considered to be incurable with current therapy with an overall 5-year survival rate of around 50%. Molecular and phenotypic characterizations, however, have allowed the definition of subsets with clinically indolent and aggressive forms of the disease (Table 8.1). These are based on studies of IgV regions, cytogenetics, cell surface phenotype, and gene expression. Of particular interest is the observation that cases with mutated IgV genes and particular biases in V gene family utilization, such as V1-69, have a much more favorable course than cases with unmutated IgV genes and biased utilization of a separate set of V regions, such as V4-34 (Chiorazzi and Ferrarini 2003). This suggests that the ability of BCRs to bind specific antigens might drive

**Table 8.1** Features of indolent and aggressive chronic lymphocytic leukemia (CLL)

Variable	Course of disease		
	Indolent	Aggressive	Ref.
IgV regions	Mutated	Not mutated	Chiorazzi and Ferrarini (2003)
IgV preference	V1–69	V4–34	Chiorazzi and Ferrarini (2003)
Chr 11, Chr 17 aberrations	Less common	More common	Dohner, Stilgenbauer et al. (2000)
Chr 13q14 deletion	More common	Less common	Dohner, Stilgenbauer et al. (2000)
CD38 expression	Low	High	Damle, Wasil et al. (1999)
BCL2 expression	Low	High	Faderl, Keating et al. (2002)
ZAP70 expression	Low	High	Crespo, Bosch et al. (2003)
Serum thymidine kinase	Low	High	Hallek, Langenmayer et al. (1999)
Serum CD23	Low	High	Sarfati, Chevret et al. (1996)

expressing cells from a state of normality to the leukemic state. Studies of antigenic specificity have shown these BCRs to be poly- and autoreactive.

### 8.2.2 *Mouse Models of CLL/SLL*

#### 8.2.2.1 Spontaneous Small B-Cell Lymphoma/Leukemia in Mice

Old mice of some strains spontaneously develop a clonal, mature B-cell disease termed SBL with many features reminiscent of CLL in humans (Fredrickson and Harris 2000; Hartley, Chattopadhyay et al. 2000). A predominant population of small lymphocytes with low mitotic activity associated with prolymphocytes and proliferation centers originates in the spleen. Advanced cases may exhibit involvement of lymph nodes, liver, and kidneys. A leukemic phase occurs in about 25% of cases and conversion to high-grade immunoblastic (IBL) lymphoma (Richter syndrome) is sometimes seen (Fredrickson and Harris 2000; Morse, Anver et al. 2002). The few cases that were phenotyped by flow cytometry were CD5<sup>lo</sup>. There is no information on V<sub>H</sub>DJ<sub>H</sub> repertoire for these mice.

#### 8.2.2.2 Models of CLL Based on Studies of New Zealand Mice

The first associations relating CD5 expression on B cells, neoplasia and NZB mice, were made in 1981 with the finding that cultured B-lymphoma lines, including several from (BALB/c × NZB)F1 mice, expressed low levels of “Lyt-1” (Lanier, Warner et al. 1981). Later studies demonstrated that a subset of normal spleen cells expressed CD5 with spleens of NZB and (NZB × NZW)F1 mice having the highest frequencies (Manohar, Brown et al. 1982; Hayakawa, Hardy et al. 1983). CD5<sup>+</sup> B cells, now termed B1a cells, were later found to comprise a high proportion of peritoneal B cells and to share a distinctive IgM<sup>hi</sup>CD11b<sup>+</sup>

phenotype with another subset of functionally similar peritoneal B cells, now designated B1b B cells. B1b cells are not found in spleen, and splenic B1a cells do not express CD11b. Both subsets of peritoneal B1 cells derive from fetal/neonatal progenitors that are distinct from the B-cell progenitors in adult bone marrow that give rise to follicular and MZ B2 cells (Hardy 2006).

In spite of their differing origins, B1 cells share many features with MZ B cells including the ability to act as front-line responders to invading pathogens from the gut or circulating in the blood (Martin and Kearney 2000, 2001). The repertoires of both B1 and MZ B cells are also enriched for poly- or autoreactive antibody specificities. Importantly, the splenomegaly of aging NZB was shown in some cases to be the result of MZ enlargement, and NZB is the strain in which splenic MZL was first described (Yumoto 1980).

CLL cells were first shown to express CD5 in 1980 (Boumsell, Coppin et al. 1980). The fact that NZB mice were known to have high levels of normal splenic CD5<sup>+</sup> B cells prompted studies of the possible relationship of these cells to NZB lymphomas (East 1970). Soon, several laboratories reported the identification of clonal populations of CD5<sup>+</sup> B cells in aging NZB mice and showed that they were readily transplanted. In some mice, these lymphomas were associated with leukemic phases. This suggested that the CD5<sup>+</sup> lymphoma/leukemias of NZB mice might serve as a model for human CLL (Okada, Takiura et al. 1991; Phillips, Mehta et al. 1992; Stall, Farinas et al. 1988).

The clonal B-cell populations were found to develop in a characteristic fashion, first appearing in the peritoneal cavity in mice 2–3 months of age; these peritoneal populations were usually oligoclonal. This was followed by successive spread to the spleen at 3–5 months, peripheral blood at 5–7 months, lymph nodes at 7–10 months, and bone marrow at 10–13 months (Stall, Farinas et al. 1988). The clones identified in spleens or lymph nodes were most often present among the several clones found in the peritoneum of the same animal. A similar pattern was seen in and NZW and (NZB × NZW)F1 mice (Stall, Farinas et al., 1988). Studies of congenic NZB and NZW and F1 mice showed that animals homozygous for *H-2<sup>Z</sup>* had the highest frequencies of peritoneal CD5<sup>+</sup> B cells (Okada, Takiura et al. 1991). Other studies of NZB mice and progeny of crosses with DBA/2 demonstrated that the CD5<sup>+</sup> B cells accumulating in the spleens of aging mice were hyperdiploid and readily transplantable (Raveche, Lalor et al. 1988). Interestingly, this group did not identify hyperdiploid cells in the peritoneum of these mice.

Changes in expression of the cytokines IL-5 and IL-10 were found to markedly affect the occurrence of CD5<sup>+</sup> lymphomas in NZB and (NZB × NZW)F1 mice. Studies of NZB mice homozygous for a null mutation of IL-10 showed that expansion of CD5<sup>+</sup> B cell outside the peritoneum and the development of clonal lymphomas was markedly reduced (Czarneski, Lin et al. 2004). In addition, (NZB × NZW)F1 mice overexpressing IL-5 from a transgene had greatly increased populations of CD5<sup>+</sup> B cells and were at increased risk for lymphoma development (Xiangshu Wen et al. 2004).

Two studies have examined the genetic basis for the lymphoma susceptibility of New Zealand mice. Analyses of backcross progeny from the crosses of (NZW × B10.NZW)F1 × B10.NZW indicated that the frequency of CD5<sup>+</sup> B cells in peripheral blood was governed by three susceptibility alleles (Hamano, Hirose et al. 1998). The first, *Bpal-1*, was closely linked to the MHC on Chr 17. This was consistent with earlier studies associating homozygosity for H-2<sup>z</sup> and CD5<sup>+</sup> B-cell frequency (Okada, Takiura et al. 1991). The second, *Bpal-2*, was located toward the centromeric end of Chr 13, and the third, *Bpal-3*, was close to the centromere on Chr 17 but not linked to the MHC (Hamano, Hirose et al. 1998). The development of leukemia in older mice was associated with elevated levels of CD5<sup>+</sup> B cells in blood at an early age. Each allele functioned independently and in an incompletely dominant fashion.

A second study examined mice from the cross (NZB × DBA/2)F1 × DBA/2, a low lymphoma strain (Raveche, Salerno et al. 2007). Lymphoproliferative disease occurred in 37% of the mice. Histologically, 94% of these cases were diagnosed as splenic MZL. Occurrence of disease was linked to three loci on chromosomes 14, 18, and 19. Sequence studies of Chr 14 identified a single base polymorphism 6 bp downstream of the pre-miR-16-1 sequence. This was associated with reduced expression of miR16 in NZB lymphoid cells and a NZB B-lymphoma cell line. Further studies suggested that miR-16-1 normally functions in B cells to retard cell cycle progression and promote apoptosis. These results were very suggestive of an important tie between the lymphomas of NZB mice and human CLL as the miR15/16 locus is frequently deleted in the malignant B cells of CLL.

The IgH V region sequences of lymphomas occurring in New Zealand mice were examined for three cases (Mahboudi, Phillips et al. 1992). All lymphomas expressed unmutated V genes and DFL16.1, a D region gene frequently used by fetal B cells. No N additions were seen. This pattern resembles that of the normal fetal/neonatal B-cell repertoire (Feeney 1990).

Taken together, these studies indicate that the peritoneal and splenic CD5<sup>+</sup> B-cell populations of young New Zealand mice are considerably larger than those of most other strains. Over time, the peritoneal and splenic populations exhibit oligo- or monoclonal expansions of CD5<sup>+</sup> B cells that can spill into the blood as leukemia, seeding lymph nodes and other tissue but rarely affecting the bone marrow. For mice that have clonal populations in both spleen and peritoneum, the clones are sometimes common to the peritoneal and splenic B-cell populations, while in others they may be restricted to one site or the other (Stall, Farinas et al. 1988; Okada, Takiura et al. 1991). Numbers of CD5<sup>+</sup> B-cell numbers in young mice are tied to later development of lymphoma. CD5<sup>+</sup> B-cell numbers and lymphoma incidence are both under polygenic control but from genes that appear to differ between NZB and NZW. Little has been done to characterize Ig mutational state, but the few available sequences are very much like those of fetal/neonatal B cells.

Importantly, the lymphomas that develop in these mice have been diagnosed almost uniformly as splenic MZL. Pseudofollicles/proliferation centers,

prolymphocytes, and para-immunoblasts are not features of the lymphomas of New Zealand mice, marking them as histologically quite distinct from human CLL.

### 8.2.2.3 The *TCL1* TG Mouse Model of CLL

The *TCL1* oncogene was initially identified at Chr 14q32.1 as the gene commonly activated by translocations or inversions involving T-cell receptor loci (Fu, Virgilio et al. 1994). The development of T-cell lymphomas in mice with *TCL1* expression driven by the *Lck* promoter in T cells established *TCL1* as a true proto-oncogene (Virgilio, Lazzeri et al. 1998). *TCL1* is also expressed in normal B-lineage cells from the pre-B to follicular B-cell stages and is then downregulated in GC B cells and extinguished in memory and plasma cells (Said, Hoyer et al. 2001; Virgilio, Narducci et al. 1994). High levels of expression have also been seen in a variety of immature and mature B-cell lymphomas (Teitell, Damore et al. 1999; Narducci, Pescarmona et al. 2000; Teitell 2005). The oncogenic effect of *TCL1* is thought to be mediated, in part, by its interactions with AKT, enhancing its activation and stimulating downstream pathways that promote proliferation and survival (Teitell 2005).

In 2002, Bichi and her collaborators described features of mice bearing an E $\mu$ -*TCL1* TG (Bichi, Shinton et al. 2002) that included the development of late onset clonal CD5 $^{+}$  lymphomas/leukemias. The general picture of this disease is remarkably similar to that described by Stall et al. for NZB mice (Stall, Farinas et al. 1988). Beginning around 2 months of age, the mice exhibited an expanded population of CD5 $^{+}$ CD11b $^{+}$  B cells in the peritoneum that became evident in the spleen at 4–5 months and then the bone marrow at 8 months. Analyses of Ig gene rearrangements in mice older than 7 months showed that the expanded populations were clonal and that clonal markers were sometimes shared between the peritoneum and spleen. All mice older than 13 months developed marked splenomegaly, hepatomegaly, lymphadenopathy, and leukemia with a mean WBC of  $180 \times 10^6/\text{ml}$ .

The B-cell expansion in mice younger than 7 months was polyclonal with a repertoire that was like that of normal CD5 $^{+}$  B cells, including the recurrent use of specific V<sub>H</sub>V<sub>L</sub> combinations (Bichi, Shinton et al. 2002). The V<sub>H</sub>11 sequences examined were essentially unmutated with only low levels of N-region additions, similar to V<sub>H</sub> regions that characterize normal CD5 $^{+}$  and fetal B cells to varying extents (Feeney 1990; Li, Hayakawa et al. 1993; Kantor, Merrill et al. 1997).

Histologic studies of mice 8 months of age and older showed a progressive enlargement of the splenic MZ by cells cytologically indistinguishable from normal MZ B cells. The phenotype of the MZ B cells was atypical, however, as they were CD21 $^{\text{lo}}$ CD5 $^{\text{lo}}$  rather than the CD21 $^{\text{hi}}$ CD5 $^{-}$  phenotype of normal MZ B cells. In older mice, these cells extended into the red pulp in a manner similar to that seen with MZL in NFS.V $^{+}$  mice, but with no cytologic progression toward high-grade disease. Even cells from leukemic mice were unchanged.

More detailed studies of Ig utilization by the malignancies of older mice (Yan, Albesiano et al. 2006) revealed several important points: (1) sequences of the expressed  $V_H$  and  $V_L$  genes were identical to or differed minimally from germline; (2) utilization of  $V_H$  families 1, 11, and 12, D segment families, and the  $J_H$  segments,  $J_H1$ , diverged from that of the normal B-cell repertoire; (3) the HCDR3 and LCDR3 regions of the clones tended to be longer than for normal adult B cells and many contained two or more charged amino acids; (3) the cases exhibited stereotypic  $V_HDJ_H$  rearrangements that resembled sequences reported previously for certain autoantibodies and antibodies reactive with microbial antigens; and (4) cloned expressed sequences that were poly- and autoreactive and bound to a variety of polysaccharides. Almost all these features, except for reactivity with non-protein antigens, are similar to those of Ig genes from aggressive, unmutated CLL.

Other studies have forwarded the E $\mu$ -TCL1 TG mouse as a tool for pre-clinical drug testing for human CLL (Johnson, Lucas et al. 2006). Leukemic mice responded initially to treatment with standard drug used in CLL, fludarabine, but then became resistant, similar to human CLL. Heightened expression of BCL2, MCL1, PDK1, and AKT1 in the transformed lymphocytes suggested that other potential drug targets were available. The importance of AKT signaling in this model was examined in studies of the effects of rapamycin, an mTOR inhibitor, on the survival of mice transplanted with expanded CD5 $^+$  B-cell populations from the TG mice. Treated mice began to die significantly later than untreated animals although all animals in both groups were dead by ~200 days after transplantation (Zanesi, Aqeilan et al. 2006).

#### 8.2.2.4 The BLC2/TRAFF Model of CLL—the NF $\kappa$ B Connection

CLL and other NHL are characterized by overexpression of BCL2, which contributes to an apoptosis-resistant phenotype. TRAF1 is also overexpressed in a spectrum of NHL and in CLL is associated with aggressive disease (Zapata, Krajewska et al. 2000). TG mice overexpressing BCL2 or a dominant negative form of TRAF2 (TRAF2DN), which mimics the signaling features of TRAF1, develop expanded populations of B cells with occasional BCL2 TG mice developing long-latency, low-grade lymphomas (Strasser, Harris et al. 1993). Mice doubly TG for BCL2 and TRAF2DN were found to die between 6 and 18 months of age with marked splenomegaly, lymphadenopathy infiltration of non-lymphoid tissues, and in many cases, ascites and pleural effusions (Zapata, Krajewska et al. 2004). Leukemias WBC counts around  $150 \times 10^6/\text{ml}$  were common. The mice were diagnosed histologically as having SBL with a leukemic phase, and analyses of Ig gene organization showed the disease to be clonal. The tumor cells were IgM $^{\text{hi}}$ CD23 $^-$ CD21 $^{\text{lo/-}}$ CD5 $^{\text{lo}}$ , consistent with an origin from B1a cells (Zapata, Krajewska et al. 2004). In addition, the cells were slowly proliferative and exhibited increased resistance to apoptosis due to effects of both BCL2 and TRAF2DN. These combined features were felt to be indicative of a CLL-like disease.

The use of double TG in preclinical drug studies was recently described (Kress, Martinez-Garcia et al. 2007). First, cells from double TG mice and humans with CLL were compared for their responses to treatment in vitro with synthetic triterpenoid derivatives. Both cell types were susceptible to induction of apoptosis. In addition, tests of the same drugs in double TG mice with leukemia resulted in marked decreases in WBC counts and reduced tissue burdens. These results were thought to support the testing of the drugs in patients with CLL.

A fascinating story that relates to that of the double TG mice comes from studies of mice bearing a mutant *NFkB2* gene (Zhang, Wang et al. 2007). Genomic alterations that result in truncation and constitutive activation of NFkB2 occur in a variety of human B- and T-cell malignancies (Zhang , Lombardi et al. 1994). TG mice developed marked splenomegaly, lymphadenopathy, and infiltration of non-lymphoid tissues and died with clonal B cell as well as some T-cell lymphomas between 5 and 18 months of age. The tumors were diagnosed histologically as SBL, but expression of CD5 was not evaluated and a leukemic phase was not described. The cells were non-proliferative and exhibited increased resistance to apoptosis. Interestingly, premalignant B cells and lymphoma exhibited significantly increased levels of TRAF1 and to a lesser extent TRAF2. TRAF1 was shown to be a direct transcriptional target of mutant NFkB2. Remarkably, TRAF1-deficient TG mice did not develop lymphoproliferation or lymphomas. These findings, together with the studies of the double TG mice, suggest a common pathway to development of SBL in mice.

### **8.2.2.5 APRIL and CLL?**

APRIL (a proliferation inducing TNF ligand), also known as TNFSF13, is a secreted member of the TNF superfamily expressed by normal T cells, neutrophils, and dendritic cells as well as by a variety of cancers. APRIL binds to two receptors, BCMA and TACI, on the surface of B-lineage cells and is known to influence plasma cell survival, Ig switching, and the function of B1 B cells (Cancro 2004; Schneider 2005). Previous studies of human NHL showed that APRIL was expressed in association with high-grade DLBCL and Burkitt lymphoma but not with low-grade NHL including mantle cell and MZ lymphoma or CLL (Schwaller, Schneider et al. 2007). In contrast, Planelles, Carvalho-Pinto et al. (2004) found APRIL transcripts to be associated with nearly 50% of the CLL cases tested and demonstrated elevated levels of APRIL in serum from these patients. To investigate a role for APRIL in CLL, they generated TG mice with APRIL expressed from T cells.

Studies of APRIL TG mice older than 9 months revealed an expanded population of peritoneal B1a cells associated with enlargement of the mesenteric LN and Peyer's patches in about 40% of mice and splenomegaly and extralymphoid spread in fewer. The cells were non-proliferative but exhibited increased resistance to apoptosis. Comparisons with non-TG mice suggested that APRIL accelerates the expansion of peritoneal B1a cells seen in NZB and other strains.

Unfortunately, no studies were done to evaluate clonality, the mice were not identified as being leukemic, and none were said to have died of their disease.

#### 8.2.2.6 Conclusions Regarding Mouse Models of CLL

As noted above, SBL, an uncommon spontaneous disease of old mice has many cytologic and histologic features in common with CLL. For mice suggested as models for CLL, some cases with histologic features of SBL are among those seen in BCL2/TRAF2DN mice (Zapata, Krajewska et al. 2004) but other lymphoma classes also develop in these mice, and the occurrence of ascites and pleural effusions is unusual. The description of the disease of mice carrying a mutant NF $\kappa$ B TG is like that of SBL, but the cells were not phenotyped or shown to be clonal. The diseases of NZB and TCL1 TG mice are histologically and cytologically quite distinct from SBL.

Many features of the malignant B-cell diseases of New Zealand mice and TCL1 TG mice are remarkably similar. They originate among oligoclonal populations of B1a cells in the peritoneum and progressively spread to spleen, peripheral blood, lymph nodes, and bone marrow. In the spleen, the histologic appearance is of MZL, but several features weigh against their origin from normal MZ B cells. First, the surface phenotype—CD5<sup>lo</sup>CD21<sup>lo/-</sup>—is not that of MZ B cells (Martin, Oliver et al. 2001; Bichi, Shinton et al. 2002). Second, the “MZL” often does not show the cytologic progression to high-grade disease seen in NFS.V<sup>+</sup> congenic mice. Instead, the cytology of cells in spleen, nodes, and even the blood of leukemic mice may differ little from those of mice with early expansion of the MZ. Third, MZL of NFS.V<sup>+</sup> almost never extend beyond the spleen. Finally, the V<sub>H</sub>DJ<sub>H</sub> sequences of the lymphomas are closer to those of normal B1a cells than to normal MZ B cells.

The process of peritoneal B1a B-cell transformation and subsequent spread may be accelerated by constitutive expression of TCL1, of the TRAF1 mimic, TRAF2DN, plus BCL2, and possibly of the mutant NF $\kappa$ B2 upstream of TRAF1 since CLL features heightened expression of TCL1, BCL2, and TRAF1. In this regard, it would be of interest to cross the TCL1 TG or mutant NF $\kappa$ B2 mice with NZB. The possible contributions of genetically determined changes in miR15/16 require confirmation but could relate the NZB disease to human CLL in yet another way. Caution is suggested by the understanding that NZW mice are like NZB in the development of clonal B1 populations but do not share the miR16 polymorphism with NZB.

The normal B-cell counterpart of the leukemic cell in CLL is not known but is clearly an issue of significant import. The candidates under consideration include resident or recirculating CD5<sup>+</sup> mantle zone B cells, MZ B cells, and lastly, the unidentified human equivalent of mouse B1a cells. Mantle cells seem unlikely as they exhibit little auto- or polyreactivity (Herve, Xu et al. 2005) while MZ and B1a cells of mice have this as a prominent feature (Martin, Oliver et al. 2001). A rationale for choosing between these cell subsets for one most like CLL is provided by extensive studies of V<sub>H</sub>DJ<sub>H</sub> sequences of purified peritoneal B1 and MZ B cells

(Kantor, Merrill et al. 1997; Schelonka, Tanner et al. 2007). Although the conclusions are based solely on analyses of the V<sub>H</sub>7183 family, the CDR3s of MZ B cells were considerably shorter than for other B-cell subsets in spleen and bone marrow, D<sub>H</sub> usage was biased toward DFL3, and J<sub>H</sub> usage toward J<sub>H</sub>2, N-region additions were fewer than for other B-cell subsets, and they had an increased proportion with charged amino acids. In contrast, B1a sequences revealed repertoires biased toward V<sub>H</sub>1, V<sub>H</sub>11 and V<sub>H</sub>12, DSP D<sub>H</sub>, and J<sub>H</sub>1 with fewer N-region additions than B1b or conventional B cells. In addition, CDR3 lengths were similar or slightly greater than those for B1b and B2 cells. These observations support a derivation of TCL1 lymphoma/leukemia from peritoneal B1a cells with the greatest discrepancy being the near-germline sequences of the clonal TCL1 TG populations. Human CLL might well derive from a parallel population of B1a cells. Efforts to develop an accelerated model of the NZB or TCL1 TG-based diseases would provide a superior preclinical model of CLL.

## 8.3 Marginal Zone Lymphomas

### 8.3.1 MZL in Humans

There are three general categories of MZL in humans: extranodal MZL of MALT lymphoma, nodal MZL, and splenic MZL (Jaffe, Harris et al. 2001). MALT and nodal MZL are characterized by accumulations of a heterogeneous population of small B cells infiltrating the marginal zones of reactive B-cell follicles. These centrocyte-like cells can be associated with occasional centroblasts and immunoblasts, and plasmacytoid differentiation is seen in some cases. In MALT lymphomas, the malignant cells characteristically infiltrate the epithelium-forming lymphoepithelial structures. Nodal MZL morphologically resembles lymph node infiltration by MALT but there is no evidence of extranodal involvement. Patients with MALT often have a history of autoimmune conditions, including Hashimoto's thyroiditis and Sjogren's syndrome, or inflammatory conditions, such as *Helicobacter pylori*-associated chronic gastritis or ocular infections with *Chlamydia psittaci*.

Splenic MZL is a rare disease in which small lymphocytes replace splenic white pulp GCs, infiltrate the surrounding marginal zones, and expand into the red pulp. Extension to splenic nodes and the bone marrow is common but involvement of peripheral nodes is not. These tumors may account for a high proportion of CD5<sup>-</sup> chronic lymphoid leukemias, sometimes featuring villous lymphocytes.

Two genes implicated in the development of MALT, *MALT1* and *BCL10*, were originally identified because of their involvement in recurring chromosomal translocations—t(11;18)(q21;q21) and t(14;18)(q32;q21) for *MALT1* and t(1;14)(p22;q32) for *BCL10*—that occur specifically in MALT lymphomas. In normal B cells, *MALT1* and *BCL10* associate with CARMA1 downstream of the BCR to activate NFkB (Thome 2004).

### 8.3.2 Mouse Models of MZL

#### 8.3.2.1 Spontaneous MZL in Mice

Splenic MZL is the only type of MZL that occurs spontaneously in mice (Fredrickson, Lennert et al. 1999). The disease, best characterized in NFS.V<sup>+</sup> mice more than a year of age (Hartley, Chattopadhyay et al. 2000), is a clonal disorder that initiates with expansion of the MZ by cells cytologically indistinguishable from normal MZ B cells and with almost no mitotic activity. Over time, these cells begin to finger into the red pulp and exhibit a more open chromatin pattern with more prominent nucleoli, and mitotic figures are more readily seen. This can progress to a high-grade lymphoma with a high mitotic index and cells cytologically indistinguishable from those of DLBCL or centroblastic (CBL) type. The lymphoma cells compress the white pulp and force out red pulp elements (Fredrickson, Lennert et al. 1999). The disease is almost always confined to the spleen but occasionally spreads to the splenic node and the liver. A leukemia phase is rare. FACS analyses have shown that the lymphomas cells are CD5<sup>lo</sup>IgM<sup>hi</sup>B220<sup>lo</sup> in the majority of cases.

Studies of NFS.V<sup>+</sup> MZL for somatically acquired proviral insertions of ecotropic MuLV identified a series of common integration sites (CIS) previously identified as candidate cancer genes (Shin, Fredrickson et al. 2004). Seven new CIS unique to MZL were also found including *Gfi1*, *Sox4*, and *Stat6* among others. Heightened expression of *Gfi1* distinguished MZL from other classes of B-cell lymphoma and was characteristic of MZL at all stages of progression suggesting a role in disease initiation.

#### 8.3.2.2 Mouse Model of MALT

Mice infected with *Helicobacter felis* for 22 months or more developed a chronic gastritis associated with the development of lymphoid follicles, the appearance of lymphoepithelial lesions, and glandular destruction (Enno, O'Rourke et al. 1995). The later development of lymphoma was shown to be antigen-dependent since the incidence and severity of disease was significantly reduced in infected mice given anti-microbial therapy (Enno, O'Rourke et al. 1998). In addition, mice immunized against *H. felis* were protected from development of lymphoma (Sutton, O'Rourke et al. 2004). None of the lymphoid lesions were tested for clonality of the expanded B-cell populations, and upregulation of MALT or BCL10 was not described in reports of expression profiling (Mueller, O'Rourke et al. 2003).

#### 8.3.2.3 Mouse Models of Splenic MZL

*Aire*-deficient mice replicate autoimmune features of patients with autoimmune polyendocrine syndrome type I, an inherited autosomal recessive disorder associated with progressive immune destruction of many tissues (Anderson,

Venanzi et al. 2002; Ramsey, Winqvist et al. 2002). More recent studies showed that mutant mice 15–24 months of age exhibited expansion of the MZ (Hassler, Ramsey et al. 2006). Analyses of IgH D–J rearrangements revealed an oligoclonal pattern suggestive of early MZL. Interestingly, the cells populating the MZ were CD21<sup>lo</sup> and secreted autoantibodies on transfer, consistent with an activated phenotype. Unfortunately, expression of CD5 was not examined. These studies suggest a role for *Aire* as a tumor suppressor gene and stimulation with autoantigens as possibly contributory to development of MZL.

Interestingly, recent studies of mice with TG-induced expression of BCL10 in B cells were found to have significantly expanded populations of splenic MZ B cells, nuclear BCL10, and constitutive activation of the canonical NFkB signaling pathway (Stephen Morris, H. Morse, unpublished observations). Mice older than 18 months have started to develop lymphomas not seen in control littermates.

### 8.3.2.4 Conclusions Regarding Mouse Models of MZL

Spontaneous splenic MZL in mice has many similarities to splenic MZL in humans, but the latency for disease of greater than a year, the occurrence with other classes of B-cell lymphoma in NFS.V<sup>+</sup> mice, and the lack of demonstrable involvement of BCL10 or MALT1 make the model impractical for preclinical studies. The *Aire*-deficient mouse model of splenic MZL also suffers from long latency and low penetrance. *Helicobacter*-associated gastric lesions similar to those of gastric MALT never appears to evolve to clonal disease but appears to be useful for understanding the role of antigenic drive in early disease. The fact that there is no unequivocal evidence in mice for MZ B cells other than those in spleen may be responsible for the lack of models for nodal MZL. Mouse disorders with similarities to Sjogren's syndrome or Hashimoto's thyroiditis exhibit B-cell infiltrates of affected tissues but have never shown progression to clonal disease.

## 8.4 Mantle Cell Lymphoma

### 8.4.1 MCL in Humans

Human MCL is a mature B-cell neoplasm of small to medium-sized lymphocytes with irregular/cleaved nuclear contours that resemble centrocytes. Synonyms are morphologically descriptive and include intermediate or poorly differentiated lymphocytic lymphoma-diffuse or nodular type centrocytic (mantle cell) lymphoma, and malignant lymphoma diffuses small cleaved cell type. Human MCL typically involves lymph nodes and less frequently the spleen, bone marrow, and GI tract and is an intermediate to aggressive, usually incurable lesion with large cell blastoid variants of ominous prognosis

(Jaffe, Harris et al. 2001d). Until recently, there were no spontaneous or genetic mouse models of MCL, precluding development of a MMHCC classification although two MCL xenotransplant models have been reported (Bryant, Pham et al. 2000; M'Kacher, Farace et al. 2003). A t(11;14)(q13;32) between *IGH* and *CYCLIN D* loci is the hallmark aberration of human MCL, with dysregulated expression of the cyclin CCND1 protein (Williams, Westermann et al. 1990; Williams, Swerdlow et al. 1993). However, *E $\mu$ -cyclin D1* TG mice have usual B-cell development and fail to develop tumors (Bodrug, Warner et al. 1994), indicating that cyclin D1 dysregulation may be necessary but not sufficient for developing a mouse model of MCL. By FACS or IHC, most human MCL are IgM<sup>+</sup>IgD<sup>+-</sup>CCND1<sup>+</sup>BCL-2<sup>+</sup>CD10<sup>-</sup>BCL6<sup>-</sup>CD23<sup>-</sup>CD43<sup>+</sup>FMC-7<sup>+</sup> (Jaffe 2001). The cell(s) of origin are unknown although most cases show unmutated *IG* genes, suggesting a naïve or extrafollicular precursor B-cell type.

### **8.4.2 Mouse Models of MCL**

#### **8.4.2.1 MCL in Genetically Engineered Mice**

Intraperitoneal injection of the tumor promoter pristane (2,6,10,14-tetramethyl-pentadecane) for 3 months into *E $\mu$ -CCND1* TG mice >9 months of age resulted in a diffusely infiltrative, clonal, IgM<sup>+</sup>CD5<sup>+</sup>CD20<sup>+</sup>CD23<sup>-</sup> B-cell lymphoma expressing the cyclin D1 transgene with intermediately sized, cleaved B cells reminiscent of MCL (Smith, Joshi et al. 2006) (Table 8.2). CD5 may represent an activation rather than differentiation marker in mouse B-cell tumors, and additional studies of tumor transplantability, aggression, and cell of origin are required to determine this model's resemblance to human MCL. Crossing *E $\mu$ -IL-14 $\alpha$*  with *E $\mu$ -Myc* TG mice results in a disseminated, blastoid variant of MCL (MCL-BV) in almost 100% of mice by 3–4 months of age (Ford, Shen et al. 2007). Tumor cells are transplantable into SCID mice and show sIgM<sup>+</sup>CD5<sup>+</sup>CD19<sup>+</sup>CD21<sup>-</sup>CD23<sup>-</sup> by flow cytometry, with increased expression of endogenous CCND1, BCL2, ATM, RelA, and NF- $\kappa$ B2, and clonal Ig gene rearrangements, providing several molecular features that are observed in human MCL. However, the histology of these tumors is that of diffuse high-grade blastic B-cell lymphoma, frequently seen as a spontaneous disease in many strains of mice as well as in some genetically engineered strains (see below).

## **8.5 Follicular B-Cell Lymphoma**

### **8.5.1 FBL in Humans**

Follicular lymphoma in humans (FBL in mice) is mature B-cell lymphoma of GC origin comprised of a mixture of centrocytes and centroblasts with at least a partially follicular pattern (Jaffe, Harris et al. 2001). The cells are embedded in a

**Table 8.2.** Mouse models of human germinal center (GC) tumors<sup>1</sup>

Mode of tumor development	Tissue site of tumor development	GC B-cell tumor type	Mouse strain	Molecular alteration	Comments/other tumors	References
Transfer of human MCL	Peritoneal cavity	MCL	SCID	None	Xenotransplant of leukemic phase MCL cells	Bryant, Pham et al. (2000)
Transfer of human MCL	Lymphoid system	MCL [BV?]	NOD/SCID	None	Xenotransplant of peritoneal MCL cells	M'Kachner, Farace et al. (2003)
de novo	Peritoneal cavity, lymphoid system, metastases	MCL	C57BL/6	<i>E<sub>μ</sub>-cyclin D1 TG</i>	Age dependent (>9-months), dependent on peritoneal inflammation (pristane)	Smith, Joshi et al. (2006)
de novo	Lymphoid system, metastases	MCL-BV	C57BL/6	<i>E<sub>μ</sub>-IL-14α X, E<sub>μ</sub>-Myc DTG</i>	Initial leukemia phase with blasts, lymphoma by 3–4 months in 100% DTG mice	Ford, Shen et al. (2007)
de novo	Lymphoid system, metastases	FBL, DLBCL	C57BL/6	<i>VavP-Bcl2 TG</i>	Other tumors include PCT, LBL, HS	Egle, Harris et al. (2004)
de novo	Lymphoid system, metastases	FBL, DLBCL-CBL	C57BL/6	<i>E<sub>μ</sub>-Pim1 TG</i>	Other tumors include pre-T-LBL, HS	van Lohuizen, Verbeek et al. (1989) and Repacholi, Basten et al. (1997)
de novo	Lymphoid system, metastases	FBL, DLBCL	C57BL/6	<i>gMCL1 TG</i>		Zhou, Levy et al. (2001)

Table 8.2. (continued)

Mode of tumor development	Tissue site of tumor development	GC B-cell tumor type	Mouse strain	Molecular alteration	Comments/other tumors	References
de novo	Lymphoid system, metastases	FBL, DLBCL, DBLL	C57BL/6 × C3H	$E_{\mu}\text{-}B29\text{-}TCL1$ TG	Other tumors include MZL, T-PLL	Hoyer, French et al. (2002); Shen, Ferguson et al. (2006); and Dawson, Hong et al. (2007)
de novo	Lymphoid system, metastases	FBL	C57BL/6 × FVB	$MLL\text{-}AF4$ knock-in	Rare erythroid or myeloid leukemia	Chen, Li et al. (2006)
de novo	Lymphoid system, metastases	FBL	C57BL/6	$Ingl$ knock-out		Kichina, Zerenski et al. (2006)
de novo	Lymphoid system, metastases	DLBCL- CBL	C57BL/6 × 129Sv	$Rizl$ knock-out	Diverse non-lymphoid tumors	Steele-Perkins, Fang et al. (2001)
de novo	Lymphoid system	FBL, DLBCL -CBL	BALB/c	$H2L^d\text{-}J16$ TG	Occasional $IgH/Myc$ gene rearrangements, co-existent PCT	Kovalchuk, Kim et al. (2002)
de novo	Lymphoid system, occasional metastases	DLBCL	C57BL/6 or 129/ SvJ	$Bad$ knock-out		Ranger, Zha et al. (2003)
de novo	Spleen, occasional lymph nodes	DLBCL, FBL	C57BL/6	$Bcl6$ knock-in to $IgH$ locus	Trisomy 13 and 15 common	Cattoretti, Pasqualucci et al. (2005)

Table 8.2. (continued)

Mode of tumor development	Tissue site of tumor development	GC B-cell tumor type	Mouse strain	Molecular alteration	Comments/other tumors	References
de novo	Lymphoid system	DLBCL, DBLL, FBL	C57BL/6	$Myc$ knock-in $S'$ of $E\mu$ locus	Other tumors include LBL, PCT	Park, Kim et al. (2005) and Zhu, Qi et al. (2005)
de novo	Lymphoid system, variable	DLBCL -CBL	C57BL/6	$AF4$ invertor knock-in to $Mll$ locus		Metzler, Forster et al. (2006)

Mice are classified according to evolving criteria established initially by report of the Mouse Models of Human Cancer Consortium (MMHCC) study (Morse, Anver et al. 2002). Classifications in parentheses are not yet confirmed by MMHCC criteria or there are no MMHCC criteria yet developed for this entity. DBLL, diffuse high-grade blastic B cell lymphoma/leukemia; DLBCL, diffuse large B cell lymphoma; DTG, double transgenic; FBL, follicular B cell lymphoma; HS, histiocytic sarcoma; LBL, lymphoblastic lymphoma; MCL, mantle cell lymphoma; MCL-BV, mantle cell lymphoma-blastoïd variant; MZL, marginal zone lymphoma; PCT, plasmacytoma; SCID, severe-combined immunodeficiency; TG, transgenic; T-PLL/CLL, T-prolymphocytic leukemia.

dense network of follicular dendritic cells. The disease appears to originate in lymph nodes, but spleen bone marrow and occasional blood involvement are not uncommon. The cells are usually IgM<sup>+</sup>CD5<sup>-</sup> and, like normal GC B cells, express BCL6 and have mutated IgV region sequences. Almost all express BCL2, usually as the result of t(14;18)(q32;p21) translocations that bring the BCL2 gene under the control of IgL regulatory sequences (Tsujimoto, Finger et al. 1984; Hockenberry, Nunez et al. 1990). Morphologic transformation to aggressive DLBCL is common and is typically the cause of death. FBL makes up about 35% of adult NHL in the United States.

### **8.5.2 *Mouse Models of FBL***

#### **8.5.2.1 Spontaneous FBL in Mice**

FBL is a mature B-cell tumor characterized by a varying mixture of neoplastic centrocytes and centroblasts. Synonyms include follicular lymphoma, follicular center cell lymphoma mixed, CBL/centrocytic lymphoma, reticulum cell sarcoma type B, and lymphoma-pleomorphic. FBL is typically a low-grade lesion that resembles human follicular lymphoma and is the most frequent B-cell tumor of aging mice in many inbred strains, including NFS.V<sup>+</sup>, CFW, and AKXD RI strains (Morse, McCarty et al. 2003). Distinct from human follicular lymphoma, spontaneous FBL is not associated with *Bcl2* gene rearrangements (Morse, Anver et al. 2002). Splenomegaly and variable enlargements of mesenteric lymph nodes and Peyer's patches are typically seen. Histologic examination reveals white pulp expansions that appear as white nodules and coalesce with advancing disease. Centroblasts and centrocytes are the main cell types present, with small follicular B cells pushed to the periphery and the T-cell zone reduced or eliminated. Blast cells should be less than 50% to distinguish FBL from diffuse high-grade blastic B-cell lymphoma/leukemia (DLBCL or DBLL). By FACS, most cases are IgM<sup>+</sup>IgD<sup>-</sup>CD5<sup>dull</sup>CD45R(B220)<sup>lo/+</sup>. By IHC, centroblasts and centrocytes are both IgM<sup>+</sup>CD45R(B220)<sup>+</sup>CD19<sup>+</sup>. Tumor cells are mono- or oligoclonal for Ig gene rearrangements, with the presumed cells of origin being GC centrocytes and centroblasts.

#### **8.5.2.2 FBL in Genetically Engineered Mice**

Two independent lines of *Eμ-BCL2* TG mice develop follicular hyperplasia but rarely develop FBL and then with greatly prolonged latencies (McDonnell and Korsmeyer 1991; Strasser, Harris et al. 1993) (Table 8.2). In contrast, *VavP-Bcl2* TG mice that do not succumb to autoimmune disease develop follicular hyperplasia, followed by FBL at 10–18 months of age in up to 50% of mice. The disease is characterized by PCNA-positive, class-switched neoplastic B cells containing mutated IgV region genes (Egle, Harris et al. 2004). GC expansion and lymphomagenesis depended upon concurrent expansion of BCL2 transgene-expressing

CD4<sup>+</sup> T cells, suggesting that microenvironmental support was required for FBL development.

*Eμ-Pim1* TG mice develop pre-T-lymphoblastic lymphoma (pre-T-LBL) at 7–10 months of age. Mice unaffected by pre-T-LBL demonstrating probable FBL and multiple subtypes of DLBCL at older ages using pre-Bethesda nomenclature criteria (van Lohuizen, Verbeek et al. 1989; Repacholi, Basten et al. 1997). A construct using the flanking regulatory elements of human *MCL1* caused lymphoma in 65% of TG mice by 24 months, with about 20% of cases diagnosed as FBL under pre-Bethesda criteria (Zhou, Levy et al. 2001).

*Eμ-B29-TCL1* TG mice, encoding the AKT co-activator *TCL1* oncogene, develop a spectrum of mature GC and non-GC B-cell lymphomas, with relatively rare FBL and more common DLBCL and DBLL generation (Hoyer, French et al. 2002; Shen, Ferguson et al. 2006). Similar to *VavP-Bcl2* TG mice, *Eμ-B29-TCL1* TG mice require concurrent *TCL1*-mediated CD4<sup>+</sup> T-cell expansion to transform GC B cells, because mice with only B-lineage *TCL1* transgene expression develop mainly a model of the aggressive form of B-CLL (Bichi, Shinton et al. 2002).

Knock-in of a human *MLL-AF4* fusion gene into the mouse *Mll* locus produces predominantly FBL with clonal sIgM<sup>+</sup>B220<sup>+</sup>Pax5<sup>+</sup>Bcl6<sup>+</sup>CD19<sup>+</sup> tumor cells arising from follicular centers following a mixed myeloid/lymphoid hyperplasia (Chen, Li et al. 2006). *MLL-AF4* tumor cells metastasized widely without a leukemic phase and are transplantable. Knock-out of the *Ing1* gene, which encodes a nuclear PHD finger-containing protein not yet associated with human lymphoid malignancies, results in 20% of mice developing lymphoma. Tumors originate in the spleen and contain a mixed population of B220<sup>+</sup> cells that histologically resemble centroblasts and centrocytes to suggest FBL (Kichina, Zeremski et al. 2006). However, additional marker studies, evaluation of Ig mutation status, and clonality and transplantation studies are required to confirm this diagnosis and to exclude a robust follicular hyperplasia instead of malignancy (Kichina, Zeremski et al. 2006).

### 8.5.2.3 Conclusions Regarding Mouse Models of FBL

Spontaneous FBL in mice and many of the disorders of TG mice models have a number of histologic and particularly cytologic features in common with the human disease. However, the true follicular pattern seen in humans is absent in all these cases including those with extensive lymph node involvement. None of the models exhibit chromosomal translocations affecting the *Bcl2* locus, and the *VavP-Bcl2* TG is the only one with constitutive *Bcl2* expression. It may be important that the major breakpoint region (MBR) in the human *BCL2* locus bears is markedly different to the same general region in the mouse locus. These differences may preclude chromosomal rearrangements in the mouse. This possibility is being tested by knocking in 2 kb around the human MBR into the mouse *Bcl2* locus.

## 8.6 Diffuse Large B-Cell Lymphoma

### 8.6.1 *DLBCL in Humans*

DLBCL is characterized by a diffuse proliferation of large neoplastic B cells with nuclear size that exceeds that of normal histiocytes. A number of cytologic variants have been described including CBL, IBL, T-cell/histiocyte rich, plasmablastic, and anaplastic with CBL being the most common. Distinction among these variants suffers from poor interobserver reproducibility, and subsets have not been reliably tied to prognosis. Consequently, a designation simply as DLBCL is felt by pathologists to be most appropriate (Jaffe, Harris et al. 2001). The tumor cells express pan-B-cell markers and surface or cytoplasmic Ig, and about 10% are CD5<sup>+</sup>. Nuclear BCL6 is expressed in almost all cases, and IgV region genes are mutated. Gene expression profiling by one group using microarrays delineated two major subsets that are related to cell of origin as activated B-cell-like and GC B-cell-like (Alizadeh, Eisen et al. 2000). The distinctions have prognostic significance as the prognosis for patients in the GC B-cell-like subset is considerably better than that of patients with the activated B-cell type. However, array-based studies of DLBCL by another group did not reproduce these associations, defining instead three discreet subgroups designated “oxidative phosphorylation”, “B-cell receptor/proliferation,” and “host response” (Monti, Savage et al. 2005).

### 8.6.2 *Mouse Models of DLBCL*

#### 8.6.2.1 Spontaneous DLBCL in Mice

DLBCL is an aggressive mature B-cell malignancy that demonstrates a diffuse proliferation of tumor cells with large nuclei and distinct cytologic features. Characteristic DLBCL variants occur spontaneously in aging mice and are classified as CBL, IBL, histiocyte-associated (HA), and primary mediastinal (PM, thymic) subtypes (Morse, Anver et al. 2002). CBL, IBL, and HA variants are common in NFS.V<sup>+</sup> mice and usually arise with splenomegaly or lymphadenopathy (Hartley, Chattopadhyay et al. 2000), whereas PM shows mainly thymic enlargement and has been seen only in mice infected with a unique replication-defective retrovirus (Morse, Anver et al. 2002).

#### 8.6.2.2 Spontaneous Variants of DLBCL in Mice

##### DLBCL-CBL Variant

CBL synonyms include large cleaved follicular center cell lymphoma and CBL lymphoma. About 12% of spontaneous lymphomas in NFS.V<sup>+</sup> and 17% in CFW mice are CBL, whereas CBL was not detected in AKXD RI lymphomas

and is not common in other inbred strains (Morse, McCarty et al. 2003). Histologically, the splenic white pulp is greatly expanded by tumor cells with round nuclei, often with one or two prominent nucleoli, basophilic cytoplasm, and numerous mitoses. These cells are admixed with varying amounts of smaller centrocytes. A diagnosis is made when >70% of the cells are blasts. When the proportion of centrocytes to blasts ranges from 40–70% and the ratio varies in different microscopic fields, a distinction between DLBCL and FBL is difficult although CBL may more completely destroy the usual GC architecture than FBL at advanced stages. CBL frequently infiltrates the lung, liver, and kidney and, less frequently, the bone marrow. By FACS, most cases are IgM<sup>+</sup> or IgG<sup>+</sup>, B220<sup>+</sup>CD5<sup>low</sup>CD19<sup>+</sup>. By IHC, they are usually BCL6<sup>+</sup>PAX5<sup>+</sup>IRF8<sup>+</sup>PU.1<sup>+</sup>CD138<sup>-</sup>XBP1<sup>-</sup>Blimp1<sup>-</sup>. Tumors are clonal for *Ig* gene rearrangements, and oligonucleotide expression microarrays have shown no clear differences between follicular or diffuse CBL subtypes, suggesting that these subtypes may represent earlier and later stages of progression (Morse, unpublished results). By expression microarray analysis, CBL is readily distinguished from the CBL form of MZL and is similar to the CBL variant of human DLBCL.

### DLBCL–IBL Variant

IBL lymphoma is the synonym ascribed to IBL. About 8% of spontaneous lymphomas in NFS.V<sup>+</sup> and 4% in CFW mice are IBL, whereas IBL was not detected in AKXD RI lymphomas (Morse, McCarty et al. 2003). Histologically, IBLs are highly aggressive and demonstrate large, round nuclei having dispersed chromatin and prominent nucleoli, abundant cytoplasm, and a high mitotic rate. A “starry sky” pattern may be seen with increased apoptotic cells. Tumor cells are clonal for Ig gene rearrangements and often admixed with centroblasts and centrocytes, which may reflect an origin from a FBL or post-GC immunoblast. By FACS, IBLs typically are sIgM<sup>low</sup>B220<sup>dull</sup> and by IHC most cases are BCL6<sup>+</sup>PAX5<sup>+</sup>IRF8<sup>-</sup>PU.1<sup>-</sup>XBP1<sup>-</sup>IRF4<sup>-</sup>Blimp1<sup>-</sup>.

### DLBCL–HA variant

DLBCL–HA (histiocyte associated) is the acronym of HA variant. About 20% of spontaneous lymphomas in AKXD RI (and 1% in NFS.V<sup>+</sup>) strain mice are HA, and HA is not common in other frequently used inbred strains (Fredrickson and Harris 2000; Morse, McCarty et al. 2003). Histologically, all mice show splenomegaly with a marked expansion of histiocytes (macrophages) that may obscure malignant B cells. Histiocytes may occupy the entire white pulp, obliterating the PALS and destroying the usual follicular architecture, thereby pushing B cells to the periphery. Malignant B cells usually have features of FBL or CBL although rare cases show tumor cells with features seen in MZL, SBL, or IBL. Lymphadenopathy is seen in half the cases, and HA may involve the liver early on. The pattern of tumor growth is mainly nodular rather than

diffuse, and this lesion resembles human histiocyte/T-cell-rich DLBCL (Jaffe 2001). It may be difficult to distinguish DLBCL-HA from histiocytic sarcoma. By IHC, the histiocytes of HA are usually EMR1 ( $F4/80^+$ )LGALS (Mac-2) $^+$ , whereas the malignant B cells express markers consistent with their origin and are typically Pax5 $^+$ . A clonal Ig gene rearrangement with PAX5 $^+$  cells in a histologic picture dominated by histiocytes is diagnostic, with the main differential diagnosis being histiocytic sarcoma. The presumed cell of origin for the malignant B-cell component is usually a GC or post-GC B cell, whereas tissue macrophages comprise the non-malignant histiocytic component.

#### 8.6.2.3 DLBCL in Genetically Engineered Mice

DLBCL may arise de novo or by aggressive transformation of FBL and possibly SMZL. Ionizing radiation causes an increased frequency of tumors with histologic features of pre-T-LBL, FBL, and DLBCL-CBL in *Eμ-Pim-1* TG mice (Repacholi, Basten et al. 1997). Targeted deletion of *Riz1*, encoding a Rb-binding zinc finger protein, results in 37% of null and 19% of heterozygous mice developing clonal, B220 $^+$  B-cell lymphomas with histologic features of DLBCL-CBL by 18–22 months of age (Steele-Perkins, Fang et al. 2001) (Table 8.2). *H2-L<sup>d</sup>-Il6* TG mice develop PCT between 6 and 19 months of age, frequently with co-existing FBL or DLBCL-CBL (Kovalchuk, Kim et al. 2002). By IHC, the lymphomas are IgM $^+$ B220 $^+$ CD19 $^+$  and several contained t(12;15) IgH/Myc gene rearrangements.

*Eμ-B29-TCL1* TG mice develop clonal IgM $^+$ B220 $^+$ CD5 $^{low}$ BCL6 $^+$  DLBCL, most often HA and occasionally CBL or IBL subtypes with somatically mutated Ig genes and widespread dissemination (Hoyer, French et al. 2002). Equally frequent DBLL and rare FBL, SMZL, and T-PLL are also formed in this model (Hoyer, French et al. 2002; Dawson, Hong et al. 2007). B-cell lymphomas were eliminated by crossing the *TCL1* TG with an *OCA-B* null mouse that fare incapable of developing GC structures (Shen, Ferguson et al. 2006).

Knock-out of the proapoptotic BH3-only *Bad* gene results in 20% of mice developing clonal sIgM $^+$  or sIgG $^+$ , B220 $^+$ CD19 $^+$ CD43 $^-$ BCL6 $^+$  DLBCL of unclear subtype by 18–24 months of age (Ranger, Zha et al. 2003). Knock-in of the murine *Bcl6* gene into the *IgH* locus results in increased GC formation in spleens of non-immunized mice, followed successively by a benign lymphoproliferative disorder with expanded white pulp and then the development of DLBCL and FBL between 13 and 20 months of age (Cattoretti, Pasqualucci et al. 2005). Tumors were clonal and IgM $^+$ IgD $^+$ B220 $^+$ CD43 CD138 $^-$  with variable Mum1/IRF4 staining by IHC. They contained mutated *IgV* region genes and frequent trisomy of Chrs 13 and 15. More recent studies showed that mice bearing the *Bcl6* knock-in do not develop DLBCL when crossed onto an AID-deficient background (Pasqualucci and Dalla-Favera, unpublished observations)

Knock-in of the mouse *Myc* gene 5' of the *Eμ* intronic enhancer results in clonal IgM $^+$ B220 $^+$ CD19 $^+$ Bcl6 $^+$  DLBCL of unclear subtype developing between 6 and 21 months of age, along with FBL, DBLL, and PCT formation (Park, Kim

et al. 2005; Zhu, Qi et al. 2005). Using an “inverter” conditional knock-in strategy to bypass embryonic lethality, a Cre-generated *Mll–AF4* fusion gene in the endogenous *Mll* locus results in IgM<sup>+</sup>B220<sup>+</sup> CBL with clonal *Ig* gene rearrangements in 60% of cases (Metzler, Forster et al. 2006). Microarray profiling of tumor cells shows strong expression of *Pax5* and *Ebf* and variable expression of *Bcl2* and *Bcl6* differentiation markers (Metzler, Forster et al. 2006). The CBL tumor cells were transplantable into *Rag1*-deficient recipient mice.

#### 8.6.2.4 Conclusions Regarding Mouse Models of DLBCL

The last several years have been marked by striking progress in the generation and validation of mouse DLBCL as shown by studies of *Eμ-B29-TCL1* TG and *Bcl6* knock-in mice. The lymphomas of these mice share histologic features with human DLBCL, carry mutated IgV regions, and are strikingly dependent on normally functioning GC for their development. They provide novel and important in vivo settings for furthering our understanding of the roles played by TCL1 and BCL6 in normal B-cell biology and lymphomagenesis.

### 8.7 Diffuse High-Grade Blastic B-cell Lymphoma/Leukemia (DBLL) in Mice

The human equivalent or parallel to this disorder is currently not known. DBLL is a highly aggressive lymphoma of medium-sized B cells that exhibit a high mitotic rate, extensive apoptosis, and sometimes a leukemic phase. Synonyms include lymphoblastic lymphoma, Burkitt and Burkitt-like lymphoma, and DLBCL of lymphoblastic lymphoma subtype [DLBCL(LL)]. About 20% of spontaneous lymphomas in NFS.V<sup>+</sup> and ~30% in AKXD RI strains and CFW mice are DBLL, but DBLL is not common in other frequently used inbred strains (Morse, McCarty et al. 2003). Cases present with lymphadenopathy, variable involvement of the spleen, and sometimes thymus, with frequent non-hematopoietic organ dissemination. Affected tissues show uniform-appearing lymphoblasts with little cytoplasm, dispersed chromatin, and indistinct nucleoli. Histologic sections show many mitotic figures typically with large numbers of tingible body macrophages ingesting apoptotic cells, leading to a “starry sky” appearance. Infiltration of the deep cortex in lymph nodes progresses to replacement of normal cells and growth outside the capsule into the fat. When involved, the spleen shows diffuse infiltration of both the red and the white pulp. Perivascular and peribronchial infiltrates of the lungs and periportal liver infiltrates are common. Histologically and cytologically, these mature B-cell neoplasms are indistinguishable from precursor T-cell lymphoblastic lymphomas that lack thymic involvement and precursor B-cell lymphoblastic lymphomas. Analyses of *Ig* and *TCR* gene organization and IHC studies provide definitive distinctions among these disorders.

There is a spectrum of IHC phenotypes for DBLL ranging from patterns similar to immature or transitional B cells ( $\text{IgM}^+\text{IgD}^-\text{C1QR1(AA4.1)}^+$ ) to that of GC-experienced B cells that are *Ig* class-switched with *IgV* region mutations. DBLL are clonal for *Ig* gene rearrangements. Structural rearrangements of cellular genes, mostly due to proviral insertions, are seen from pooled studies of NFS.V<sup>+</sup> (Hartley, Chattopadhyay et al. 2000) and AKXD RI (Morse, Qi et al. 2001) lymphomas for *Zfp521* (*Evi3*) (11.9%), *Pim1* (5.6%), *Evi1* (4.8%), and *Myc* (0.8%). Lymphomas of  $\lambda$ -*MYC* were characterized by chromosomal instability and frequent biallelic deletions of *Cdkn2a* (p16) (Kovalchuk, Qi et al. 2000). Lymphomas of *E $\mu$ -Myc* TG mice had frequent changes in the p19<sup>ARF</sup>-MDM2-p53 tumor suppressor axis (Park, Kim et al. 2005). Immature or transitional B cells are the presumed cells of origin for *E $\mu$ -Myc*, *IgH/c-myc YAC*, *E $\mu$ IgH/c-myc YAC*, and  $\lambda$ -*MYC* TG mice. Probable GC or early post-GC B cells are the cells of origin for those with features similar to the DBLL of *E $\mu$ -B29-TCL1* TG mice.

### **8.7.1 DBLL in Genetically Engineered Mice**

An immature or transitional cell immunophenotype is characteristic of most lymphomas of *E $\mu$ -Myc*, *IgH/c-Myc YAC*, *E $\mu$ IgH/c-Myc YAC*, and  $\lambda$ -*MYC* TG mice. More mature immunophenotypes occur in spontaneous DBLL of NFS.V<sup>+</sup> mice and some lymphomas of *E $\mu$ -B29-TCL1* TG and *E $\mu$ -Myc* knock-in mice, along with many other genetically engineered mice. B-lineage lymphomas with lymphoblastic cytology but distinct from precursor B-lymphoblastic neoplasms are seen at low to high frequency in many strains of genetically engineered mice and a number of conventional inbred strains. The lymphomas of  $\lambda$ -*MYC* TG mice were originally designated Burkitt lymphoma (Morse, Anver et al. 2002), but *Ig* genes are not mutated and tumor cells have an immunophenotype of transitional or immature B cells indicating that they differ from human Burkitt cases. A change in nomenclature is clearly warranted. Mouse cases with similar histology and cytology occurring in mice other than the  $\lambda$ -*MYC* TGs were previously designated Burkitt-like (Morse, Anver et al. 2002). The findings that these tumors rarely have structural alterations in *Myc* and do not overexpress *Myc* distinguish them from human Burkitt-like lymphomas (Jaffe 2001).

## **8.8 Plasma Cell Neoplasms**

### **8.8.1 Human PCN Including Multiple Myeloma**

The evaluation of present mouse models of human PCN, and attempts to devise improved models, should be guided by insights into the natural history of PCN

development in human beings (Mitsiades, Mitsiades et al. 2007) and the biologic and molecular genetic features of frank, untreated PCN at the time of clinical presentation (Carrasco, Tonon et al. 2006). In analogy to cancer development in general (Hanahan and Weinberg 2000), human PCN including MM are thought to be initiated by somatic mutations in oncogenes and/or tumor suppressor genes, followed by the stepwise accumulation of genetic and epigenetic alterations that comprise tumor progression events (Bergsagel and Kuehl 2005; Kuehl and Bergsagel 2005). The later changes alter the phenotype of the incipient tumor cell as well as its interactions with the local microenvironment until fully malignant transformation has occurred. As the acronym suggests, PCN comprise a spectrum of malignancies that share the rather uniform histopathology of the aberrant, neoplastic plasma cell. However, despite their morphologic similarities, PCN demonstrate a great deal of diversity at the molecular level associated with major differences in epidemiology, clinical behavior, and treatment options.

The classification of PCN in the World Health Organization (WHO) nomenclature includes the following:

1. *Plasma cell myeloma.* This is a bone marrow-based PCN, usually multifocal, thus commonly referred to as MM. MM is incurable with a survival rate of ~40% at 5 years after diagnosis. MM is defined by monoclonal Ig protein (M spike) in serum, bone destruction, hypercalcemia, and anemia. The standard of care includes low- and high-dose chemotherapy, bone marrow transplantation, and novel drugs. Tumor variants include non-secretory myeloma (no serum monoclonal Ig ), indolent myeloma, smoldering myeloma, and plasma cell leukemia.
2. *PCT.* A solitary, localized, monoclonal PCN that grows either in bone (solitary bone PCT) or soft tissue (solitary extraosseous or extramedullary PCT). PCT is rare but curable with moderate-dose radiotherapy as the preferred treatment. The most common pattern of relapse is systemic, indicating progression to MM.
3. *Ig deposition diseases.* Primary amyloidosis and systemic IgL chain and IgH chain deposition diseases.
4. *Osteosclerotic myeloma (POEMS syndrome).* POEMS, defined by polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes is very rare.
5. *Heavy chain diseases.* Distinguished according to the isotype ( $\gamma$ ,  $\mu$ , and  $\alpha$ ) of the monoclonal IgH chain produced by neoplastic plasma cells.

Among human PCN, MM is by far the most important and well-studied disease. MM is a neoplasm of mature post-GC, Ig-secreting, isotype-switched plasma cells (Zojer, Ludwig et al. 2003) that accumulate in the bone marrow and cause bone destruction (Roodman 2006). Recent molecular and cytogenetic studies have shown that MM is remarkably a heterogeneous disease that can be divided into a number of distinct categories based on global gene expression profiles, detection of reciprocal chromosomal translocations

that recombine Ig loci with oncogenes, and ploidy status of tumor cells (Zhan, Hardin et al. 2002; Shaughnessy and Barlogie 2003). Depending on the criteria applied by different laboratories, it is possible to distinguish 5–8 subcategories of MM. Regardless of how these differences are resolved, the present molecular and cytogenetic subdivision of MM is already of clinical relevance because it predicts significant differences in the prognosis and response to therapy of MM patients, no matter whether treatment relies on standard, high-dose, or novel therapies (Mulligan, Mitsiades et al. 2007).

No definitive cause of MM has been identified. Genetic risk factors include gender (male > female), race (the incidence in African Americans in the United States is twice that of US whites), and age (median age at diagnosis is ~70 years). Familial clustering points to a hereditary predisposition consistent with an autosomal-dominant mode of inheritance, but tumor susceptibility alleles or “MM genes” have not been identified. Ionizing radiation is thought to be the strongest environmental risk factor for MM, but definitive studies have not been described. Although the evidence is not conclusive, there are reports of associations between MM and occupational exposure to various metals (nickel), chemical compounds (aromatic hydrocarbons, silicone, and petrochemical agents), pesticides and animal viruses (farming), protracted infections that can lead to sustained B-cell activation by microbial antigens (*H. pylori*, HHV8), acquired immunodeficiency syndromes, such as HIV/AIDS, that result in reduced immune surveillance by T cells, and autoimmune diseases, such as rheumatoid arthritis.

The neoplastic cell in MM appears to derive from an antigen-experienced isotype-switched post-GC B-lymphocyte that has undergone somatic hypermutation of the expressed *IgH* and *IgL* genes. Pathogenic factors implicated in MM include cytogenetic and molecular genetic alterations that result in the deregulated expression of oncogenes, such as *CCND1* (encoding cyclin D1), *FGFR3* (fibroblast growth factor receptor 3), and *WHSC1* (Wolf-Hirschhorn syndrome candidate 1; also known as *MMSET* or MM SET domain containing protein type III). The interaction of tumor cells with the bone marrow microenvironment is of crucial importance, as it leads to the production of cytokines including IL-6, IGF-1, VEGF, SDF-1 $\alpha$ , TNF- $\alpha$ , and TGF- $\beta$ .

The subgroups of MM are presently distinguished based on recurrent *IGH* translocations, ploidy status of tumor cells, and global gene expression patterns. Hyperdiploid tumors (40% of cases) contain 48–75 chromosomes and are characterized by multiple trisomies of Chr 3, 5, 7, 9, 11, 15, 19, and 21. Non-hyperdiploid tumors (nearly 50% of cases) carry one of seven recurrent chromosomal translocations that recombine *IGH* at 14q32 with seven different oncogenes. These translocations are thought to be very early if not the initiating oncogenic events and are caused by errors in Ig switch recombination or somatic hypermutation during the GC reaction. They fall into one of three groups.

1. D-type cyclins: *CCND1* (cyclin D1) at 11q13 in 15% of cases, *CCND2* (cyclin D2) at 12p13 in <15 of cases, and *CCND3* (cyclin D3) at 6p21 in 2% of cases.

2. MAF family genes: *MAF* (c-Maf) at 16q23 in 5% of cases, *MAFB* (Maf B) at 20q12 in 2% of cases, and *MAFA* (Maf A) at 8q24.3 in 1% of cases.
3. MMSET/FGFR3: *MMSET* (formally designated *WHSC1*) and *FGFR3* at 4p16 in 15% of cases.

Tumor progression events include chromosomal translocations that affect the *MYC* gene at 8q24 (15% of primary MM, 45% of advanced MM, and >90% of MM-derived cell lines) but do not involve aberrant isotype switching or somatic hypermutation and exhibit a similar prevalence in hyperdiploid and non-hyperdiploid tumors. They also include activating mutations of *NRAS*, *KRAS*, or *FGFR3*; amplifications of 1q; deletions of 13q and p53; constitutive activation of NF $\kappa$ B—e.g., via inactivation of TRAF3, constitutive overexpression of NIK (NF $\kappa$ B inducing kinase), or activation of NFKB2—perturbation of the RB pathway—e.g., via methylation of the p16<sup>INK4a</sup> promoter—and deletion of p18<sup>INK4c</sup>.

MM is preceded in a sizable fraction of cases by a premalignant disorder that is characterized by the abnormal persistence, sometimes for decades, of a clone of Ig-producing plasma cells that are lodged in the bone marrow without causing osteoporosis or osteolytic lesions. This disorder is referred to as MGUS (Rajkumar, Lacy et al. 2007). MGUS is defined by a monoclonal serum Ig of <30 g/l, the presence of 10% or fewer plasma cells in the bone marrow, the absence of anemia and lytic bone lesions, and the absence of hypercalcemia and renal insufficiency related to the clonal plasma cell proliferation. The prevalence of MGUS in elderly patients is ~5%. The progression from MGUS to MM occurs at a slow but remarkably steady rate of 1% per year. The etiology of MGUS is not known, but epidemiologic evidence points to age, gender, and race as risk factors. The pathogenesis of MGUS is poorly understood. Approximately 50% of MGUS cell clones carry chromosomal translocations that rearrange *IGH* at 14q32 with oncogenes on one of five partner chromosomes also identified in MM: *CCND1*, *CCND3*, *FGFR3* and *MMSET*, *MAF*, and *MAFB*. These translocations are thought to play an important role in the initiation of MM. Approximately 40% of the plasma cell clones in MGUS smoldering MM and frank MM are hyperdiploid. This consistency suggests that hyperdiploid MM originates from hyperdiploid MGUS. Likewise, deletions of Chr 13q, which have an adverse prognostic association in MM, are found in similar frequencies in MGUS and MM, indicating a direct precursor–product relationship of 13q<sup>-</sup> preneoplastic and neoplastic states. Empirical observations of this kind suggest that MGUS occurs as distinct molecular subtypes, which lead, in turn, to different forms of MM.

Evidence suggests that MGUS and MM develop along one of two distinct pathways that result in either non-hyperdiploid tumors that usually carry one of the seven recurrent *IGH* translocations or hyperdiploid tumors that usually are not associated with *IGH* translocations. Despite enormous progress in the past decade in our understanding of MM pathogenesis, many important questions remain. What are the molecular and microenvironmental mechanisms

that drive the transition from MGUS to MM? How does hyperdiploidy contribute to plasma cell transformation? What genetic lesions underlie recurrent cytogenetic changes, such as gain of chromosome 1q or loss of chromosome 13q? Mouse models of human PCN may help to provide answers to these and other important questions.

### **8.8.2 *Established and Newly Emerging Mouse Models of Human PCN***

The presently available mouse models of human PCN can be divided into de novo and transplantation models. Although none of the genetically engineered strains recapitulate all features of a particular human PCN, several strains have emerged as useful platforms for mechanistic and therapeutic studies of alterations in signaling pathway found in human PCN (e.g., IL-6, Abl, and Myc). Furthermore, although the succession of oncogenic processes responsible for tumor development that occurs de novo does not completely match those in humans, the incipient tumor cells in mice interact with their microenvironment (immune cells, vascular and lymphatic networks, and extracellular matrix) in ways that mirror the interactions of neoplastic plasma cells in humans with their specific tissue microenvironment. Similarly, mouse models that have been developed to permit the outgrowth of fully transformed transplanted tumor cells in vivo are not suitable for studying mechanisms of tumor development. Nonetheless, they are highly valuable for many other purposes including preclinical drug testing. Table 8.3 shows mouse models of human PCN in chronological order of development, beginning with strains in which tumor development occurs de novo, followed by models that rely on tumor cell transfer.

The first mouse model of de novo PCN, peritoneal PCT in strain BALB/c, was discovered 50 years ago by Dr. Michael Potter of the National Cancer Institute and has been progressively developed and refined by him over the last half century Anderson and Potter (1969). Salient features of this model include dependency on chronic inflammation (usually induced by intraperitoneal application of pristane), genetic background (BALB/cAnPt is highly susceptible, NZB and BALB/cJ are weakly susceptible, and all other tested strains including DBA/2, C57BL/6, CBA/J, C3H, and 129 are solidly resistant), maintenance of mice in an antigen-rich conventional facility (SPF mice are refractory to tumor development), and the acquisition of *Myc*-deregulating chromosomal translocation in early tumor precursors. The penetrance of peritoneal PCT at 65% is incomplete and the average latency of 220 days is long. However, tumor development can be greatly accelerated by infection of mice with retrovirus expressing any of the series of oncogenes alone or in combination—Abelson-(*v-abl*), RIM (*v-Ha-Ras* and *Eμ-c-Myc*), J3V1 (*v-Raf1* and *v-myc*), and ABL-MYC (*v-abl* and human *MYC*).

Unfortunately, this model of PCN has been largely dismissed by the myeloma community as artificial and irrelevant for human MM, mainly due to

**Table 8.3.** Mouse models of human plasma cell neoplasms (PCN) including extraskeletal plasmacytoma (PCT) and multiple myeloma (MM)

Mode of tumor development	Tissue site of tumor development	Tumor type	Mouse strain	Transgene	Comment	Reference
de novo	Peritoneal cavity	PCT	BALB/c	None	High impact on immunology and cancer research	Potter (2003)
					Dependent on peritoneal inflammation (pristane)	Potter and MacCardle (1964) and Anderson and Potter (1969)
					Dependent on <i>Myc</i> translocation	Potter and Wiener (1992) and Janz (2006)
					Accelerated by retroviruses, such as A-MuLV, RIM (c-myc + v-ras), J3V1 (v-myc + v-raf), and ABL-MYC	Potter, Sklar et al. (1973), Ohno, Migita et al. (1984), Clynes, Wax et al. (1988), Troppmair, Huleihel et al. (1988), Weissinger, Mischak et al. (1991)
de novo	Bone marrow	MM	C57BL/Ka	None	Spontaneous tumors Impractical due to long latency and low incidence Source of transplantable 5T tumors (see below)	Radl, Croese et al. (1988), and Radl, Van Arkel et al. (1996)
de novo	Lymphoid system	PCT	BALB/c	Eμ-v-abl	No <i>Myc</i> translocations Mice develop peritoneal tumors upon treatment with pristane	Rosenbaum, Harris et al. (1990)
de novo	Lymphoid system, GALT	PCT	BALB/c	H2-L <sup>d</sup> -IL-6	Mice develop peritoneal tumors upon treatment with pristane	Kovalchuk, Kim et al. (2002)

Table 8.3. (continued)

Mode of tumor development	Tissue site of tumor development	Tumor type	Mouse strain	Transgene	Comment	Reference
de novo	Lymphoid system	PCT	BALB/c	E $\mu$ -Bcl-2	Mice develop peritoneal tumors upon treatment with pristane	Silva, Kovalchuk et al. (2003)
de novo	Bone marrow, lymphoid system	MM > PCT	C57BL/6 and BALB/c	NPM-ALK	Transgene targeted to T lymphocytes	Chiarle, Gong et al. (2003)
de novo	Peritoneal cavity, lymphoid system, and bone marrow	PCT > MM	Mixed BALB/c	iMyc	Mice develop peritoneal tumors upon treatment with pristane	Park, Kim et al. (2005), Park, Shaffer et al. (2005), and Kim, Han et al. (2006)
de novo	Lymphoid system, bone marrow	PCT > MM	Mixed	iMyc + Bcl-X <sub>L</sub>	3'KE-Bcl-X <sub>L</sub> transgene	Cheung, Kim et al. (2004)
de novo	Lymphoid system, bone marrow	PCT > MM	FVB/N	Bcl-X <sub>L</sub>	Accelerated tumorigenesis upon crossing in the E $\mu$ /c-Myc transgene	Linden, Kirchhoff et al. (2004) and Adams et al. (1985)
de novo	Bone marrow and lymphoid system	MM > PCT	C57BL/6	Bcl-X <sub>L</sub> + virus		Linden, Kirchhoff et al. (2005)
de novo	Bone marrow	MM > PCT	C57BL/6	Xbp1		Carrasco, Sukhadia et al. (2007)
de novo	Not yet reported	Not yet reported		TVA/TVB	Unpublished	F. Asimakopoulos and H.E. Varmus <sup>1</sup>
de novo	Bone marrow	MM		Kappa <sup>*</sup> Myc	Unpublished	M. Chesi, A.K. Stewart, and P.L. Bergsagel et al. <sup>1</sup>

Table 8.3. (continued)

Mode of tumor development	Tissue site of tumor development	Tumor type	Mouse strain	Transgene	Comment	Reference
Transfer of mouse MM	Bone marrow	MM	C57BL/6	None	Relies on 5T tumor cells homing to mouse bone, extensively studied	Vanderkerken, Goes et al. (1996)
Transfer of human MM	Bone marrow	MM	SCID-hu	None	Human MM cells homing to human fetal bone implanted s.c. in mice, well established	Yaccoby, Barlogie et al. (1998)
Transfer of human MM	Bone marrow	MM	SCID-hu	None	Human MM bone cores implanted i.m. in mice	Campbell, Manyak et al. (2006)
Transfer of human MM	Bone marrow	MM	NOD/SCID-hu	None	Immunodeficiency more pronounced than SCID	Pilarski, Hipperson et al. (2000) and Huang, Tien et al. (2004)
Transfer of human MM	Bone marrow	MM	NOG = NOD/ SCID/ gammac(null)	None	Immunodeficiency more pronounced than NOD/ SCID	Miyakawa, Ohnishi et al. (2004)
Transfer of human MM	Bone marrow	MM	SCID-rab	None	Human MM cells homing to rabbit fetal bone implanted s.c. in mice	Yata and Yaccoby (2004)

<sup>†</sup>Unpublished. Presented at the XI International Workshop on Multiple Myeloma, Kos Island, Greece, June 2007. NPM, nucleophosmin; PCT, plasmacytomas; SCID, severe-combined immunodeficiency.

the lack of bone marrow involvement. The dismissal may be premature, as certain properties of peritoneal PCT may be of great relevance for human MM. For example, peritoneal PCT formation is profoundly inhibited by anti-inflammatory agents, such as corticosteroids, which play an important role in the standard therapy of human MM. BALB/cAnPt mice deficient in IL-6 or treated with the cyclooxygenase inhibitors, indomethacin and sulindac, are also resistant to tumor induction. This suggests an intriguing parallel to the postulated tumor-promoting role of chronic inflammatory processes in the pathogenesis of human MM. Just as MM is preceded by MGUS, peritoneal PCT is preceded by a well defined and easily studied preneoplastic lesion, namely foci of aberrant plasma cells that reside in the inflammatory granulomas of the peritoneum where they can persist for months. Plasma cell foci of this sort may provide a good experimental opportunity to elucidate the enigmatic transition from preneoplastic to neoplastic plasma cell growth in a genetically defined and environmentally controlled study.

The 5T mouse myeloma of strain C57BL/KaLwRij was developed more than a quarter century ago by Dr. Jiri Radl (Radl 1981; Radl, Croese et al. 1988). In a survey of 2-year-old C57BL/Ka mice, he estimated that 0.5% of the mice developed aggressive PCN originating in the bone marrow. These tumors, designated as 5T myelomas, produced copious amounts of monoclonal Ig, were readily transplanted when injected intravenously into syngeneic mice, and, importantly, produced osteolytic lesions in recipient animals. Two serially transplanted neoplasms, called 5T2 and 5T33, are now in common use and are widely considered as the only mouse model that accurately recapitulates key properties of human MM [reviewed in Vanderkerken, Asosingh et al. (2003)]. 5T2 and 5T33 offer the unique advantage of testing new strategies for the treatment of MM in a neoplastic plasma cell that resides in the appropriate microenvironment of the bone marrow in an immunocompetent host. The 5T33 system has recently been adapted to tissue culture in presence of an adherent layer of stromal cells, further enhancing the suitability of this preclinical model system for drug testing. Another enhancement is provided by the continuous improvement of bioimaging methods that allow a more accurate evaluation of myeloma bone disease and tumor burden than was previously possible. Relevant methods include micro-CT of whole bone or bone explants and imaging techniques that detect 5T cells labeled with fluorochromes (GFP), luciferases (bioluminescence), or sodium iodide symporter (SPECT) with great sensitivity *in vivo*. Unlike BALB/c PCT, 5T myeloma does not harbor a chromosomal *c-Myc* translocation. This defines another intriguing parallel to human MM, in which *MYC* translocations, if they occur at all, appear as so-called secondary translocations involved in tumor progression.

The classic model of BALB/c PCT has been refined and accelerated by the development of a number of TGs that target oncogenes to the B-cell lineage. Among these are the H2-L<sup>d</sup>-hu-IL-6, E $\mu$ -Bcl-2, iMyc, and E $\mu$ -v-abl transgenes. The Bcl2 and v-abl TG takes advantage of the intronic IgH enhancer, E $\mu$ , to enforce the expression of the target genes in B-lineage cells. PCT incidence in

$\text{E}\mu\text{-v-abl}$  mice approaches 100%, does not require treatment with inflammatory agents, is independent of genetic background, and has been reported to extensively involve the bone marrow. Some  $\text{E}\mu\text{-v-abl}$  mice were shown to present with only bone marrow PCT, developing hind limb paralysis as the result of tumor growth in vertebral marrow cavities. If further modification of this mouse were to succeed in reproducing this primary bone marrow manifestation of PCN growth more consistently, strain  $\text{E}\mu\text{-v-abl}$  may evolve into a true counterpart of human MM.

Among many attempts to express the plasma cell growth, differentiation and survival factor, IL-6, in the B cell and other cell lineages of TG mice, the H2-L<sup>d</sup>-hu-IL-6 TG has emerged as the most promising for studying PCN. Deregulated expression of IL-6 in young TG mice causes progressive plasma cell hyperplasia in lymphoid tissues, hypergammaglobulinemia, kidney damage, and a histologic picture that resembles human multicentric Castleman's disease (Kovalchuk, Kishimoto et al. 2000). The transition from plasma cell hyperplasia to neoplasia occurs in older mice, usually in enlarged lymph nodes of the gut-associated lymphoid tissue, GALT (Kovalchuk, Kim et al. 2002). Bone marrow infiltration in mice bearing advanced tumors is often extensive. This mouse model may be useful for elucidating the molecular and cellular mechanisms of IL-6-driven plasma cell neoplasia and to test new treatments that target the IL-6 receptor (Yoshio-Hoshino, Adachi et al. 2007) or downstream elements of the IL-6 signaling pathway (Bhutani, Pathak et al. 2007; Hausherr, Tavares et al. 2007; Loffler, Brocke-Heidrich et al. 2007).

The  $\text{E}\mu\text{SV-Bcl-2-22}$  TG contains a human *BCL2* cDNA driven by  $\text{E}\mu$  (Strasser, Whittingham et al. 1991). Transfer of the TG from PCT-resistant C57BL/6 mice onto the PCT-susceptible BALB/c genetic background resulted in a 24-fold increase in tumor incidence and a two-fold reduction in tumor latency. Similar to their IL-6 TG counterparts, *BCL2* TG PCT harbor *Myc*-deregulating T(12;15) translocations (Silva, Kovalchuk et al. 2003). Accelerated plasmacytogenesis in strain  $\text{E}\mu\text{-Bcl-2}$  may facilitate the design and testing of *BCL2* inhibition strategies of potential relevance to *BCL2*-overexpressing human PCN, such as Waldenström's macroglobulinemia and MM (Kline, Rajkumar et al. 2007).

TG mice, designated iMyc, contain a His<sub>6</sub>-tagged mouse c-Myc cDNA, Myc<sub>His</sub>, inserted head-to-head into different sites of the mouse *IgH* locus in ways that mimic the Myc-activating T(12;15) translocations of BALB/c PCT. A strain carrying the iMyc TG just 5' of  $\text{E}\mu$  is the most thoroughly characterized to date (Park, Kim et al. 2005). In analogy to the experience with the IL-6 and Bcl-2 TG mentioned above, the transfer of the iMyc TG onto BALB/c rendered the mice hyper-susceptible to inflammation-induced peritoneal PCT (Park, Shaffer et al. 2005; Kim, Han et al. 2006). As expected, the PCT overexpressed Myc<sub>His</sub>, produced monoclonal Ig, and exhibited a unique plasma cell signature upon gene expression profiling on mouse lymphochip.

A somewhat surprising observation was made in mice harboring a T-cell-targeted fusion gene joining nucleophosmin (NPM) and anaplastic lymphoma

kinase (ALK). Predictably, these mice developed T-cell lymphomas; however, 20% developed PCN instead of T-cell lymphomas. The PCN arose in peripheral lymphoid tissues or the bone marrow. In the latter case, tumor growth resulted in peripheral neuropathy and hind leg paralysis. NPM-ALK TG mice are currently used primarily for studies on the T-cell neoplasm, anaplastic large cell lymphoma (Amin and Lai 2007). Further modification of this strain for modeling human MM has not been attempted.

With several newly developed TG models at their disposal, researchers began to generate double TG mice in efforts to develop robust models of human PCN. Other modifications, such as infecting TG mice with oncogenic virus or changing the genetic background, were also pursued to further accelerate tumor development and/or shift the tumor pattern from B-cell lymphoma to PCN. PCN formation is dramatically accelerated in double TG mice that carry the H2-L<sup>d</sup>-hu-IL-6 TG and a Bcl-2 TG (Janz, unpublished finding), a Bcl-X<sub>L</sub> TG (Fang, Mueller et al. 1996; Potter 2003) (Janz, unpublished finding) or iMyc TG (Janz, unpublished finding). The same findings were obtained in iMyc/Bcl-2 double TG mice (Janz, unpublished finding). An interesting alternative approach involved the infection of Bcl-X<sub>L</sub> TG mice with ABL-MYC virus (Linden, Kirchhof et al. 2005). This resulted in a unique model of MM that recently was acknowledged by a panel of MM experts as holding promise for the validation of new therapeutics (Dalton and Anderson 2006). Additional research is warranted to better characterize double TG and virally accelerated mouse models of human PCN before recommendations can be made as to which model may be most suitable for elucidating specific aspects of the human disease.

Recent studies have shown that X-box-binding protein-1 (XBP1), a differentiation and unfolded protein/ergoplasmatic reticulum stress response factor essential for normal plasma cell development in mice, may also be implicated in human PCN. This prompted the development of a new model of human MM that relies on enforced expression of Xbp-1s ORF in the B-cell lineage under the control of the IgV<sub>H</sub> promoter and Eμ enhancer. The mice are prone to a MGUS-like disorder followed by a type of PCN with many similarities to MM. Like all other TG strains susceptible to PCN, Eμ-Xbp1 TG also develop extraosseous PCN, either together with the MM-like tumors or on their own. Nonetheless, a number of features indicate that the Eμ-Xbp1 mice offer an attractive model of human MM. Among other applications, they may be useful to uncover the elusive genetic changes responsible for the transition of MGUS to MM.

A fresh, unorthodox approach to recapitulating the natural history of MM in a relevant cellular and physiologic milieu is a mouse model system that enables the delivery of stochastic, sequential, somatic mutations to precisely defined plasma cell precursors *in vivo*. Asimakopoulos and Varmus used BAC TG technology to express two distinct types of avian leukosis virus (ALV) receptors, TVA and TVB, in the expanding centroblasts of the GC dark zone and the committed plasmablasts of the light zone. Mouse cells are refractory to

infection by retroviruses of the ALV family unless they ectopically express the cognate avian-derived receptors. To that end, TG mice were genetically developed that express TVA driven by regulatory elements of *Myb11* (*A-Myb*), a transcription factor expressed in dividing blasts of the GC dark zone, and TVB under control of Blimp-1, a master regulator of plasma cell differentiation. As a result, the mice express TVA in dividing follicular B cells and TVB in cells of the GC light zone, extrafollicular plasma cells, and mature plasma cells in the bone marrow. Viral vectors have been engineered to carry dominant oncogenes or various inactivators of tumor suppressor genes, permitting the introduction of sequential oncogenic lesions in putative precursors of PCN.

Chesi, Bergsagel, and associates reported recently on a new mouse model of MM designated VK\*MYC. This model is based on the VK\*MYC TG that contains an inactive, non-coding human *c-MYC* gene under the transcriptional control of the *Vk* promoter. TG MYC is activated sporadically in GC B cells undergoing somatic hypermutation. This approach has two potential benefits. First, in contrast to all previously generated MYC TG, expression of the VK\*MYC TG occurs only at the GC stage of B-cell development. This circumvents the unwanted transformation of less mature B-lineage cells that occurs when Myc is expressed earlier in differentiation. Second, B cells in which MYC becomes activated are likely to participate in an ongoing T-cell-dependent immune response, because they were part of a GC reaction. This further restricts the pool of MYC target cells to those that define the postulated MM precursor pool in humans. Evidence indicates that virtually all *Vk\*MYC* mice develop MGUS-like disease by 50 weeks of age. Plasma cells are fully differentiated (CD19<sup>-</sup>CD138<sup>+</sup>), have a very low proliferation index, and are found exclusively in the bone marrow. *Vk\*MYC* mice also develop anemia, bone disease with low trabecular density as well as sporadic lytic bone lesions and hind limb paralysis. Similar to the *Xbp1* TG mice, 30% of the *Vk\*MYC* mice exhibit extramedullary disease. Importantly, *VK\*MYC* mice responded to drugs known to be active against MM while demonstrating no response to drugs with little or no clinical activity. These features indicate that strain *VK\*MYC* will be useful in the study of MM biology and the development of new pharmacological and immunological therapies.

### ***8.8.3 Xenograft Models of Human Myeloma in Mice***

In contrast to the models of de novo PCN formation in mice, xenograft models use MM cells or cell lines that are transplanted into SCID-Hu, NOD/SCID, or SCID-Rab mice. These systems offer the unique ability to test therapeutics *in vivo* against true MM (Tassone, Neri et al. 2005). The SCID-Hu mouse model that employs fetal bone permits studies on the interaction of MM cells with the microenvironment of human bone marrow. The NOD/SCID has been adapted to myeloma cells labeled with green fluorescent protein. Intravenous injection

of these cells creates a model in which diffuse PCN dissemination can be visualized using whole-body, real-time fluorescence imaging to reproducibly quantify tumor burden. This allows serial, noninvasive monitoring of drug treatment. The SCID-Rab model avoids the ethical concerns about the use of human fetal bone in the SCID-Hu model by using rabbit bones. This model supports the growth of MM cells in a non-myelomatous, non-human, and non-fetal microenvironment. Although xenograft models are the current work-horses in preclinical testing of efficacy and mechanism of action of novel myeloma drugs, the xenograft implants have their own severe limitations, including the lack of an intact immune system, inability to model premalignant neoplastic stages, and imperfections in recapitulating the interactions between myeloma cells and surrounding stroma.

The SCID-hu mouse, which was originally developed for studies on human hematopoiesis in mice (Shultz, Ishikawa et al. 2007), has been adapted by Dr. J. Epstein, Y. Yaccoby, and their associates to investigate human MM cells in their native microenvironment of the human bone marrow (Yaccoby, Barlogie et al. 1998). In this system, myeloma growth is restricted to and dependent on human bone marrow and leads to osteolytic lesions in the transplanted human bone. The SCID-hu model contributed to our understanding of myeloma biology by demonstrating that myeloma alters the balanced expression of the osteoclast differentiation factor, RANKL/OPG, in the bone marrow (Pearse, Sordillo et al. 2001), depends on osteoclasts for growth and survival (Yaccoby, Pearse et al. 2002), relies, in part, on IL-6 to avoid programmed cell death (Yaccoby, Pearse et al. 2002), abolishes osteoblasts in the course of tumor progression (Yaccoby, Wezeman et al. 2006), and uses the serine phosphatase, fibroblast activation protein, to interact with stromal cells (Ge, Zhan et al. 2006). Importantly, the SCID-hu model was instrumental in showing that the anti-myeloma activity is dependent on its metabolism by liver microsomes (Yaccoby, Wezeman et al. 2006).

Progress in research on humanized mice (Shultz, Ishikawa et al. 2007) led to additional modifications of the SCID-hu model, such as NOD/SCID-hu (Huang, Tien et al. 2004), NOG (NOD/SCID/ $\gamma_c^{null}$ ) (Miyakawa, Ohnishi et al. 2004), and SCID-rab (Yata and Yaccoby 2004), which permit the engraftment of human myeloma cells in endogenous human bone or implanted rabbit bone, respectively. The addition of the NOD genetic background to SCID mice enhances the immunodeficiency conferred by the SCID background, resulting in lack of B and T cells, lack of circulating complement, defective macrophage function, and low natural killer cell activity. The  $\gamma_c^{null}$  phenotype causes the complete loss of natural killer cells. Just like the original SCID-hu model, the newer models enhance our understanding of myeloma biology and are useful for evaluating novel drugs and drug candidates in a preclinical setting. The utility of the SCID-rab system to evaluate effects of antibody to DKK1, of bortezomib, and of bone anabolic agents on bone remodeling and myeloma growth illustrates this point (Yaccoby, Ling et al. 2007).

### 8.8.4 Conclusions Regarding Mouse Models of Human PCN

Recent progress in the design and development of genetically engineered mouse models of human PCN has resulted in two categories of experimental models systems. In the first or de novo category, PCN arise either spontaneously or are induced in inbred or TG mice. Tumor development occurs in predictable stages and is preceded by the expansion of premalignant plasma cells resembling benign monoclonal gammopathy (BMG), MGUS, Castleman's disease, or similar non-malignant human plasma cell disorders. The mouse models in this category are indispensable for mechanistic studies of plasma cell transformation and the design and testing of strategies for tumor prevention. The greatest weakness among these models is their failure to recapitulate the bone marrow manifestations of tumor growth that are typical of MM. Two recent models offer a glimmer of hope along this line (Cheung, Kim et al. 2004; Linden, Kirchhof et al. 2005; Boylan, Gosse et al. 2007; Carrasco, Sukhdeo et al. 2007). In the second or transplantation-based category, fully transformed plasma cells of mouse origin (5T) or human origin (primary MM cells and myeloma cell lines) are transferred into syngeneic, immunocompetent mice (5T) or immunodeficient SCID mice (xenotransplant system) that frequently harbor human or rabbit bone as a nesting ground for the incoming plasma cells. The mouse models in this category have made and continue to make important scientific contributions to the preclinical assessment of myeloma therapeutics and our understanding of myeloma bone disease. Nonetheless, technical and logistic barriers have prevented these models from having a significant economic effect on the process of anti-myeloma drug discovery. They are not extensively used in preclinical trials and have not gained wide acceptance in industry. In addition, transplantation models are not useful for studies on tumor development and prevention.

## References

- Alizadeh, A. A., M. B. Eisen, et al. (2000). "Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling." *Nature* **403**: 503.
- Amin, H. M. and R. Lai(2007). "Pathobiology of ALK+ anaplastic large-cell lymphoma." *Blood* **110**: 2259–2267
- Anderson, P. N. and M. Potter(1969). "Induction of plasma cell tumours in BALB-c mice with 2,6,10,14-tetramethylpentadecane (pristane)." *Nature* **222**: 994–5.
- Anderson, M. S., E. S. Venanzi, et al. (2002). "Projection of an Immunological Self Shadow Within the Thymus by the Aire Protein." *Science* **298**(5597): 1395–1401.
- Aronoff, R. and C. C. Petersen (2006). "Controlled and localized genetic manipulation in the brain." *J Cell Mol Med* **10**(2): 333–52.
- Bergsagel, P. L. and W. M. Kuehl (2005). "Molecular pathogenesis and a consequent classification of multiple myeloma." *J Clin Oncol* **23**(26): 6333–8.
- Bhutani, M., A. K. Pathak, et al. (2007). "Capsaicin is a novel blocker of constitutive and interleukin-6-inducible STAT3 activation." *Clin Cancer Res* **13**(10): 3024–32.
- Bichi, R., S. A. Shinton, et al. (2002). "Human chronic lymphocytic leukemia modeled in mouse by targeted TCL1 expression." *Proc Natl Acad Sci USA* **99**: 6955.

- Bodrug, S. E., B. J. Warner, et al. (1994). "Cyclin D1 transgene impedes lymphocyte maturation and collaborates in lymphomagenesis with the myc gene." *EMBO J* **13**(9): 2124–30.
- Boumsell, L., H. Coppin, et al. (1980). "An antigen shared by a human T cell subset and B cell chronic lymphocytic leukemic cells. Distribution on normal and malignant lymphoid cells." *J Exp Med* **152**(1): 229–34.
- Boylan, K. L., M. A. Gosse, et al. (2007). "A transgenic mouse model of plasma cell malignancy shows phenotypic, cytogenetic, and gene expression heterogeneity similar to human multiple myeloma." *Cancer Res* **67**(9): 4069–78.
- Bryant, J., L. Pham, et al. (2000). "Development of intermediate-grade (mantle cell) and low-grade (small lymphocytic and marginal zone) human non-Hodgkin's lymphomas xenotransplanted in severe combined immunodeficiency mouse models." *Lab Invest* **80**(4): 557–73.
- Calin, G. A., C. Sevignani, et al. (2004). "Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers." *Proc Natl Acad Sci USA* **101**(9): 2999–3004.
- Cancro, M. P. (2004). "The BLYS family of ligands and receptors: an archetype for niche-specific homeostatic regulation." *Immunol Rev* **202**(1): 237–49.
- Carrasco, D. R., K. Sukhdeo, et al. (2007). "The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis." *Cancer Cell* **11**(4): 349–60.
- Carrasco, D. R., G. Tonon, et al. (2006). "High-resolution genomic profiles define distinct clinicopathogenetic subgroups of multiple myeloma patients." *Cancer Cell* **9**(4): 313–25.
- Cattoretti, G., L. Pasqualucci, et al. (2005). "Deregulated BCL6 expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice." *Cancer Cell* **7**(5): 445–55.
- Chen, W., Q. Li, et al. (2006). "A murine Mll-AF4 knock-in model results in lymphoid and myeloid deregulation and hematologic malignancy." *Blood* **108**(2): 669–77.
- Cheung, W. C., J. S. Kim, et al. (2004). "Novel targeted deregulation of c-Myc cooperates with Bcl-X(L) to cause plasma cell neoplasms in mice." *J Clin Invest* **113**(12): 1763–73.
- Chiarle, R., J. Z. Gong, et al. (2003). "NPM-ALK transgenic mice spontaneously develop T-cell lymphomas and plasma cell tumors." *Blood* **101**(5): 1919–27.
- Chiorazzi, N. and M. Ferrarini (2003). "B cell chronic lymphocytic leukemia: Lessons learned from studies of the B cell antigen receptor." *Annu Rev Immunol* **21**: 841–94.
- Clynes, R., J. Wax, et al. (1988). "Rapid induction of IgM-secreting murine plasmacytomas by pristane and an immunoglobulin heavy-chain promoter/enhancer-driven c-myc/v-Ha-ras retrovirus." *Proc Natl Acad Sci USA* **85**: 6067–71.
- Crespo, M., Bosch, et al. (2003). "ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia." *N Engl J Med* **348**(18): 1764–1775.
- Czarneski, J., Y. C. Lin, et al. (2004). "Studies in NZB IL-10 knockout mice of the requirement of IL-10 for progression of B-cell lymphoma." *Leukemia* **18**(3): 597–606.
- Dalton, W. and K. C. Anderson (2006). "Synopsis of a roundtable on validating novel therapeutics for multiple myeloma." *Clin Cancer Res* **12**(22): 6603–10.
- Damle, R. N., Wasil, et al. (1999). "Ig V gene mutation status and CD38 expression as novel prognostic factors in chronic lymphocytic leukemia." *Blood* **94**: 1837–1839.
- Dawson, D. W., J. S. Hong, et al. (2007). "Global DNA methylation profiling reveals silencing of a secreted form of EphA7 in mouse and human germinal center B-cell lymphomas." *Oncogene* **26**(29): 4243–52.
- Dohner, H., S. Stilgenbauer, et al. (2000). "Genomic aberrations and survival in chronic lymphocytic leukemia." *N Engl J Med* **343**(26): 1910–6.
- Du, Z., K. Podsypanina, et al. (2006). "Introduction of oncogenes into mammary glands in vivo with an avian retroviral vector initiates and promotes carcinogenesis in mouse models." *Proc Natl Acad Sci USA* **103**(46): 17396–401.
- East, J. (1970). "Immunopathology and neoplasms in New Zealand Black (NZB) and SJL/J mice." *Prog Exp Tumor Res* **13**: 84–134.

- Egle, A., A. W. Harris, et al. (2004). "VavP-Bcl2 transgenic mice develop follicular lymphoma preceded by germinal center hyperplasia." *Blood* **103**(6): 2276–83.
- Enno, A., J. L. O'Rourke, et al. (1995). "MALToMa-like lesions in the murine gastric mucosa after long-term infection with Helicobacter felis. A mouse model of Helicobacter pylori-induced gastric lymphoma." *Am J Pathol* **147**(1): 217–22.
- Enno, A., J. L. O'Rourke, et al. (1998). "Antigen-dependent progression of mucosa-associated lymphoid tissue (MALT)-type lymphoma in the stomach. Effects of antimicrobial therapy on gastric MALT lymphoma in mice." *Am J Pathol* **152**(6): 1625–32.
- Faderl, S., Keating, et al. (2002). "Expression profile of 11 proteins and their prognostic significance in patients with chronic lymphocytic leukemia (CLL)." *Leukemia* **16**(6): 1045–1052.
- Fang, W., D. L. Mueller, et al. (1996). "Frequent aberrant immunoglobulin gene rearrangements in pro-B cells revealed by a bcl-xL transgene." *Immunity* **4**(3): 291–9.
- Feeney, A. J. (1990). "Lack of N regions in fetal and neonatal mouse immunoglobulin V-D-J junctional sequences." *J Exp Med* **172**(5): 1377–90.
- Ford, R. J., L. Shen, et al. (2007). "Development of a murine model for blastoid variant mantle-cell lymphoma." *Blood* **109**(11): 4899–906.
- Fredrickson, T. H., A. W. Harris (2000). *Atlas of Mouse Hematopathology*. Amsterdam, Harwood Academic Publishers.
- Fredrickson, T. N., K. Lennert, et al. (1999). "Splenic marginal zone lymphomas of mice." *Am J Pathol* **154**(3): 805–12.
- Fu, T. B., L. Virgilio, et al. (1994). "Characterization and localization of the TCL-1 oncogene product." *Cancer Res* **54**: 6297.
- Ge, Y., F. Zhan, et al. (2006). "Fibroblast activation protein (FAP) is upregulated in myelomatous bone and supports myeloma cell survival." *Br J Haematol* **133**(1): 83–92.
- Hallek, M., Langenmayer, et al. (1999). "Elevated serum thymidine kinase levels identify a subgroup at high risk of disease progression in early, nonsmoldering chronic lymphocytic leukemia." *Blood* **93**(5): 1732–1737.
- Hamano, Y., S. Hirose, et al. (1998). "Susceptibility alleles for aberrant B-1 cell proliferation involved in spontaneously occurring B-cell chronic lymphocytic leukemia in a model of New Zealand white mice." *Blood* **92**(10): 3772–9.
- Hanahan, D. and R. A. Weinberg (2000). "The hallmarks of cancer." *Cell* **100**(1): 57–70.
- Hardy, R. R. (2006). "B-1 B cell development." *J Immunol* **177**(5): 2749–54.
- Hartley, J. W., S. K. Chattopadhyay, et al. (2000). "Accelerated appearance of multiple B cell lymphoma types in NFS/N mice congenic for ecotropic murine leukemia viruses." *Lab Invest* **80**(2): 159–69.
- Hassler, S., C. Ramsey, et al. (2006). "Aire-deficient mice develop hematopoietic irregularities and marginal zone B-cell lymphoma." *Blood* **108**(6): 1941–48.
- Hausherr, A., R. Tavares, et al. (2007). "Inhibition of IL-6-dependent growth of myeloma cells by an acidic peptide repressing the gp130-mediated activation of Src family kinases." *Oncogene* **26**(34): 4987–98.
- Hayakawa, K., R. R. Hardy, et al. (1983). "The -Ly-1 B— cell subpopulation in normal immunodefective, and autoimmune mice." *J Exp Med* **157**(1): 202–18.
- Herve, M., K. Xu, et al. (2005). "Unmutated and mutated chronic lymphocytic leukemias derive from self-reactive B cell precursors despite expressing different antibody reactivity." *J Clin Invest* **115**(6): 1636–43.
- Hockenberry, D., G. Nunez, et al. (1990). "Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death." *Nature* **348**(6299): 334–6.
- Hoyer, K. K., S. W. French, et al. (2002). "Dysregulated TCL1 promotes multiple classes of mature B cell lymphoma." *Proc Natl Acad Sci USA* **99**(22): 14392–7.
- Hu, Y., S. Swerdlow, et al. (2006). "Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph+ leukemia in mice." *Proc Natl Acad Sci USA* **103**(45): 16870–5.

- Huang, S. Y., H. F. Tien, et al. (2004). "Nonirradiated NOD/SCID-human chimeric animal model for primary human multiple myeloma: a potential in vivo culture system." *Am J Pathol* **164**(2): 747–56.
- Jaffe, E. S., N. L. Harris, et al. (2001a). *World Health Organization Classification of Tumours. Pathology and Genetics of Haematopoietic and Lymphoid Tissues*. Lyon, IARC Press.
- Jaffe, E. S., N. L. Harris, et al. (2001b). "Burkitt lymphoma." *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. P. a. S. Kleihues, L.H. Lyon, IARC Press: 181–4.
- Jaffe, E. S., N. L. Harris, et al. (2001c). "Diffuse large B cell lymphoma." *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. P. a. S. Kleihues, L.H. Lyon, IARC Press: 171–4.
- Jaffe, E. S., N. L. Harris, et al. (2001d). "Mantle cell lymphoma." *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. P. a. S. Kleihues, L.H. Lyon, IARC Press: 168–70.
- Janz, S. (2006). "Myc translocations in B cell and plasma cell neoplasms." *DNA Repair (Amst.)* **5**(9–10): 1213–24.
- Johnson, A. J., D. M. Lucas, et al. (2006). "Characterization of the TCL-1 transgenic mouse as a preclinical drug development tool for human chronic lymphocytic leukemia." *Blood* **108**(4): 1334–8.
- Kantor, A. B., C. E. Merrill, et al. (1997). "An unbiased analysis of V(H)-D-J(H) sequences from B-1a, B-1b, and conventional B cells." *J Immunol* **158**(3): 1175–86.
- Kichina, J. V., M. Zeremski, et al. (2006). "Targeted disruption of the mouse *ing1* locus results in reduced body size, hypersensitivity to radiation and elevated incidence of lymphomas." *Oncogene* **25**(6): 857–66.
- Kim, J., S. Han, et al. (2006). "Plasma cell tumour progression in iMyc(Emicro) gene-insertion mice." *J Pathol* **209**(1): 44–55.
- Kline, M. P., S. V. Rajkumar, et al. (2007). "ABT-737, an inhibitor of Bcl-2 family proteins, is a potent inducer of apoptosis in multiple myeloma cells." *Leukemia* **21**(7): 1549–60.
- Kovalchuk, A. L., J. S. Kim, et al. (2002). "IL-6 transgenic mouse model for extraosseous plasmacytoma." *Proc Natl Acad Sci USA* **99**(3): 1509–14.
- Kovalchuk, A. L., T. Kishimoto, et al. (2000). "Lymph nodes and Peyer's patches of IL-6 transgenic BALB/c mice harbor T(12;15) translocated plasma cells that contain illegitimate exchanges between the immunoglobulin heavy-chain mu locus and c-myc." *Leukemia* **14**: 1127–35.
- Kovalchuk, A. L., C. F. Qi, et al. (2000). "Burkitt lymphoma in the mouse." *J Exp Med* **192**(8): 1183–90.
- Kress, C. K., M. Martinez-Garcia, et al. (2007). "Triterpenoids display single agent anti-tumor activity in a transgenic mouse model of chronic lymphocytic leukemia and small B cell lymphoma." *PLoS ONE* **2**: e559.
- Kuehl, W. M. and P. L. Bergsagel (2005). "Early genetic events provide the basis for a clinical classification of multiple myeloma." *Hematology Am Soc Hematol Educ Program* : 346–52.
- Kyle, R. A., T. M. Therneau, et al. (2002). "A Long-Term Study of Prognosis in Monoclonal Gammopathy of Undetermined Significance." *N Engl J Med* **346**(8): 564–9.
- Lanier, L. L., N. L. Warner, et al. (1981). "Expression of Lyt-1 antigen on certain murine B cell lymphomas." *J Exp Med* **153**(4): 998–1003.
- Li, Y. S., K. Hayakawa, et al. (1993). "The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver." *J Exp Med* **178**(3): 951–60.
- Linden, M., N. Kirchhof, et al. (2004). "Targeted overexpression of Bcl-XL in B-lymphoid cells results in lymphoproliferative disease and plasma cell malignancies." *Blood* **103**(7): 2779–86.
- Linden, M., N. Kirchhof, et al. (2005). "ABL-MYC retroviral infection elicits bone marrow plasma cell tumors in Bcl-X(L) transgenic mice." *Leuk Res* **29**(4): 435–44.

- Loffler, D., K. Brocke-Heidrich, et al. (2007). "Interleukin-6-dependent survival of multiple myeloma cells involves the Stat3-mediated induction of microRNA-21 through a highly conserved enhancer." *Blood* **110**: 1330–1333.
- McDonnell, T. J. and S. J. Korsmeyer (1991). "Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14; 18)." *Nature* **349**(6306): 254–6.
- Mahboudi, F. P., J. A. Phillips, et al. (1992). "Immunoglobulin gene sequence analysis of B1 (CD5+B) cell clones in a murine model of chronic lymphocytic leukemia and Richter's syndrome." *Int J Oncol* **1**: 459–65.
- Manohar, V., E. Brown, et al. (1982). "Expression of Lyt-1 by a subset of B lymphocytes." *J Immunol* **129**(2): 532–8.
- Martin, F. and J. F. Kearney (2000). "B-cell subsets and the mature preimmune repertoire. Marginal zone and B1B cells as part of a "natural immune memory"." *Immunol Rev* **175**: 70–79.
- Martin, F. and J. F. Kearney (2001). "B1 cells: similarities and differences with other B cell subsets." *Cur Opin Immunol* **13**(2): 195–201.
- Martin, F., A. M. Oliver, et al. (2001). "Marginal zone and B1B cells unite in the early response against T-independent blood-borne particulate antigens." *Immunity* **14**(5): 617–29.
- Metzler, M., A. Forster, et al. (2006). "A conditional model of MLL-AF4 B-cell tumourigenesis using invertor technology." *Oncogene* **25**(22): 3093–103.
- Mikkers, H. and A. Berns (2003). "Retroviral insertional mutagenesis: tagging cancer pathways." *Adv Cancer Res* **88**: 53–99.
- Mitsiades, C. S., N. S. Mitsiades, et al. (2007). "Multiple myeloma: a prototypic disease model for the characterization and therapeutic targeting of interactions between tumor cells and their local microenvironment." *J Cell Biochem* **101**(4): 950–68.
- Miyakawa, Y., Y. Ohnishi, et al. (2004). "Establishment of a new model of human multiple myeloma using NOD/SCID/gammac(null) (NOG) mice." *Biochem Biophys Res Commun* **313**(2): 258–62.
- M'Kacher, R., F. Farace, et al. (2003). "Blastoid mantle cell lymphoma: evidence for nonrandom cytogenetic abnormalities additional to t(11;14) and generation of a mouse model." *Cancer Genet Cytogenet* **143**(1): 32–8.
- Monti, S., K. J. Savage, et al. (2005). "Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response." *Blood* **105**(5): 1851–61.
- Morse, H. C., 3rd, M. R. Anver, et al. (2002). "Bethesda proposals for classification of lymphoid neoplasms in mice." *Blood* **100**(1): 246–58.
- Morse, H. C., 3rd, T. McCarty, et al. (2003). "B lymphoid neoplasms of mice: characteristics of naturally occurring and engineered diseases and relationships to human disorders." *Adv Immunol* **81**: 97–121.
- Morse, H. C., 3rd, C. F. Qi, et al. (2001). "Combined histologic and molecular features reveal previously unappreciated subsets of lymphoma in AKXD recombinant inbred mice." *Leuk Res* **25**(8): 719–33.
- Mueller, A., J. O'Rourke, et al. (2003). "Distinct gene expression profiles characterize the histopathological stages of disease in Helicobacter-induced mucosa-associated lymphoid tissue lymphoma." *Proc Natl Acad Sci USA* **100**(3): 1292–7.
- Mulligan, G., C. Mitsiades, et al. (2007). "Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib." *Blood* **109**(8): 3177–88.
- Narducci, M. G., E. Pescarmona, et al. (2000). "Regulation of TCL1 expression in B- and T-cell lymphomas and reactive lymphoid tissues." *Cancer Res* **60**: 2095.
- Ohno, S., S. Migita, et al. (1984). "Chromosomal translocations activating myc sequences and transduction of v-abl are critical events in the rapid induction of plasmacytomas by pristane and abelson virus." *J Exp Med* **159**: 1762–77.

- Okada, T. T., F; Tokushige, et al. (1991). "Major histocompatibility complex controls clonal proliferation of CD5+ B cells in H-2-congenic New Zealand mice: a model for B cell chronic lymphocytic leukemia and autoimmune disease." *Eur J Immunol* **21**: 2743–8.
- Park, S. S., J. S. Kim, et al. (2005). "Insertion of c-Myc into IgH induces B-cell and plasma-cell neoplasms in mice." *Cancer Res* **65**(4): 1306–15.
- Park, S. S., A. L. Shaffer, et al. (2005). "Insertion of Myc into IgH accelerates peritoneal plasmacytomas in mice." *Cancer Res* **65**(17): 7644–52.
- Pearse, R. N., E. M. Sordillo, et al. (2001). "Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression." *Proc Natl Acad Sci USA* **98**(20): 11581–6.
- Phillips, J. A., K. Mehta, et al. (1992). "The NZB mouse as a model for chronic lymphocytic leukemia." *Cancer Res* **52**(2): 437–43.
- Pilarski, L. M., G. Hipperson, et al. (2000). "Myeloma progenitors in the blood of patients with aggressive or minimal disease: engraftment and self-renewal of primary human myeloma in the bone marrow of NOD SCID mice." *Blood* **95**(3): 1056–65.
- Planelles, L., C. E. Carvalho-Pinto, et al. (2004). "APRIL promotes B-1 cell-associated neoplasm." *Cancer Cell* **6**(4): 399–408.
- Potter, M. (2003). "Neoplastic development in plasma cells." *Immunol Rev* **194**: 177–95.
- Potter, M. and R. C. MacCardle (1964). "Histology of developing plasma cell neoplasia induced by mineral oil in BALB/c mice." *J Natl Cancer Inst* **33**: 497.
- Potter, M., M. D. Sklar, et al. (1973). "Rapid viral induction of plasmacytomas in pristane-primed BALB-c mice." *Science* **182**: 592–4.
- Potter, M. and F. Wiener (1992). "Plasmacytomagenesis in mice: model of neoplastic development dependent upon chromosomal translocations." *Carcinogenesis* **13**: 1681–97.
- Radl, J. (1981). "Animal model of human disease. Benign monoclonal gammopathy (idiopathic paraproteinemia)." *Am J Pathol* **105**(1): 91–3.
- Radl, J., J. W. Croese, et al. (1988). "Animal model of human disease. Multiple myeloma." *Am J Pathol* **132**(3): 593–7.
- Radl, J., C. Van Arkel, et al. (1996). "Tenfold increased incidence of spontaneous multiple myeloma in long-term immunosuppressed aging C57BL/KaLwRij mice." *Clin Immunol Immunopathol* **79**: 155–62.
- Rajkumar, S. V., M. Q. Lacy, et al. (2007). "Monoclonal gammopathy of undetermined significance and smoldering multiple myeloma." *Blood Rev* **21**: 255–265.
- Ramsey, C., O. Winqvist, et al. (2002). "Aire deficient mice develop multiple features of APECED phenotype and show altered immune response." *Hum Mol Genet* **11**(4): 397–409.
- Ranger, A. M., J. Zha, et al. (2003). "Bad-deficient mice develop diffuse large B cell lymphoma." *Proc Natl Acad Sci USA* **100**(16): 9324–9.
- Raveche, E. S., P. Lalor, et al. (1988). "In vivo effects of hyperdiploid Ly-1+ B cells of NZB origin." *J Immunol* **141**(12): 4133–9.
- Raveche, E. S., E. Salerno, et al. (2007). "Abnormal microRNA-16 locus with synteny to human 13q14 linked to CLL in NZB mice." *Blood* **109**(12): 5079–86.
- Repacholi, M. H., A. Basten, et al. (1997). "Lymphomas in E mu-Pim1 transgenic mice exposed to pulsed 900 MHZ electromagnetic fields." *Radiat Res* **147**(5): 631–40.
- Roodman, G. D. (2006). "New potential targets for treating myeloma bone disease." *Clin Cancer Res* **12**(20 Pt 2): 6270s–6273s.
- Rosenbaum, H., A. W. Harris, et al. (1990). "An E mu-v-abl transgene elicits plasmacytomas in concert with an activated myc gene." *EMBO J* **9**: 897–905.
- Said, J. W., K. K. Hoyer, et al. (2001). "TCL1 oncogene expression in B cell subsets from lymphoid hyperplasia and distinct classes of B cell lymphoma." *Lab Invest* **81**(4): 555–64.
- Sarfati, M., Chevret, et al. (1996). "Prognostic importance of serum soluble CD23 level in chronic lymphocytic leukemia." *Blood* **88**(11): 4259–4264.

- Schelonka, R. L., J. Tanner, et al. (2007). "Categorical selection of the antibody repertoire in splenic B cells." *Eur J Immunol* **37**(4): 1010–21.
- Schneider, P. (2005). "The role of APRIL and BAFF in lymphocyte activation." *Cur Opin Immunol* **17**(3): 282–9.
- Schwaller, J., P. Schneider, et al. (2007). "Neutrophil-derived APRIL concentrated in tumor lesions by proteoglycans correlates with human B-cell lymphoma aggressiveness." *Blood* **109**(1): 331–8.
- Shaughnessy, J. D. and B. Barlogie (2003). "Interpreting the molecular biology and clinical behavior of multiple myeloma in the context of global gene expression profiling." *Immunol Rev* **194**: 140–63.
- Shen, R. R., D. O. Ferguson, et al. (2006). "Dysregulated TCL1 requires the germinal center and genome instability for mature B-cell transformation." *Blood* **108**(6): 1991–8.
- Shin, M. S., T. N. Fredrickson, et al. (2004). "High-throughput retroviral tagging for identification of genes involved in initiation and progression of mouse splenic marginal zone lymphomas." *Cancer Res* **64**(13): 4419–27.
- Shultz, L. D., F. Ishikawa, et al. (2007). "Humanized mice in translational biomedical research." *Nat Rev Immunol* **7**(2): 118–30.
- Silva, S., A. L. Kovalchuk, et al. (2003). "BCL2 accelerates inflammation-induced BALB/c plasmacytomas and promotes novel tumors with coexisting T(12;15) and T(6;15) translocations." *Cancer Res* **63**(24): 8656–63.
- Smith, M. R., I. Joshi, et al. (2006). "Murine model for mantle cell lymphoma." *Leukemia* **20**(5): 891–3.
- Stall, A. M., M. C. Farinas, et al. (1988). "Ly-1 B-cell clones similar to human chronic lymphocytic leukemias routinely develop in older normal mice and young autoimmune (New Zealand Black-related) animals." *Proc Natl Acad Sci USA* **85**: 7312.
- Steele-Perkins, G., W. Fang, et al. (2001). "Tumor formation and inactivation of RIZ1, an Rb-binding member of a nuclear protein-methyltransferase superfamily." *Genes Dev* **15**(17): 2250–62.
- Strair, R. K., W. Sheay, et al. (2002). "Adenovirus infection of primary malignant lymphoid cells." *Leuk Lymphoma* **43**(1): 37–49.
- Strasser, A., A. W. Harris, et al. (1993). "E mu-bcl-2 transgene facilitates spontaneous transformation of early pre-B and immunoglobulin-secreting cells but not T cells." *Oncogene* **8**(1): 1–9.
- Strasser, A., S. Whittingham, et al. (1991). "Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease." *Proc Natl Acad Sci USA* **88**: 8661–5.
- Sutton, P., J. O'Rourke, et al. (2004). "Immunisation against Helicobacter felis infection protects against the development of gastric MALT Lymphoma." *Vaccine* **22**(20): 2541–6.
- Tassone, P., P. Neri, et al. (2005). "Combination therapy with interleukin-6 receptor super-antagonist Sant7 and dexamethasone induces antitumor effects in a novel SCID-hu In vivo model of human multiple myeloma." *Clin Cancer Res* **11**(11): 4251–8.
- Teitel, M. A. (2005). "The TCL1 family of oncoproteins: co-activators of transformation." *Nat Rev Cancer* **5**(8): 640–8.
- Teitel, M., M. A. Damore, et al. (1999). "TCL1 oncogene expression in AIDS-related lymphomas and lymphoid tissues." *Proc Natl Acad Sci USA* **96**(17): 9809–14.
- Thome, M. (2004). "CARMA1, BCL-10 and MALT1 in lymphocyte development and activation." *Nat Rev Immunol* **4**(5): 348–59.
- Troppmair, J., M. Huleihel, et al. (1988). "Plasmacytoma induction by J series of v-myc recombinant retroviruses: evidence for the requirement of two (raf and myc) oncogenes for transformation." *Curr Top Microbiol Immunol* **141**: 110–4.
- Tsujimoto, Y., L. R. Finger, et al. (1984). "Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation." *Science* **226**(4678): 1097–9.

- van Lohuizen, M., S. Verbeek, et al. (1989). "Predisposition to lymphomagenesis in pim-1 transgenic mice: cooperation with c-myc and N-myc in murine leukemia virus-induced tumors." *Cell* **56**(4): 673–82.
- Vanderkerken, K., K. Asosingh, et al. (2003). "Multiple myeloma biology: lessons from the STMM models." *Immunol Rev* **194**: 196–206.
- Vanderkerken, K., E. Goes, et al. (1996). "Follow-up of bone lesions in an experimental multiple myeloma mouse model: description of an in vivo technique using radiography dedicated for mammography." *Br J Cancer* **73**: 1463–5.
- Victor Hoffbrand, A. and T. J. Hamblin (2007). "Is "leukemia" an appropriate label for all patients who meet the diagnostic criteria of chronic lymphocytic leukemia?" *Leukemia Res* **31**(3): 273–5.
- Virgilio, L., C. Lazzeri, et al. (1998). "Deregulated expression of TCL1 causes T cell leukemia in mice." *Proc Natl Acad Sci USA* **95**(7): 3885–9.
- Virgilio, L., M. G. Narducci, et al. (1994). "Identification of the TCL1 gene involved in T-cell malignancies." *Proc Natl Acad Sci USA* **91**(26): 12530–4.
- Weissinger, E. M., H. Mischak, et al. (1991). "Induction of plasmacytomas secreting antigen-specific monoclonal antibodies with a retrovirus expressing v-abl and c-myc." *Proc Natl Acad Sci USA* **88**: 8735–9.
- Williams, M. E., S. H. Swerdlow, et al. (1993). "Chromosome 11 translocation breakpoints at the PRAD1/cyclin D1 gene locus in centrocytic lymphoma." *Leukemia* **7**(2): 241–5.
- Williams, M. E., C. D. Westermann, et al. (1990). "Genotypic characterization of centrocytic lymphoma: frequent rearrangement of the chromosome 11 bcl-1 locus." *Blood* **76**(7): 1387–91.
- Xiangshu Wen, D. Z., Yuji Kikuchi, et al. (2004). "Transgene-mediated hyper-expression of IL-5 inhibits autoimmune disease but increases the risk of B cell chronic lymphocytic leukemia in a model of murine lupus." *Eur J Immunol* **34**(10): 2740–9.
- Yaccoby, S., B. Barlogie, et al. (1998). "Primary myeloma cells growing in SCID-hu mice: a model for studying the biology and treatment of myeloma and its manifestations." *Blood* **92**(8): 2908–13.
- Yaccoby, S., W. Ling, et al. (2007). "Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo." *Blood* **109**(5): 2106–11.
- Yaccoby, S., R. N. Pearse, et al. (2002). "Myeloma interacts with the bone marrow micro-environment to induce osteoclastogenesis and is dependent on osteoclast activity." *Br J Haematol* **116**(2): 278–90.
- Yaccoby, S., M. J. Wezeman, et al. (2006). "Inhibitory effects of osteoblasts and increased bone formation on myeloma in novel culture systems and a myelomatous mouse model." *Haematologica* **91**(2): 192–9.
- Yan, X.-j., E. Albesiano, et al. (2006). "B cell receptors in TCL1 transgenic mice resemble those of aggressive, treatment-resistant human chronic lymphocytic leukemia." *Proc Natl Acad Sci USA* **103**(31): 11713–8.
- Yata, K. and S. Yaccoby (2004). "The SCID-rab model: a novel in vivo system for primary human myeloma demonstrating growth of CD138-expressing malignant cells." *Leukemia* **18**(11): 1891–7.
- Yoshio-Hoshino, N., Y. Adachi, et al. (2007). "Establishment of a new interleukin-6 (IL-6) receptor inhibitor applicable to the gene therapy for IL-6-dependent tumor." *Cancer Res* **67**(3): 871–5.
- Yumoto, T. Y., Y. Yoshida, et al. (1980). "Prelymphomatous and lymphomatous changes in splenomegaly of New Zealand Black mice." *Acta Pathol Japan* **30**(2): 171–6.
- Zanesi, N., R. Aqeilan, et al. (2006). "Effect of rapamycin on mouse chronic lymphocytic leukemia and the development of nonhematopoietic malignancies in E{micro}-TCL1 transgenic mice." *Cancer Res* **66**(2): 915–20.
- Zapata, J. M., M. Krajewska, et al. (2000). "TNFR-associated factor family protein expression in normal tissues and lymphoid malignancies." *J Immunol* **165**(9): 5084–96.

- Zapata, J. M., M. Krajewska, et al. (2004). "TNF receptor-associated factor (TRAF) domain and Bcl-2 cooperate to induce small B cell lymphoma/chronic lymphocytic leukemia in transgenic mice." *Proc Nat Acad Sci USA* **101**(47): 16600–5.
- Zhan, F., J. Hardin, et al. (2002). "Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells." *Blood* **99**(5): 1745–57.
- Zhang, B., Z. Wang, et al. (2007). "NF- $\kappa$ B2 mutation targets TRAF1 to induce lymphomagenesis." *Blood* **110**(2): 743–51.
- Zhang, J. C., CC; Lombardi, et al. (1994). "Rearranged NFKB2 gene in the HUT78 T-lymphoma cell line codes for a constitutive nuclear factor lacking transcriptional repressor functions." *Oncogene* **9**(7): 1931–7.
- Zhou, P., N. B. Levy, et al. (2001). "MCL1 transgenic mice exhibit a high incidence of B-cell lymphoma manifested as a spectrum of histologic subtypes." *Blood* **97**(12): 3902–9.
- Zhu, D., C. F. Qi, et al. (2005). "Deregulated expression of the Myc cellular oncogene drives development of mouse "Burkitt-like" lymphomas from naive B cells." *Blood* **105**(5): 2135–7.
- Zoer, N., H. Ludwig, et al. (2003). "Patterns of somatic mutations in VH genes reveal pathways of clonal transformation from MGUS to multiple myeloma." *Blood* **101**(10): 4137–9.

# **Chapter 9**

## **Genetic and Virological Predisposition to Pre-B Lymphomagenesis in SL/Kh**

**Hiroshi Hiai**

### **Contents**

9.1	Introduction . . . . .	228
9.2	Origin of SL/Kh and Related Strains of Mice . . . . .	228
9.3	Immunopathology of SL/Kh Pre-B Lymphomas . . . . .	229
9.4	Host Genetic Factors Affecting Types of Lymphomas . . . . .	231
9.4.1	Pre-B Lymphoma vs. Follicular Center Cell Lymphoma . . . . .	231
9.4.2	T Lymphoma vs. B Lymphoma . . . . .	232
9.4.3	Unusual Mixed-Type Lymphoma in Thymectomized (SL/Kh × AKR)F1 Mice . . . . .	235
9.4.4	Other Host Loci Affecting Lymphomagenesis . . . . .	236
9.4.4.1	Two Dominant Resistance Loci in MSM/Ms Mice . . . . .	236
9.4.4.2	Lymphoma Resistance of the SL/Ni Strain . . . . .	236
9.5	Bone Marrow Pre-B-Cell Expansion . . . . .	237
9.5.1	Genetically Determined Pre-B-cell Expansion . . . . .	237
9.5.2	Pre-B-Cell Expansion is Not Sufficient for Lymphomagenesis . . . . .	238
9.6	Molecular Pathogenesis: Retrovirus Integrations in Lymphoma . . . . .	239
9.7	Discussion . . . . .	240

### **List of Abbreviations**

BM	bone marrow
<i>Bomb-1</i>	<i>bone marrow pre-B-1</i>
<i>Esl-1</i>	<i>early lymphoma in SL/Kh-1</i>
<i>Foc-1</i>	<i>follicular center lymphoma-1</i>
Ig	immunoglobulin
<i>Lla</i>	<i>lymphoma latency acceleration</i>
MCF	mink cell focus

---

H. Hiai

Shiga Medical Center Research Institute, 5-4-30 Moriyama, City of Moriyama,  
Shiga 524-8524, Japan  
hiai6029@shigamed.jp

MHC	major histocompatibility complex
MMMTV	mouse mammary tumor virus
MRF	maternal resistance factor
MuLV	murine leukemia virus
PMA	phorbol myristic acetate
RI	recombinant inbred
QTL	quantitative trait locus
<i>Svi-1</i>	<i>SL/Kh virus integration-1</i>
<i>Tlsm-1</i>	<i>thymic lymphoma susceptible mouse-1</i>

## 9.1 Introduction

Spontaneous pre-B lymphoma is a relatively infrequent hematopoietic malignancy in mice. Pre-B lymphomas are occasionally observed in some mouse models such as AKXD recombinant inbred (RI) strains, Eμ-myc transgenic mice, and Abelson virus-injected mice. In an inbred strain SL/Kh, pre-B lymphomas develop at an unusually high incidence (>90%) by 6 months of age [10]. Reintegration of the endogenous ecotropic murine leukemia virus (MuLV) provirus to host DNA is a pathogenetic mechanism [16, 36], but lymphoma development is largely dependent on a number of host loci as well as on epigenetic factors [8, 13]. Therefore, SL/Kh pre-B lymphoma is an excellent multifactorial disease model. In this article, we will review the origin of SL/Kh mice, the biological features of lymphomas in this strain, the virology and genetics of lymphomagenesis, the unusual transient expansion of pre-B cells in pre-cancerous bone marrow (BM), and provirus integration in lymphoma DNA leading to the activation of cancer-related genes. Contained in this report are historical descriptions of our previous research; however, many important questions remain to be answered as technology and genome informatics advance.

## 9.2 Origin of SL/Kh and Related Strains of Mice

The SL has been established in Japan as a mouse strain prone to spontaneous leukemia. Their origin, history of establishment, and genetic interrelationship have been extensively studied [1]. The ancestors of the SL family were outbred Swiss mice and probably A2G mice, which were imported to Japan from an anonymous US source in approximately 1941 and have been maintained at several institutions. To date, four substrains with distinctive biological properties have been reported, that is, SL/Am, SL/Ni, SL/Kh, and SL/QDg. Some parameters relevant to lymphomagenesis are listed in Table 9.1. At present, the SL/Kh and SL/Ni strains are deposited at the RIKEN Bioresource Center at Tsukuba, but SL/Am and SL/QDg have been terminated.

**Table 9.1** Parameters for lymphomagenesis in AKR and SL family mice

	AKR/Ms	SL/Kh	SL/Ni	SL/Am	SL/QDj
Lymphoma type, incidence	T, 85%	Pre-B, >95%	Mature B, <10%	Mature B and myelogenous, 56.4%	None
Ecotropic MuLV genome (kb size of EcoRI fragments)	27, 14.5, 10.5	27, 21, 19, 14.5, 13, <sup>a</sup> 10.5	21, 19	21, 19	21, 14.5, 8.8
ecotropic virus expression	High	High	High or None	High	None
xenotropic virus expression	High	High	None	High	None
MHC	k	q	q	q	Q
Thy1	Thy1.1	Thy1.1	Thy1.2	Thy1.2	Thy1.2
Mx	Type-2	Type-2	Type-1	Type-1	Type-2

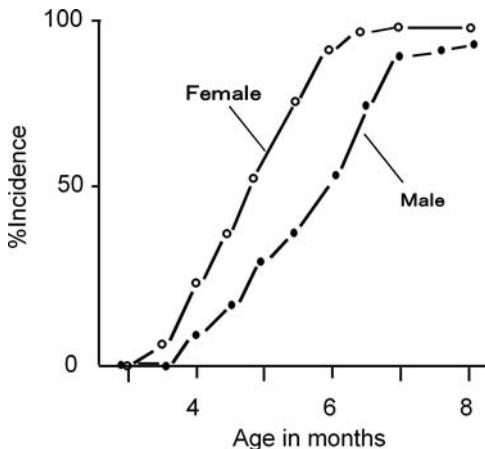
<sup>a</sup>A duplet in Southern blot (37)

The SL/Kh strain originated from a pair of SL mice introduced to the Aichi Cancer Center Research Institute (Nagoya, Japan) from Kyushu University (Fukuoka, Japan) by Nishizuka in the 1970s. SL/Kh mice share alleles at 59% microsatellite loci with SL/Am and SL/Ni, showing a closer relation than any other unrelated inbred strains [1]. However, the genetic profiles of the endogenous MuLV, the mammary tumor virus (MMTV), and the Mx gene allele are different from those of other SL family members. Moreover, many distinct microsatellite alleles are shared with AKR mice. For instance, Thy1, a marker antigen for T-cells, is Thy1.1 in SL/Kh, a rare genotype carried by AKR. When EcoRI-digested genomic DNA is Southern blotted with an *Akv1 env* probe, SL/Kh shows seven copies of ecotropic virus fragments, of which the 27-kb band is shared with the AKR endogenous ecotropic virus Emv11. SL/Ni and SL/Am do not have this band. These observations suggest that the SL/Kh strain is a RI strain derived from an intercross of proto-SL mice and AKR at an earlier stage of maintenance. The provirus yielding the 27-kb band is presumably acquired genetically from the AKR ancestor. We have shown that a high level of the infectious ecotropic virus is produced from two provirus loci in crosses with NFS mice without an endogenous ecotropic provirus and that one of the viruses producing the loci is at the proximal end of chromosome 7, where Emv11 is located [37].

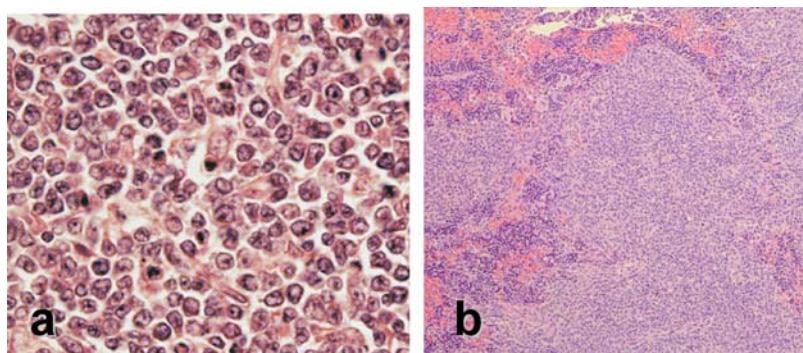
### 9.3 Immunopathology of SL/Kh Pre-B Lymphomas

The most prevalent form of hematopoietic neoplasm in the mouse is the T lymphoma, which develops spontaneously or is induced by a virus, a chemical, radiation, or gene manipulation. SL/Kh mice are unique, as they develop

**Fig. 9.1** Cumulative percent incidence of spontaneous pre-B lymphomas in SL/Kh mice [10]



exclusively pre-B lymphomas early in life at a high incidence (Fig. 9.1) [10]. There are two clinically distinct types among them. In the major type (83.7%), lymph node swelling and hepatosplenomegaly are the main features, while, in the minor type (16.3%), no macroscopically overt lymphoid organ involvement was evident although spinal paraplegia and incontinence were remarkable. BM is the main site of lymphoma cell proliferation in both types. In the minor type, the growth of lymphoma cells is limited in BM, and it compresses the spinal cord to cause spinal paralysis. Both types of lymphomas are readily transplantable in syngenic adult mice and kill recipients in as little as 2 weeks. The macroscopic types of lymphoma remain the same as primary lymphomas for at least three generations of *in vivo* passage. Both types of lymphomas are diffuse lymphoblastic lymphomas (Fig. 9.2a) but indistinguishable from each other except for tissues of infiltration.



**Fig. 9.2** (a) Histopathology of a major type pre-B lymphoma in a SL/Kh mouse.  $\times 400$ , H.E. (b) Follicular center cell lymphoma in a (SL/Kh  $\times$  NFS)F1  $\times$  NFS mouse [8].  $\times 100$ , H.E. (See color insert)

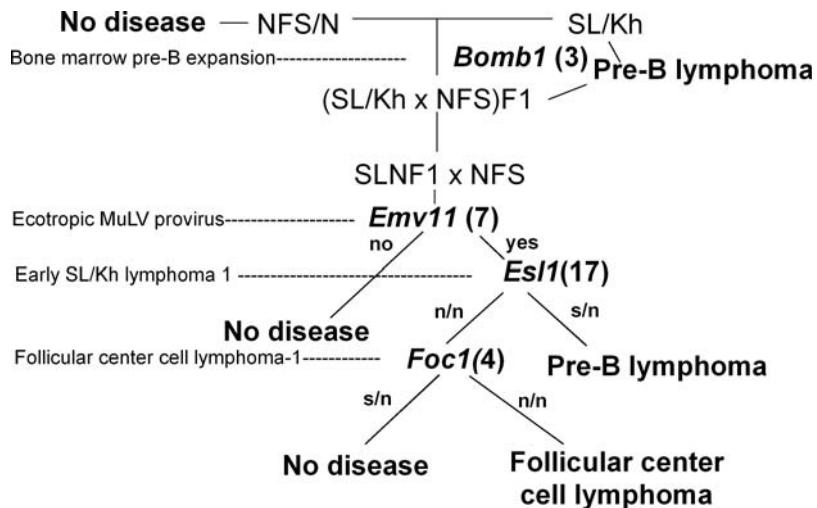
Both types of lymphomas show typical pre-B-cell phenotypes, that is, positive for B220, a pan-B cell antigen, and BP1, a pre-B cell antigen, and negative for surface immunoglobulin (Ig) [32]. The Ig heavy-chain gene is rearranged either mono- or oligoclonally, but the light-chain gene remains in a germ-line configuration. Products of the *lambda 5* and *V<sub>pre-B</sub>* gene form a receptor-like complex on the cell surface by which pre-B lymphocytes transmit a signal for proliferation. *RAG-1* and *RAG-2* genes code for key enzymes inducing the Ig gene recombination. SL/Kh lymphomas express *Lambda 5*, *V<sub>pre-B</sub>*, *RAG-1*, and *RAG-2* genes. Unlike normal pre-B cells, SL/Kh neoplastic pre-B cells express adhesion molecules LECAM-1 and LFA-1, but their expression is also shared by both types of lymphomas [24]. To date, no parameter has been found to explain the distinctive in vivo behavior of these two types of pre-B lymphomas.

## 9.4 Host Genetic Factors Affecting Types of Lymphomas

### 9.4.1 *Pre-B Lymphoma vs. Follicular Center Cell Lymphoma*

It is well known that lymphoma development is significantly affected by host genes. Among particularly important genes, *Fv1* determines the tropism of MuLV and *H-2*, the major histocompatibility complex gene, affects the immune responsiveness to MuLV and lymphoma cells. Investigations to find loci affecting lymphomagenesis have been extensive in chemical- and radiation-induced lymphomagenesis.

In order to analyze host genetic predisposition to pre-B lymphomas in SL/Kh, we studied lymphomagenesis in F1 and a backcross to NFS, a mouse strain without the endogenous ecotropic MuLV genome and without spontaneous lymphoma by 12 months of age [37]. In (SL/Kh × NFS)F1, all lymphomas were of the pre-B type, but their incidence was lower (53.8%). Furthermore, the latent period was longer (8.4 months). In 83 backcross mice, 22 (26.5%) developed hematopoietic malignancies, including 8 pre-B lymphomas, 2 myeloid leukemias, and 12 follicular center cell lymphomas (Fig. 9.2b). All individuals developing lymphomas expressed a high level of ecotropic MuLV. Genome-wide screening with microsatellite and biochemical genetic markers revealed that all the backcross mice developing any tumor had an SL/Kh-derived allele at *Gpi1* at the proximal end of chromosome 7. It is known that *Gpi1* is closely linked with *Akv1* (*Env11*), which is an endogenous ecotropic MuLV in AKR mice. As previously stated, one of the seven ecotropic provirus in SL/Kh is genetically acquired from an AKR ancestor. Further genetic analysis showed that, without exception, the backcross mice developing pre-B lymphomas had a dominant SL/Kh-derived allele on the chromosome 17 segment bearing the MHC locus. This locus is called *Esl-1* (*early lymphoma in SL/Kh-1*). We concluded that the presence of an *Akv1*-like provirus and the SL/Kh-derived allele at *Esl-1* is required for pre-B lymphomas to develop. On the other hand, the



**Fig. 9.3** Genetic determination of lymphomagenesis in a cross of SL/Kh × NFS [13]. Chromosome numbers for relevant loci are shown in parentheses

backcross mice developing follicular center lymphomas have a recessive NFS-derived allele at *foc-1* (*follicular center lymphoma-1*) on the proximal segment of chromosome 4. Therefore, the requirements for follicular center cell lymphomas are the presence of the *Akv1*-like provirus and the homozygosity of the NFS-derived allele at *foc-1*. The majority of hematopoietic neoplasms developing in NFS mice into which *Akv1* was introgressed by extensive backcrossing are mature B-cell type [3, 6]. The observations of the two groups of researchers indicated above are consistent with our findings. Two myeloid leukemias have genotypes *Esl-1<sup>SL/Kh/NFS</sup>* and *foc-1<sup>NFS/NFS</sup>*. Thus, the forms of lymphomas are determined by combinations of the host loci genotype as summarized in Fig. 9.3. Unfortunately, the genes corresponding to these loci have not been identified; therefore, the mechanism of the disease type determination remains hypothetical.

#### 9.4.2 T Lymphoma vs. B Lymphoma

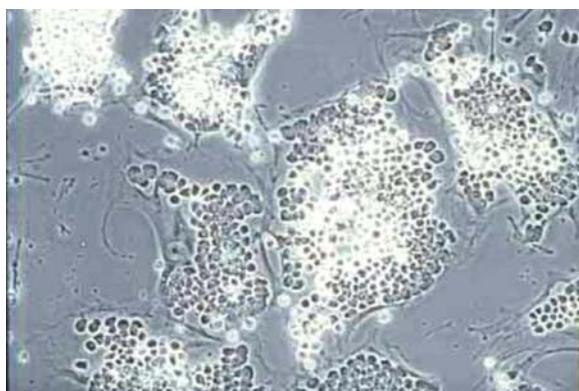
Subsequently, we investigated lymphomagenesis in the cross between SL/Kh and AKR mice to analyze the possible genetic mechanism determining the type of lymphomas to the B- or T-cell type. Before discussion of the primary topic, a brief review of the role of the thymus in T-lymphomagenesis will be presented.

The lymphomas developing spontaneously in AKR mice are T lymphomas arising in the thymus and secondarily infiltrating into other lymphoid organs. These lymphoma cells show an immunocytochemical profile of primitive thymic

cortical lymphocytes. Gross first found that cell-free extracts of AKR lymphoma induced T lymphomas when injected into newborn C3H mice as reviewed in 1958 [5]. A pathogenetic virus is derived from endogenous ecotropic MuLVs; however, it was later shown that, ultimately, a leukemogenic virus is generated in the pre-leukemic thymus through complicated recombination events among endogenous MuLVs [7, 21, 34]. This virus is called a mink cell focus (MCF)-forming virus, as it induces morphological changes in infected mink lung cells. The MCF virus is thymotropic, has a dualtropic host range, and induces T lymphomas by injection to appropriate recipients. Similar viruses have been detected in high leukemia strains, such as C58 and HRS/J.

The thymus is essential for T lymphomagenesis in the mouse. Thymectomy remarkably suppresses lymphoma development, and a thymus graft from an appropriate donor restores lymphomagenesis. There are three explanations for the essential role of the thymus. First, the thymus provides the target cells for lymphomagenesis, since lymphoma cells have the properties of primitive thymic cortical lymphocytes. However, whether or not a thymic lymphocyte per se is the direct target of a carcinogen is controversial. It is possible that the initial transformation may occur at the level of pre-T or lymphoid precursors residing in BM and that the cells later colonize and grow in the thymic microenvironments. Second, recombination events among endogenous MuLVs and their infection to target cells take place in the thymus. The thymus is also required for T lymphomagenesis induced by radiation or chemical carcinogens, in which direct involvement of recombinant viruses is not likely. Third, the thymus physiologically provides tissue microenvironments to support the growth and differentiation of thymic lymphocytes. This function is applicable to transformed thymic lymphocytes. From AKR primary lymphoma thymuses, we could consistently isolate cellular complexes of neoplastic thymocytes and thymic stromal cells [11] and maintain them in tissue culture for a long term. Neoplastic lymphocytes survive and grow in close contact with stromal cells in the form of pseudo-emperipoleisis (Fig. 9.4) for a few weeks in culture. When

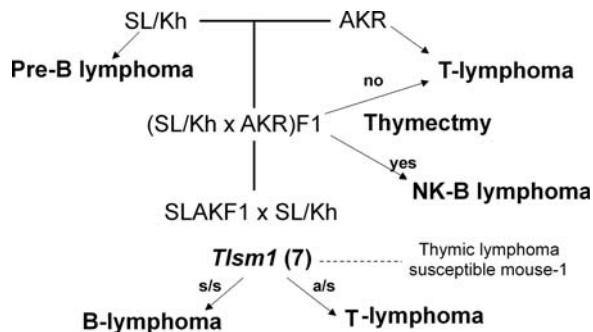
**Fig. 9.4** Symbiotic complexes isolated from a primary lymphoma thymus of an AKR mouse. Note most T-lymphoma cells are crawling under cytoplasm of thymic stromal cells (pseudo-emperipoleisis). Phase contrast,  $\times 200$



they are separated from the stromal cells, they quickly become apoptotic. However, they can survive and grow when phorbol myristic acetate (PMA) or a related tumor promoter is supplemented to the culture [12, 18]. It is possible to maintain microenvironment-dependent lymphoma cells in a PMA-containing medium for more than a year [19]. Lymphoma cells complexed with stromal cells gradually acquire the capability of autonomic growth. The cellular complex of neoplastic lymphocytes and stromal cells is called symbiotic complex. Stromal cells are one of the subsets of thymic epithelial cells residing in the thymic subcapsular zone and medulla [11, 20]. Based on these observations, we postulate that developing T lymphomas requires some support of thymic microenvironments. In their natural history, they form a symbiotic complex with thymic stromal cells. Under close cell-to-cell interaction in the complex, lymphoma cells survive, grow, and are selected for autonomic growth. These three functions of thymus may not be mutually exclusive, but they contribute to thymic lymphomagenesis by a variety of etiologies.

The lymphomas in AKR and SL/Kh are distinct in immunocytology; therefore, they provide an excellent tool to study the genetic factor determining types of lymphomas [38]. Of 84 lymphomas developing in 91 (AKR × SL/Kh)F<sub>1</sub> mice, 75 were T lymphomas. In 75 (AKR × SL/Kh)F<sub>1</sub> × SL/Kh backcross mice, 74 developed lymphomas, including 35 T (47.3%), 34 B (45.9%), and 5 mixed T and B lymphomas (6.8%). Of 144 lymphomas in the (AKR × SL/Kh)F<sub>2</sub> intercross, 96 (66.7%) were T lymphomas, 38 (26.4%) B lymphomas, and 10 (6.8%) mixed T and B lymphomas. The segregation data are best explained by assuming a single dominant *AKR* gene determining the type of lymphoma to the T-cell type. We call this gene *Tlsm-1* (*thymic lymphoma susceptible mouse-1*) (Fig. 9.5). By genome-wide screening with microsatellite genetic markers, *Tlsm-1* is mapped on a 7 cM segment between *D7Mit8* and *D7Mit13* on chromosome 7.

The AKXD strains are a set of RI strains between AKR/J and DBA/2J. AKXD RI mice develop spontaneous lymphomas of various pathological forms [4]. We tried to determine whether the above locus is involved in the determination of the type of lymphoma by examining the allele type of microsatellite loci in the *Tlsm-1* segment on chromosome 7. Out of 20 AKXD RI



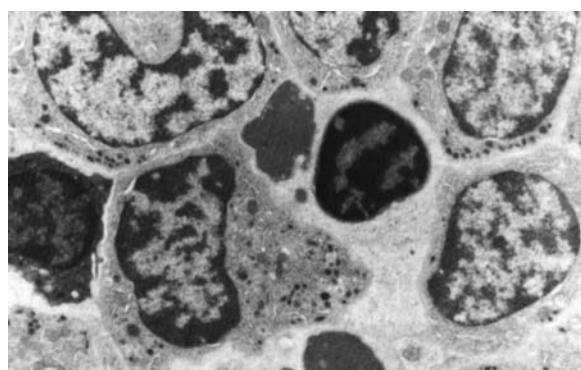
**Fig. 9.5** Genetic and epigenetic determination of types of lymphomas in a cross of SL/Kh × AKR [13]. Chromosome number for *Tlsm-1* is shown in parentheses

strains, 9 strains with high T-lymphoma incidence had an AKR-derived allele between *D7Mit71* and *D7Mit13*, where *Tlsm-1* is located [38]. It is highly likely that AKR mice have a dominant locus for susceptibility to T lymphomas rather than B lymphomas not only in crosses to SL/Kh but also in those to DBA/2J.

The length of the latent period is determined by *MHC* rather than by *Tlsm-1* [38]. By quantitative trait locus (QTL) analysis of the latent period of lymphomas in the F2 intercross, a locus on chromosome 17 was mapped with an LOD score of 7 and called *Lla* (*lymphoma latency acceleration*) [17]. The QTL peak for *Lla* is located in the MHC class II. Homozygosity of the SL/Kh recessive allele of *Lla* is associated with significant shortening of lymphoma latency. The MHC haplotype of SL/Kh is q, which has a defect in I-E. SL/Kh mice responded well to the I-A-dependent antigen but not to the I-E-dependent antigen (Shimada, unpublished observation). The MHC class II molecule is involved in an immune response to a retrovirus-enveloping antigen. A defect in I-E may reduce the immune response to an infectious virus.

#### 9.4.3 Unusual Mixed-Type Lymphoma in Thymectomized (SL/Kh × AKR)F1 Mice

To study the role of the thymus in T lymphomagenesis in (SL/Kh × AKR)F1 mice, we compared the lymphomas in F1 mice intact or thymectomized at 3–5 days of age [23]. In AKR, thymectomy remarkably reduces the incidence of lymphomas except for the low incidence of B1 lymphomas after a long latent period [31]. In contrast, thymectomy does not reduce lymphoma development in (SL/Kh × AKR)F1. Of 39 intact F1 mice, 36 developed lymphomas (92.3%), of which 30 were T lymphomas. In contrast, 39 of 41 thymectomized mice (95.1%) developed lymphomas with an unusual phenotype, NK1.1<sup>+</sup>Mac1<sup>+</sup> CD16<sup>+</sup>. Resembling large granular lymphocytes (Fig. 9.6), these lymphoma cells had large lysosomal granules and expressed IL4, perforin, and interferon-γ. To our knowledge, unusual NK1<sup>+</sup> B1 lymphomas have not been reported.



**Fig. 9.6** Large granular-cell lymphomas with unusual NK-B phenotype in thymectomized (SL/Kh × AKR)F1 mice [23]. Electron microscopy,  $\times 5000$

An increase of NK or NK-T cells is known in thymectomized mice, suggesting that the thymus may well play a role in negative regulation to NK-related cells. However, grafting of a newborn thymus from AKR or SL/Kh to thymectomized F1 mice at 4 weeks of age did not prevent NK1<sup>+</sup> B1 lymphomas from developing (Lu, unpublished data). Further study is thus indicated to understand the role of the thymus in modulating the type of lymphomas.

#### **9.4.4 Other Host Loci Affecting Lymphomagenesis**

Subsequently, we explored other host loci affecting lymphomagenesis in crosses to a variety of inbred laboratory mice [2]. F1 mice between SL/Kh and low-lymphoma strains, such as BALB/c, B10, NZB, A/J, C3H, CBA, SJL, DBA/2, and MSM/Ms, expressed endogenous ecotropic MuLV depending on the allelotype of *Fv1*. F1 mice with BALB/c (*Fv1<sup>b</sup>*), B10 (*Fv1<sup>b</sup>*), or NZB (*Fv1<sup>m</sup>*) did not show virus expression, whereas those with C3H, CBA, SJL, DBA/2, or MSM/Ms (*Fv1<sup>n</sup>*) did. In these F1 mice, lymphoma incidence was very low or null. For lymphoma development, the *Fv1* allelotype and ecotropic MuLV expression are not sufficient, but there should be dominant resistance to lymphomagenesis in low-lymphoma strains.

##### **9.4.4.1 Two Dominant Resistance Loci in MSM/Ms Mice**

The MSM/Ms is an inbred mouse strain established from Japanese wild mice, *Mus. Molossinus*. This strain is the most remote from laboratory mice from a genetic standpoint [1]. During 2 years of observation, no spontaneous tumor was observed. As stated above, (SL/Kh × MSM)F1 expressed ecotropic virus expression, but lymphoma development was tightly suppressed [2]. A genome-wide screening for the resistant loci was carried out in 60 SL/Kh × (SL/Kh × MSM)F1, and we mapped two loci *Msmr1* and *Msmr2* on chromosomes 17 and 18, respectively. Of 14 backcross mice developing pre-B lymphomas, 13 were homozygous for the SL/Kh allele at both loci. The map location of *Msmr1* showed overlapping with those of *MHC*, *Esl-1*, and *Lla*. In *Esl-1* and *Lla*, the SL/Kh allele was associated with dominant susceptibility. Judging from the mode of inheritance, *Msmr1* is distinct from *Esl-1* and *Lla*. This, however, does not exclude the possibility that *Msmr1* is a part of *MHC*. Candidate genes for *Msmr2*, *li*, and *CD14* are being considered, but so far, no direct evidence has been obtained.

##### **9.4.4.2 Lymphoma Resistance of the SL/Ni Strain**

The SL/Ni strain is a member of the SL family and is closely related to the SL/Am strain. They have been carefully maintained as an inbred strain, but from 1970 to 1990, a variety of changes in the virus–host relationship were observed. When

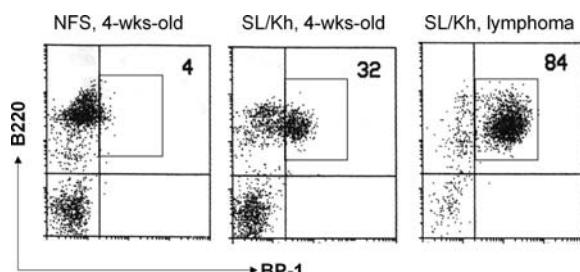
SL/Ni mice [28] were obtained from Nishizuka at the Aichi Cancer Center Research Institute (Nagoya, Japan), some SL/Ni mice expressed a high level of endogenous ecotropic virus, but others did not [9]. We found that the virus-negative individuals had a maternal resistance factor (MRF), namely, a natural antibody to ecotropic virus Gp70. A similar factor has been described regarding the RF strain [26, 27]. This factor is transmitted from mother to pups via maternal milk. Therefore, newborn mice from a highly viremic mother became virus-free when they were nursed by a virus-negative foster mother or injected with the serum of virus-free SL/Ni mice immediately after birth. When SL/Kh newborn mice were injected with an MRF-containing serum, ecotropic virus expression was intensely inhibited, but xenotropic virus expression was not affected. Pre-B-lymphoma development was remarkably delayed [9]. This is another clue providing evidence that the expression of an ecotropic virus is essential for SL/Kh pre-B lymphomas.

SL/Ni mice without MRF express an ecotropic virus as highly as SL/Kh mice, but they develop follicular center cell lymphomas or myeloid leukemias at a very low incidence (<10% at 18 months of age). Genetic analysis of (SL/Kh × SL/Ni)F1 × SL/Ni backcross mice revealed that lymphoma resistance of SL/Ni is associated with the homozygosity of recessive SL/Ni allele at locus *nir1* on chromosome 4 weakly but significantly [33]. The map position of *nir1* shows overlapping with that of *foc-1* described previously [37], but it remains obscure whether or not these are identical.

## 9.5 Bone Marrow Pre-B-Cell Expansion

### 9.5.1 Genetically Determined Pre-B-cell Expansion

Studying the characteristics of a hemo-lymphopoietic system of SL/Kh, we noted that young SL/Kh mice have an unusually high proportion of pre-B cells among BM lymphocytes [30]. In most laboratory strain mice, BP1<sup>+</sup>B220<sup>+</sup> pre-B cells in BM are 2–5%, whereas the percentage in SL/Kh is nearly 30% (Fig. 9.7). The percentage of pre-B cells changes by age, with a peak at 4–6 weeks. Thereafter, it somehow declines, but after 12 weeks of age, it increases,



**Fig. 9.7** Bone marrow pre-B cells in NFS (4-week-old), SL/Kh (4-week-old), and a SL/Kh lymphoma [8]. Percentage of BP1<sup>+</sup>B220<sup>+</sup> cells is shown in right upper quadrant

and pre-B lymphomas ultimately develop. These observations suggest that pre-B expansion in BM may be a precursor lesion of lymphoma or abnormal differentiation of early B cells predisposing to lymphomagenesis, as suggested in Abelson virus-injected mice [35] and  $E\mu$ -*myc* transgenic mice [22]. When either of these hypotheses is correct, pre-B lymphomagenesis in SL/Kh mice should occur in two steps.

We next set out to determine whether pre-B expansion is induced by ecotropic MuLV essentially required for lymphomagenesis. To examine this hypothesis, we prepared F1 hybrids of SL/Kh and C4W, a BALB/c congenic for *Fv4*, and used (SL/Kh × BALB/c)F1 as a control. *Fv4* is a fragment of the Gp70 gene derived from wild-mice MuLV and is inherited as a dominant host gene [29]. The product of *Fv4* binds a cellular receptor for an ecotropic virus and thus, intensely inhibits infection by an ecotropic virus [15]. The level of an endogenous ecotropic virus is also suppressed as horizontal infection is inhibited. In (SL/Kh × C4W)F1, an ecotropic virus was not detectable, but the expansion of pre-B cells was as strong as that in (SL/Kh × BALB/c)F1 mice. Furthermore, pre-B cells in SL/Kh BM were not reduced when the expression of ecotropic MuLV was inhibited by neonatal injection of the MRF. These observations exclude the hypothesis that pre-B cell expansion is induced by ecotropic MuLV.

To determine whether the pre-B cell expansion is a genetic property of BM stem cells or is induced by BM microenvironments, we produced reciprocal radiation chimeras between SL/Kh and BALB/c mice by transferring BM cells and one month later, evaluating the percentage of pre-B cells. The lethally irradiated and BALB/c and SL/Kh mice receiving SL/Kh BM cells showed a high level of pre-B cells, whereas those receiving BALB/c BM cells did not. Therefore, the genetic property of BM stem cells is essential for pre-B-cell expansion [30].

The levels of pre-B cells in BM of 4-week-old (SL/Kh × NFS)F1 and (NFS × SL/Kh)F1 mice are comparable and an intermediate between those of SL/Kh and NFS, suggesting that the mode of inheritance is semidominant or polygenic. We measured the percentage of BP1<sup>+</sup>B220<sup>+</sup> cells in BM of 4-week-old (SL/Kh × NFS)F2 and SL/Kh × (SL/Kh × NFS)F1 mice. A genome-wide screening for QTL responsible for increased pre-B cells revealed a QTL peak on chromosome 3 with an LOD score 22.0. It was named as *Bomb-1* (*bone marrow pre-B-1*) [25].

### 9.5.2 Pre-B-Cell Expansion is Not Sufficient for Lymphomagenesis

Determining the significance of pre-B-cell expansion in SL/Kh pre-B lymphomagenesis is important. The fact that expanded pre-B cells show a similar phenotype to lymphoma cells suggests the hypothesis that they represent an early lymphoma and start to grow with an appropriate second hit, for instance, the insertion of a retrovirus genome, but are destined to die without this second hit. The second hypothesis is that the transient pre-B-cell expansion is due to a

genetic abnormality that expands the target cells for virus insertion. To examine these possibilities, we generated NFS mice into which a chromosomal segment carrying SL/Kh *Bomb-1* was introgressed using a speed congenic procedure [14]. The NFS.SL/Kh-*Bomb-1* mice showed BM pre-B expansion at an equivalent level to that of SL/Kh mice. However, none of the NFS.SL/Kh-*Bomb-1* mice developed lymphomas within 1 year of observation. Injection of infectious ecotropic MuLV from an SL/Kh lymphoma to neonates induced viremia but not lymphoma.

To determine whether or not BM pre-B cells in the prelymphoma stage are clonal, BP1<sup>+</sup>B220<sup>+</sup> cells were collected from BM of SL/Kh and NFS.SL/Kh-*Bomb-1* at 4, 10, and 15 weeks of age using a cell sorter, and the Ig heavy-chain gene was examined for recombination by PCR Southern blot. The pre-B cells in NFS.SL/Kh-*Bomb-1* remained polyclonal at all ages, whereas in SL/Kh, a monoclonal population appeared in SL/Kh at 10 weeks of age and thereafter, gradually prevailed. Therefore, the pre-B expansion induced by *Bomb-1* is polyclonal in nature and not sufficient in itself for lymphoma development [14]. As previously reported, in the cross between SL/Kh and NFS, a dominant SL/Kh allele at *Esl-1* [37] is required for a pre-B lymphoma to occur. This may explain why NFS.SL/Kh-*Bomb-1* mice failed to develop lymphomas.

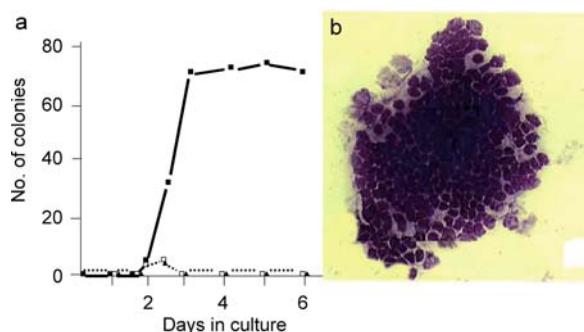
Although the gene for the *Bomb-1* locus has not been identified, it is a useful target for the investigation of the growth requirement of the normal early B lymphocytes as well as their lymphomagenesis.

## 9.6 Molecular Pathogenesis: Retrovirus Integrations in Lymphoma

The etiologic role of endogenous ecotropic MuLV in SL/Kh lymphomagenesis has been recognized since earlier genetic analysis [37], as has the inhibition of lymphomagenesis by MRF [9]. However, its molecular mechanism remained obscure until the start of systemic study on viral genome integration at strategic sites in the DNA of target cells. After the introduction of inverse PCR technology, we amplified the virus–host junctions and characterized the host flanking sequence very effectively. In this way, a number of cancer-related genes, including *Stat5a*, *Evi3*, *c-myc*, *N-myc*, and *Stat5b*, were identified as genes activated by provirus integration. The plethora of genes activated by retrovirus in SL/Kh pre-B lymphomas was beyond our expectations to some extent.

In SL/Kh pre-B lymphomas, *Stat5a* is the first gene found to be activated by provirus integration [36]. *Stat5a* is translated from the third exon, and the integrations are concentrated in the second intron of *Stat5a*; therefore, it is called *Svi-1* (*SL/Kh virus integration-1*). In *Svi-1* lymphomas, *Stat5a* mRNA and the STAT5a protein are expressed at a high level. The intensely phosphorylated STAT5a protein is translocated to the nucleus, where it binds to an interferon-γ-activated sequence (GAS) element on host genomic DNA, as

**Fig. 9.8** (a) Kinetics of colony formation by pre-B cells after transfection of SL/Kh BM cells with constitutively activated mutant Stat5a cDNA [36]. Closed square, transfected with mutant Stat5a cDNA; open square, with wild type Stat5a cDNA; closed triangle, mock transfection. (b) A colony of pre-B cells in semisolid medium [36]. (See color insert)



shown by a gel-shift assay. Genes with a GAS element, such as *c-myc*, *pim1*, and *Bcl<sub>XL</sub>*, increase their expression. Such genes are known to have anti-apoptotic activity.

The *Svi-1* lymphomas express B220, BP1, CD19, CD24, CD43, Ig $\alpha$ , and IL7R and thus share the characteristics of pre-B lymphocytes. On the other hand, many non-*Svi-1* lymphomas are CD43 $^+$ IL7R $^-$ ; thus, they seem to be pre-B lymphocytes in a later stage of maturation. The activation of STAT5a in *Svi-1* lymphoma occurs via the IL7-signaling pathway because the addition of IL7 to cultured *Svi-1* lymphoma cells intensifies the phosphorylation of STAT5a.

Evidence for the direct contribution of activated STAT5a to lymphomagenesis is given by the fact that transfection of constitutively activated mutant *Stat5a* cDNA to normal SL/Kh BM cells induces selective colonial growth of pre-B cells in a semisolid medium (Fig. 9.8a, 9.8b) [36]. By transfection of wild-type *Stat5a* cDNA or mock transfection, pre-B cells die and colonial growth fails to occur. The presence of IL7 is essential for the early stage of in vitro growth of the transfectant. On transfer to an IL7-free medium, within the first 48 hours in the culture, the transfected BM cells quickly become apoptotic, but after 96 hours, they lose dependence on IL7.

The transfectants growing in colonies express STAT5a intensely and also B220, BP1, CD19, CD24, CD43, and IL7R. The phenotype is common to *Svi-1* lymphoma cells and early pre-B cells in normal BM. Colonial growth of pre-B cells by constitutively activated mutant *Stat5a* cDNA occurs exclusively in SL/Kh BM, but not in NFS, C57BL/6, C3H, or AKR BM. This indicates that some host factor proper to SL/Kh is essential for this phenomenon, but its nature has not been clarified.

## 9.7 Discussion

We have shown that pre-B lymphomagenesis in SL/Kh strain mice is an excellent model of multifactorial diseases. Reintegration of endogenous ecotropic MuLV to several strategic sites for pre-B cell growth, including *Stat5a*,

*Evi3*, *c-myc*, *N-myc*, and *Stat5b*, is pathogenetic. From the viewpoint of a pathobiologist, the attention here has been focused on host genetic and epigenetic factors affecting pre-B lymphomagenesis. As reviewed in this article, virus expression, immunity to virus and their spread, types of lymphomas, and growth regulation of the BM pre-B cells, which are possible target cells of lymphomagenesis, are under host control. In SL/Kh, all these steps are destined to favor the development of pre-B lymphomas. Most of this study was conducted in the 1990s, before the author's retirement from Kyoto University. Regrettably, none of these host loci have been cloned; therefore, their functions have not been clarified at the molecular level. It is obvious that future study should be directed to the molecular cloning of these genes. Research involving *Esl-1*, *foc-1*, *Tlsm-1*, and *Bomb-1* would be particularly rewarding. SL/Kh, SL/Ni, SL/Kh.AKR-*Tlsm-1*, NFS.SL/Kh-*foc-1*, and NFS.SL/Kh-*Bomb-1* mice are maintained either as live stocks or frozen embryos at the National Bioresource Center in RIKEN Tsukuba Institute (<http://www.brc.riken.go.jp/lab/animal/>) and are available on request.

**Acknowledgments** I am very grateful to all of my colleagues who have contributed to the understanding of pre-B lymphomagenesis in SL/Kh mice. This study was supported by grants-in-aid for Scientific Research by the Ministry of Education, Culture, Sports, and Science and Grants for Cancer Research by the Ministry of Health, Labor, and Welfare, Japan.

## References

1. Abujiang P, Yamada Y, Haller O, Kobayashi K, Kamoto T, Lu L-M, Ogawa M, Ishimoto A, Katoh H, Kanehira K, Ikegami S, Fukumoto M, Hiai H (1996a) The origin of the SL family mice. *Lab Anim Sci* 46: 410–417
2. Abujiang P, Kamoto T, Lu L-M, Yamada Y, Hiai H (1996b) Two dominant resistance genes to pre-B lymphoma in a wild-derived inbred mouse strain MSM/Ms. *Cancer Res* 56: 3716–3720
3. Fredrickson TN, Morse HC 3rd, Yetter RA, Rowe WP, Hartley JW, Pattengale PK (1985) Multiparameter analyses of spontaneous nonthymic lymphomas occurring in NFS/N mice congenic for ecotropic murine leukemia viruses. *Am J Pathol* 121:349–360.
4. Gilbert DJ, Neumann PE, Taylor BA, Jenkins NA, Copeland NG (1993) Susceptibility of AKXD recombinant inbred mouse strains to lymphoma. *J Virol* 67: 2083–2090.
5. Gross L (1958) Viral etiology of spontaneous leukemia: a review. *Cancer Res* 18: 371–381.
6. Hartley JW, Chattopadhyay SK, Lander MR, Tadesse-Heath L, Naghashfar Z, Morse HC 3rd, Fredrickson TN (2000) Accelerated appearance of multiple B cell lymphoma types in NFS/N mice congenic for ecotropic murine leukemia viruses. *Lab Invest* 80:159–169
7. Hartley JW, Wolford NK, Old LJ, Rowe WP (1977) A new class of murine leukemia virus associated with development of spontaneous lymphomas. *Proc Natl Acad Sci USA* 74: 789–792.
8. Hiai H (1996) Genetic predisposition to lymphomas in mice. *Pathol Intern* 46: 707–718
9. Hiai H, Buma YO, Ikeda H, Moriwaki K, Nishizuka Y (1987) Epigenetic control of endogenous ecotropic virus expression in SL/Ni strain mice. *J Natl Cancer Inst* 79: 781–787.

10. Hiai H, Kaneshima H, Nakamura H, Oguro BY, Moriwaki K, Nishizuka Y (1982) Unusually early and high rate of spontaneous occurrence of non-thymic leukemias in SL/Kh mice, a subline of SL strain. *Jpn J Cancer Res* 73: 603–613.
11. Hiai H, Nishi Y, Miyazawa T, Matsudaira Y, Nishizuka Y (1981) Mouse lymphoid leukemias: Symbiotic complexes of neoplastic lymphocytes and their microenvironments. *J Natl Cancer Inst* 66: 703–722.
12. Hiai H, Nishizuka Y (1981) Growth stimulation of microenvironment-dependent mouse leukemias by tumor-promoting phorbol esters. *J Natl Cancer Inst* 67: 1333–1340.
13. Hiai H, Tsuruyama T, Yamada Y (2003) Pre-B lymphomas in SL/Kh mice: A multi-factorial disease model. *Cancer Sci* 94: 847–850
14. Hiratsuka T, Tsuruyama T, Kaszynski R, Kometani K, Minato N, Nakamura T, Tamaki K, Hiai H (2008) Bone marrow pre-B expansion by SL/Kh Bomb1 locus: Not sufficient for lymphomagenesis. *Leuk Res* 32: 309–14
15. Ikeda H, Odaka T (1983) Cellular expression of murine leukemia virus gp70-related antigen on thymocytes of uninfected mice correlates with *Fv4* gene-controlled resistance to Friend leukemia virus infection. *Virology* 128: 127–139
16. Jin G, Tsuruyama T, Yamada Y, Hiai H (2003) *Svi3*: a provirus common integration site in c-myc of SL/Kh pre-B lymphomas. *Cancer Sci* 94: 791–795
17. Kamoto T, Shisa H, Abujiang P, Lu L-M, Yoshida O, Yamada Y, Hiai H (1996) A quantitative trait locus in major histocompatibility complex determining latent period of mouse lymphomas. *Jpn J Cancer Res* 87: 401–404
18. Kaneshima H, Hiai H, Fujiki H, Iijima S, Sugimura T, Nishizuka Y (1983) Teleocidine-induced modulation of growth and cell interaction in microenvironment-dependent mouse leukemias. *Leuk Res* 7: 287–293.
19. Kaneshima H, Hiai H, Fujiki H, Oguro BY, Iijima S, Sugimura T, Nishizuka Y (1983) Tumor-promoter dependent mouse leukemia cell line. *Cancer Res* 43: 4676–4680
20. Kaneshima H, Ito M, Asai J, Taguchi O, Hiai H (1987) Thymic epithelial reticular cell sub-populations in mice defined by monoclonal antibodies. *Lab Invest* 56: 372–380
21. Kawashima K, Ikeda H, Hartley JW, Stockert E, Rowe WP, Old LJ (1976) Changes in expression of murine leukemia virus antigens and production of xenotropic virus in the late preleukemic period in AKR mice. *Proc Natl Acad Sci USA* 73: 4680–4684
22. Langdon WY, Harris AW, Cory S, Adams JM (1986) The *c-myc* oncogene perturb B lymphocyte development in *Eμ-myc* transgenic mice. *Cell* 47: 11–18
23. Lu L-M, Hiai H (1999) Mixed phenotype lymphomas in thymectomized (AKR x SL/Kh) F1 mice. *Jpn J Cancer Res* 90: 1218–1223
24. Lu L-M, Ogawa M, Kamoto T, Yamada Y, Abujiang P, Hiai H (1997) Expression of LECAM-1 and LFA-1 on pre-B lymphoma cells but not on preneoplastic pre-B cells in SL/Kh mice. *Leuk Res* 21: 337–342
25. Lu L-M, Shimada M, Higashi S, Zeng Z-Z, Hiai H (1999) Bone marrow pre-B-1 (Bomb1): a quantitative trait locus inducing bone marrow pre-B cell expansion in lymphoma-prone SL/Kh mice. *Cancer Res* 59: 2593–2595
26. Mayer A, Struick FD, Duran-Reynals ML, Lilly F (1980) Maternally transmitted resistance to lymphoma development in mice of reciprocal crosses of the RF/J and AKR/J strains. *Cell* 19: 431–436.
27. Melamedoff M, Lilly F, Duran-Reynals ML (1983) Suppression of endogenous murine leukemia virus by maternal resistance factor. *J Exp Med* 158; 506–14
28. Nishizuka Y (1979) Origin and use of SL strain mice: an animal model of disease. *Exp Anim* 28: 185–191 (In Japanese)
29. Odaka T, Ikeda H, Yoshikura H, Moriwaki K, Suzuki S (1981) *Fv-4*: Gene controlling resistance to NB-tropic Friend leukemia virus. Distribution in wild mice, introduction into genetic background of BALB/c mice and mapping of chromosome. *J Natl Cancer Inst* 67: 1123–1127

30. Okamoto K, Yamada Y, Shimada MO, Nakakuki Y, Nomura H, Hiai H (1994) Abnormal bone marrow B-cell differentiation in pre-B lymphoma-prone SL/Kh mice. *Cancer Res* 54:399–402
31. Peled A, Haran-Ghera N (1985) High incidence of B cell lymphomas derived from thymectomized AKR mice expressing TL.4 antigen. *J Exp Med* 162:1081–1086.
32. Shimada MO, Yamada Y, Nakakuki Y, Okamoto K, Fukumoto M, Honjo T, Hiai H. (1993) SL/Kh strain mice: A novel animal model of pre B lymphomas. *Leuk Res* 17: 573–578
33. Shisa H, Yamada Y, Kawarai A, Terada N, Kawai M, Matsushiro H, Hiai H (1996) Genetic and epigenetic resistance of SL/Ni mice to lymphomas. *Jpn J Cancer Res* 87: 258–262
34. Stoye JP, Moroni C, Coffin JM (1991) Virological events leading to spontaneous AKR thymomas. *J Virol* 65: 1273–1285.
35. Tidmarsh G, Dailey MO, Whitlock CA, Pilemer E, Weissman IL (1985) Transformed lymphocytes from Abelson-diseased mice express levels of a lineage transformation associated antigen elevated from that found on normal lymphocytes. *J Exp Med* 162: 1421–1343
36. Tsuruyama T, Nakamura T, Jin G, Ozeki M, Yamada Y, Hiai H (2002) Constitutive activation of Stat5a by retrovirus integration in early pre-B lymphomas of SL/Kh strain mice. *Proc. Natl. Acad. Sci. USA* 99: 8253–8258
37. Yamada Y, Shimada MO, Toyokuni S, Okamoto K, Fukumoto M, Hiai H (1994) Genetic predisposition to pre-B lymphoma in SL/Kh strain mice. *Cancer Res* 54:403–407
38. Yamada Y, Shisa H, Matsushiro H, Kamoto T, Kobayashi Y, Kawarai A, Hiai H (1994) T-lymphomagenesis is determined by a dominant host gene Thymic Lymphoma Susceptible Mouse-1 (Tlsm-1) in murine models. *J Exp Med* 180: 2155–2162

# **Chapter 10**

# **Animal Cancer Models in Anticancer Drug Discovery and Development**

**Francis Lee and Roberto Weinmann**

## **Contents**

10.1	Introduction .....	245
10.2	Identifying the Main Causes of Failure of Anticancer Drug Candidates .....	246
10.3	The Modern Paradigm for Anticancer Drug Discovery .....	247
10.4	Typical Protocol for Drug Evaluation Utilizing Tumor Xenografts-Commonality and Variables .....	248
10.4.1	Site of Implant .....	248
10.4.2	Origin of the Model .....	249
10.4.3	Study Endpoints .....	250
10.5	The Value of the Xenograft Models in Contemporary Cancer Drug Discovery .....	252
10.6	Taxane Resistance in Breast Cancer .....	253
10.7	Imatinib Resistance in Chronic Myeloid Leukemia .....	255
10.8	Summary .....	257

### **10.1 Introduction**

The prevention and treatment of cancer continues to pose great challenges to modern medical science. Long dreaded, cancer remains one of most lethal diseases in the United States and is poised to overtake heart diseases as the most common cause of death in the very near future [1]. Despite great advances over the last 50 years in our understanding of the cause (the genetics of cancer) and pathological progression (the physiology of cancer), efforts in the clinic to effectively control the disease have met with uneven successes in prolonging patients survival, ranging from the highly effective (in the treatment of even advanced stages of choriocarcinoma, testicular cancers, and some lymphomas), to the partially effective (when used as an adjuvant to surgery in the early stages of some common carcinomas of adults), to the modest at best (in the treatment

---

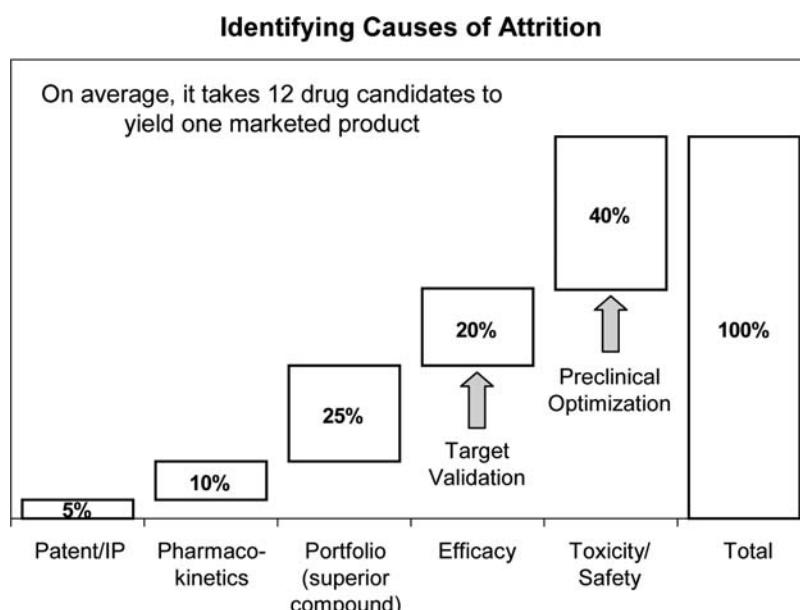
F. Lee

Bristol-Myers Squibb Research and Development, Oncology Discovery, Princeton,  
NJ 08543, USA  
francis.lee@bms.com

of the most common advanced stages adult solid malignancies). In recent years, through unprecedented collaborative efforts between academic- or government-funded laboratories and the pharmaceutical industries, the discovery and development of anticancer drugs has undergone a drastic change. The commonly held perception regarding the slow pace of therapeutic advances is rapidly changing with the recent successful drugs such as Herceptin (trastuzumab), Gleevec (imatinib), Erbitux (cetuximab), Avastin (bevacizumab), and Sprycel (dasatinib). Nonetheless, the overall success with oncology drug discovery and development in recent years has been mixed. Indeed, the failure rates for oncology drugs in the clinic are among the worst overall in comparison with other disease areas, with only 5% of drugs that enter the clinic make it to marketing approval [2] compared with the pharmaceutical industry overall success rate of 11%. Although this book focuses on blood cancers, it is necessary to use some examples for solid tumors to help express our points.

## 10.2 Identifying the Main Causes of Failure of Anticancer Drug Candidates

Why do drug development candidates fail? Some of the main reasons for failure have been identified and it is informative to review this (Fig. 10.1). Patents and intellectual property issues are responsible for 5% of the total causes of attrition.



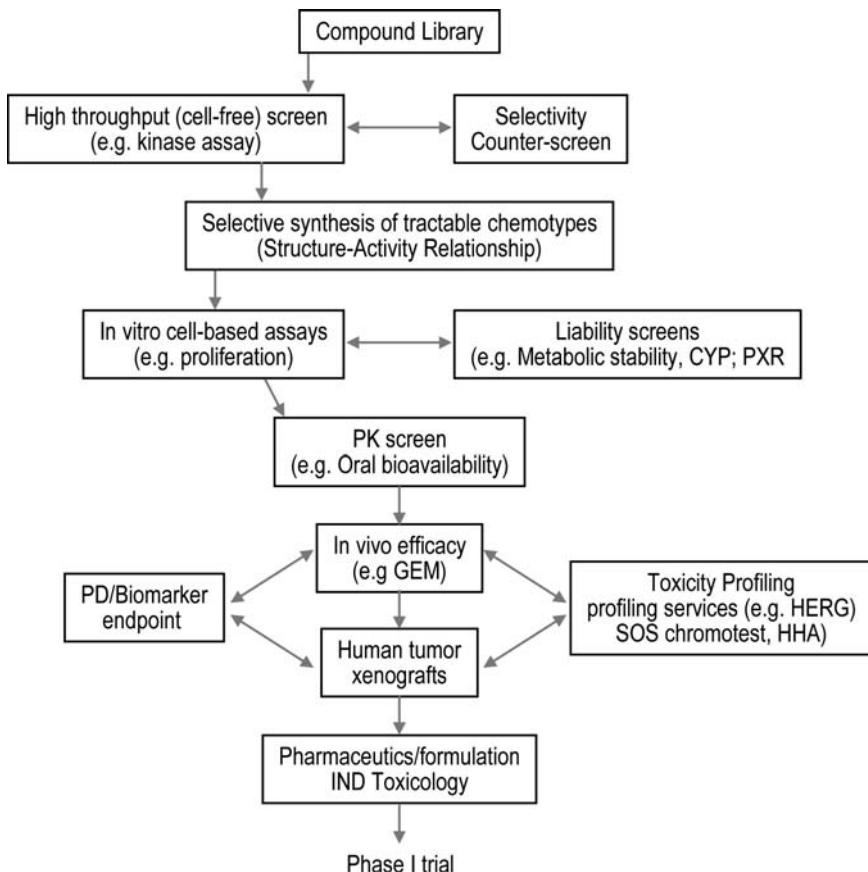
**Fig. 10.1** Rates and causes for attrition of clinical candidates during discovery and development in oncology

In addition, approximately 25% of compounds are terminated for portfolio reasons. Some of this attrition represents changes in internal strategy, while other terminations are due to changes in the commercial environment. Before the 1990s, drug candidates frequently failed (at a rate of 25%) due to poor pharmacokinetic behavior, that is, the inability to deliver the required amount of drug to the target site for the required amount of time. This may be due to insufficient oral bioavailability or to differences in pharmacokinetics or metabolism between test animals and human subjects. With the availability of sophisticated technology of monitoring drug fate in biologic samples (e.g., HPLC-MS) and powerful PK modeling tool [3], this deficiency in drug development had been greatly improved and accounts for approximately only 10% of attrition for the last 10 years compared to over 25% 15 years ago. The last two causes of attrition, namely lack of efficacy and/or toxicity and safety concerns, represent approximately 60 percent of attrition. Moreover, lack of efficacy in the target disease at maximum-tolerated dose (MTD), which usually only becomes evident late in development and is therefore costly, is a failure of on-target hypotheses and should be addressable by rigorous target identification and validation during the drug discovery process. The inability to gain signal during early development about the efficacy of a candidate compound, clearly points to a deficiency of the animal models utilized to evaluate its developmental potential. Thus, a last portion of the risks can be reduced by identifying better predictive cancer models.

### 10.3 The Modern Paradigm for Anticancer Drug Discovery

To gain a better understanding of how more predictive animal models may help to reduce risk, it is instructive to gain an understanding of the sea of change that has taken place in the pharmaceutical industry with regard to drug discovery approach. The modern paradigm for anticancer drug discovery comprises a series of carefully constructed steps that are designed to rapidly and efficiently allow the demonstration of the so-called proof of principle of a particular target, through the evaluation of large number of pharmaceutically tractable molecules, culminating in phases I and II clinical trials of the final drug candidate (Fig. 10.2). This cascade of steps, called a decision network, can be envisioned as molecules feeding into a series of iterative stop/go tests of increasing biological complexity. The concept of “therapeutic index,” that is the demonstration of antitumour efficacy at doses well below those causing severe toxicities, is also a long-established paradigm in preclinical drug discovery [4]. A key part of the preclinical stage of the process, and often representing a significant bottleneck, is the demonstration of antitumour efficacy in a “relevant” tumour model *in vivo*.

Mouse cancer models are critical tools for elucidating mechanisms of cancer development, as well as for assessment of putative cancer therapies. However, validation studies of these model systems for their ability to adequately predict therapeutic responses in patients have been rare [5]. Consequently, design and interpretation of preclinical studies for tumor modeling must be undertaken



**Fig. 10.2** Example of the flow of compounds in a decision network for drug discovery in oncology

carefully. This chapter briefly reviews the most commonly used transplanted tumor models (the human tumor xenografts). It also reviews commonly utilized *in vivo* study endpoints. Even small improvements in predictive value achieved through careful selection of models and endpoints have the potential to have large impacts on productivity and overall drug development costs.

## 10.4 Typical Protocol for Drug Evaluation Utilizing Tumor Xenografts—Commonality and Variables

### 10.4.1 Site of Implant

Most transplantable human tumor xenografts are placed heterotopically (ectopically) in host systems. Tumor lines in use have been specifically selected

for mutations that allow heterotopic growth in mice. Although these tumors will grow and respond to therapeutics, the selection of the transplantation site may modulate tumor growth [6] and success of therapeutic intervention [7]. The subcutaneous (SC) site is by far the most commonly utilized for primary tumors for reasons of accessibility, lack of distress and interference with mobility in mice, and visibility for monitoring. Generally, SC refers to placement by injection or surgical implantation in the flank, a region referring to the posterior lateral abdominal quadrant. Placement is generally done in fat and mammary gland tissues near popliteal, inguinal, or accessory axillary lymph nodes.

#### ***10.4.2 Origin of the Model***

From the early days, human tumour xenografts were established either by direct implantation of patient biopsy material or via inoculation of continuous human tumour cell lines. A particularly important large panel of xenografts, derived directly from biopsies, has been established by HH Fiebig and colleagues at the University of Freiburg in Germany [8]. More than 1600 tumors have been transplanted SC into nude mice and more than 300 xenografts established, representative of all of the major tumour types [8].

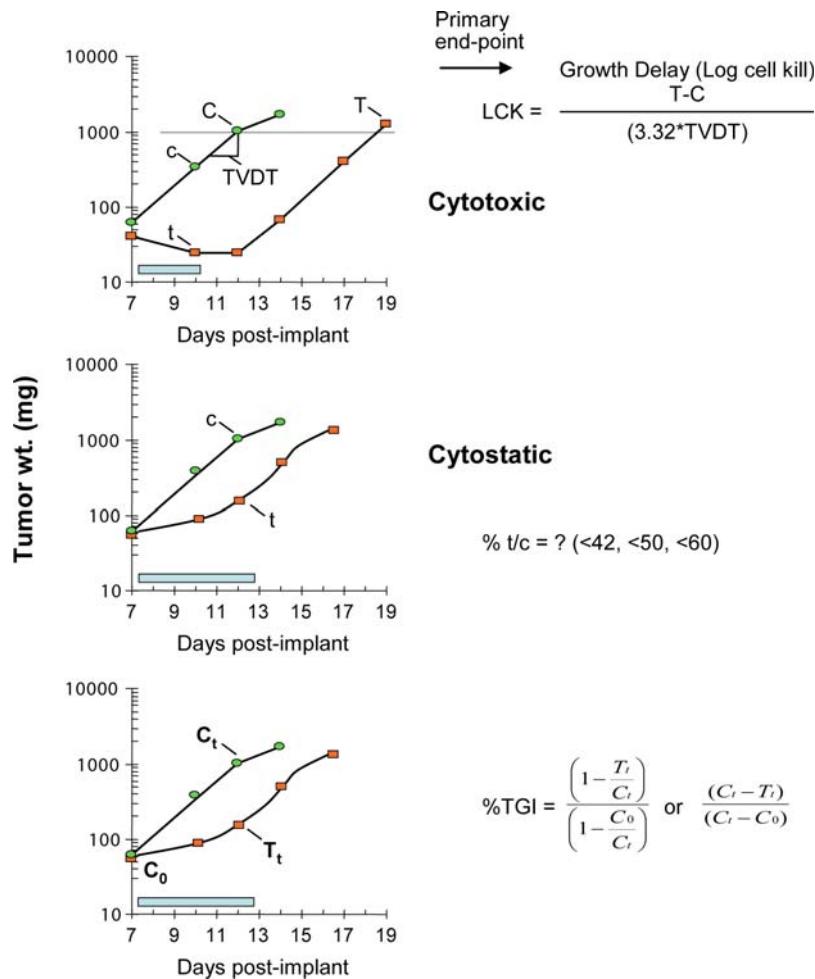
A comparison of drug response in the xenograft compared with that in the patient was made in 80 cases in 55 xenografts using either an *in vivo* assay (a comparison of treated versus control tumour volumes) or *ex vivo* using a soft agar clonogenic assay from disaggregated tumors. In accordance with the earlier studies of Steel and colleagues alluded to above, the xenografts predicted correctly for clinical response in 19/21 (90%) of occasions when using the *in vivo* assay (this was reduced a little to 60% using the clonogenic assay) and predicted for resistance in 57/59 (97%) of occasions when using the *in vivo* assay (92% for the clonogenic assay) [8].

In addition, the response pattern of more recently discovered clinically active drugs, paclitaxel, gemcitabine, docetaxel, vindesine and topotecan, was determined in 187 xenografts. Overall, the five drugs induced remissions in 24% (45/187) of the xenografts studied, whereas minor regressions or no change occurred in 13% of cases while 63% (117/187) of xenografts progressed on treatment. These findings are similar to the overall response rates recorded for monotherapy clinical trials with these agents. In addition, more responses (37%) were seen in a subgroup of tumors classified by the authors as clinically sensitive (small cell and non-small cell lung, breast, head and neck, leukaemia, melanoma, non-Hodgkin's lymphoma, gastric, testis) in comparison with those designated as resistant [4%; bladder, colon, cervix, central nervous system (CNS), hepatoma, mesothelioma, ovary, pancreas, prostate, renal soft tissue sarcoma].

### ***10.4.3 Study Endpoints***

The ultimate goal of cancer chemotherapy is to reduce the tumor burden to the lowest possible level without intolerable toxicity, improve survival, and quality of life. More stringent criteria than curability of experimental tumors in mice need to be assessed to determine therapeutic efficacy. Measurement of tumor burden is the easiest and most frequently used outcomes of efficacy in preclinical studies. In reality, endpoints need to be matched to type of tumor (solid, leukemia, or metastatic), context of the study, accessibility of the implantation site, type of implantation, and therapeutic class. Simplistic criteria used in mouse models do not match criteria of partial and complete responses used in clinical oncology, and they contribute to the conflicting opinions about the relevance and predictability of mouse models. Therefore, use of other metrics and evaluations of angiogenesis, immunomodulation, metastases, and detailed histopathology need to be incorporated into most study designs for preclinical testing. Despite the technical difficulty, labor-intensive nature, and expense commonly cited as limitations for detailed examinations, the utility of mouse models is improved by multiple and appropriate endpoints.

Tumor growth inhibition studies where treatment is prophylactically administered before or on the day of tumor induction are not realistic for preclinical evaluation of clinical responses. Typically, preclinical efficacy studies use tumor growth delay in which tumors are initiated by injection or surgical implantation of cells or tissue fragments and allowed to establish for a number of days prior to initiation of treatment. Solid tumors in accessible sites are amenable to a variety of metrics that are not applicable to primary and metastatic tumors of internal organs and hematologic neoplasms. Spontaneous regressions and failure of tumors to become established may account for a small percentage of false cures. For this reason, tumor onset and progression should be monitored frequently to ensure adequate and similar tumor masses prior to treatment and monitor onset of regression in each group of a sufficient number of animals. In models with log-phase tumor growth, animals can be randomized into treatment groups after a predictable period of development, usually 4–45 days. Conversely, less well-developed tumor models may require enrollment of individual animals into the study when the tumor burden reaches a minimum size. Such enrollment studies are more difficult to evaluate. Allowing extra days for tumor growth may be as misleading as starting treatment on small tumor masses that have not yet established and show enhanced regression after onset of treatment. In vivo progression of tumor burden should be evaluated on a frequent basis. Tumor size estimates (e.g.,  $\text{length} \times \text{width}^2/2$ ) is performed with a caliper at least twice a week. Tumor response endpoint is expressed in terms of tumor growth delay (T–C value), defined as the difference in time (days) required for the treated tumors (T) to reach a predetermined target size, usually 0.5–1.0 cm<sup>3</sup> (Fig. 10.3), compared to those of the control group (C). This value has been suggested to mimic clinical endpoints and disease progression. Tumor cell kill



**Fig. 10.3** Examples of xenograft tumor response to a cytotoxic drug (*upper panel*) and the calculations required to determine log cell kill (LCK). In the case of cytostatic drugs (*two lower panels*), the relative difference between treated and controls is smaller and a therapeutic growth index (TGI) can be calculated

(net and total) can also be estimated based on assumed exponential tumor growth kinetics as follows:

And,

$$\text{Log cell kill (LCK)} = T - C \div (3.32 \times \text{tumor volume doubling time})$$

Today, in contemporary oncological therapy, there is a far less emphasis on the development of cytotoxic agent. Instead, modern chemotherapy includes

diverse approaches, with particular focus on attacking specific molecular targets where often cytostatic rather than cytotoxic effects may be predicted. Thus, a slowing of tumour growth rather than shrinkage may occur. This may require a re-evaluation of the *in vivo* models developed and validated using cytotoxic drugs when testing such agents. Targeted compounds are not potent to produce tumor regressions by themselves, as the tumor may be driven by multiple changes in its genetic makeup and only result in stasis or delay in tumor growth. Moreover, changes in response to the agent may only occur in a subset of models in which the target(s) of the agent is the driving force for tumor growth.

For targeted agents that induce cytostasis, tumor response is more appropriately measured in terms of relative growth inhibition and may be expressed as % *t/c* or more rigorously as percent tumor growth inhibition (%*TGI*) and calculated as follows:

$$\%TGI = \frac{(1 - \frac{T_t}{C_t})}{(1 - \frac{C_0}{C_t})} \text{ or } \frac{(C_t - T_t)}{(C_t - C_0)}$$

where,  $C_t$  is the median control tumor size at end of treatment,  $C_0$ , median control tumor size at treatment initiation,  $T_t$ , median tumor size of treated group at end of treatment, and  $T_0$ , median tumor size of treated group at treatment initiation.

## 10.5 The Value of the Xenograft Models in Contemporary Cancer Drug Discovery

In the post-genomic era, there has been a considerable move away from the “black-box” approach to phase I clinical trials where many agents of unknown mechanisms of action and poorly defined preclinical pharmacokinetics were introduced into the clinic. Does this necessarily mean that the xenograft model is of no further value in contemporary mechanism-directed cancer drug development? This may not be the case as long as care is taken to ensure the xenografts used are a faithful representation of the pathophysiology of the tumor of origin. In careful mechanism-based studies, combined with sound pharmacological principles (as described above), xenograft model remains of great value, both for assisting in the selection of leads for clinical evaluation and for guiding clinical studies (e.g., scheduling and combination strategies). Some of the other advantages are the speed of tumor development and response, as well as the easier timing of large cohorts of experimental animals for testing purposes.

Here, two examples will be given of where xenograft studies have proven invaluable in selecting candidate agents that ultimately received FDA approval in the last 2 years and, moreover, potentially guiding their clinical utility. It should be pointed out here that the number of new cancer drugs entering clinical

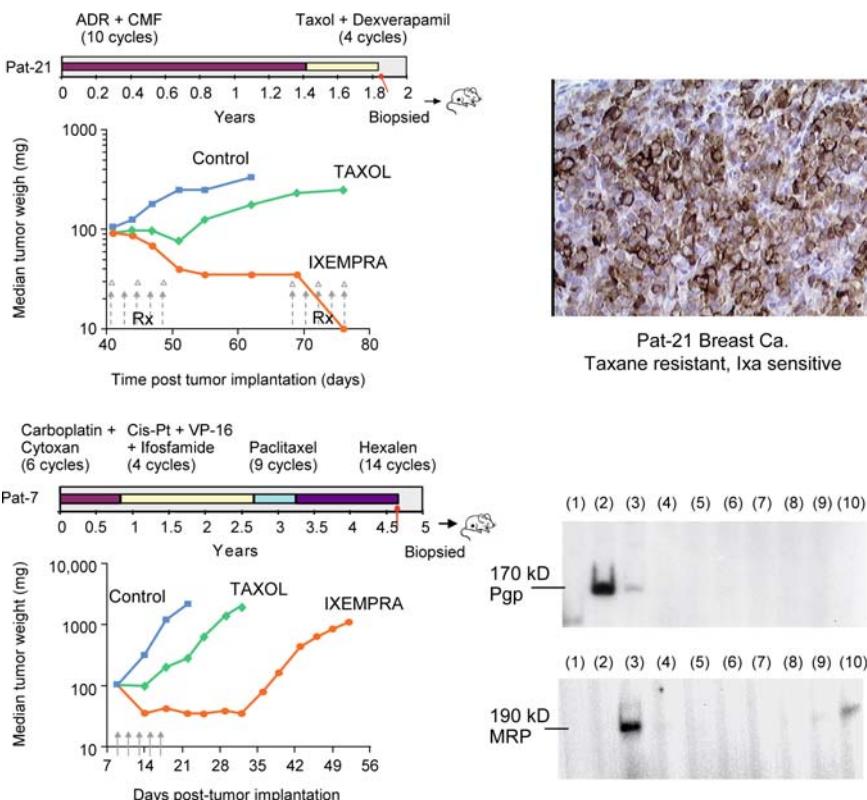
development more than doubled between the early 1990s and the mid-2000s, from 33/year to 73/year, respectively. At the same time, patients with diseases such as breast cancer where in the past effective therapy was lacking now have multiple options and will likely receive multiple lines of therapy. Thus, the hurdle for developing a successful agent that can gain regulatory approval has been raised considerably and the competition for eligible patients for clinical trials has dramatically increased. This has adversely affected approval rate in recent years and lengthened the time taken to complete the clinical development and approval process [9]. It is thus highly advantageous, if at all possible, to define the medical need (i.e., the patient population) at the outset of a drug development program. Moreover, with the increasing move of clinical oncology practice and the regulatory process toward evidence-based approach to treatment, the ability to identify the patient population that is likely to benefit and then to develop tumor models that faithfully reflect that population is likely to be attractive to investigators and regulatory agencies. Such approaches remove much of the uncertainties regarding the predictability of the models being used to predict outcome and increase the confidence that the agent under investigation may recapitulate similar degree of activity in clinical trials.

## 10.6 Taxane Resistance in Breast Cancer

The first example concerns the development of ixabepilone (Ixempra) for the treatment of metastatic breast cancer in patients who have failed prior therapy, primarily taxanes. Since the antitumor activity of the taxanes was discovered in the 1990s, the rationale for using microtubule-stabilizing agents in the treatment of cancer is undisputed. Taxanes are clinically active against a wide range of tumor types and play a key role in the treatment of both primary and metastatic breast cancer. However, resistance to cytotoxic drugs (including taxanes) is common and results in reduced response rates and ultimately disease progression in up to 90% of patients with metastatic cancer. While some tumors display intrinsic resistance to chemotherapeutic drugs, and thus show no response, others are initially responsive to chemotherapy, but subsequently develop acquired resistance. Both intrinsic and acquired resistance lead to a requirement for alternative treatment options [10].

In the laboratory, a major mechanism by which tumors display resistance to commonly used agents such as taxanes and anthracyclines is through overexpression of multidrug resistance (MDR) proteins including *P*-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP)-1 [11]. Overexpression of these efflux pump proteins causes retention of subtherapeutic concentrations of drug in tumor cells, which results in a lack of efficacy. In some tumors that are intrinsically resistant to chemotherapy, expression of MDR proteins reflects the constitutive expression of these proteins by the tissues from which the tumors are derived (e.g., liver and kidney). However, in tumors derived from tissue types that do not express MDR proteins physiologically, treatment with chemotherapy can

induce expression of these proteins. This results in acquired resistance to the chemotherapy agent used, in addition to drugs of the same class and, on occasion, of different classes. In addition, at least two additional mechanisms of drug resistance are known to exist for taxanes, both of which are related to tubulin, the therapeutic target for the taxanes. Firstly, mutations in beta-tubulin can prevent taxanes from binding to their target. Secondly, overexpression of the beta-III tubulin isoform in preference to the beta-II isoform reduces the efficacy of taxanes, as these drugs specifically target the beta-II isoform [12]. Thus, as described above, multiple mechanisms can lead to taxane resistance in preclinical laboratory models; however, it is not at all clear which of the potential mechanisms play key roles in clinical resistance found in patients.



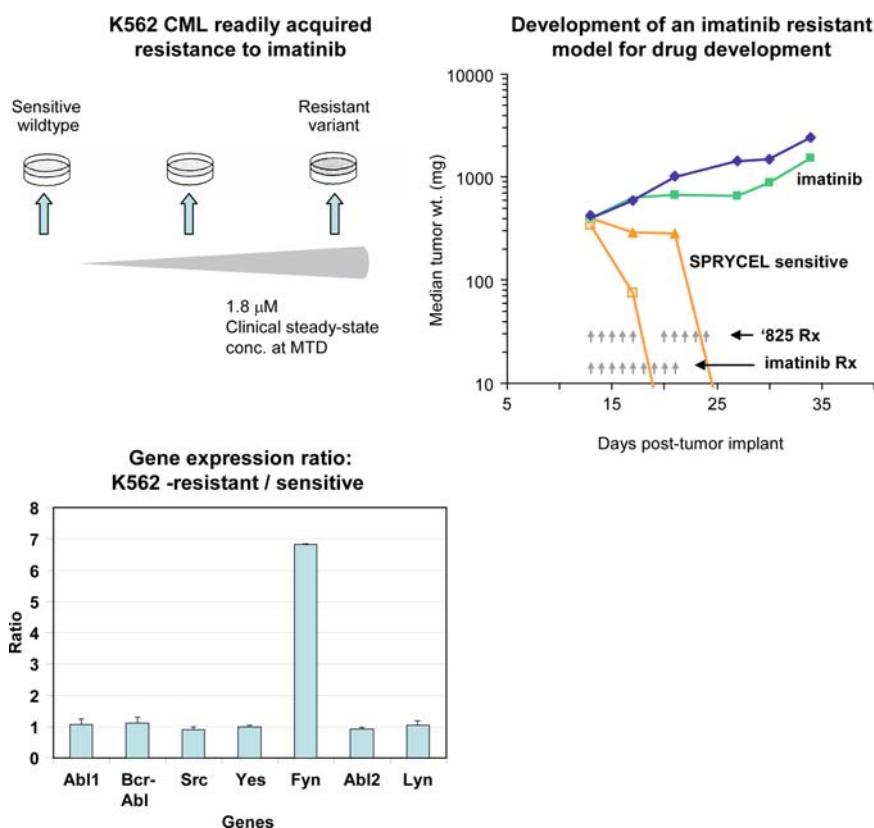
**Fig. 10.4** Examples of xenografts used for development of Ixempra. The history of treatment for the tumor of Pat-21 (patient-21) and Pat-7 (patient-7) followed by biopsy and transplant to mice is shown in the bar graph. The *upper graph* shows the response in xenografts to paclitaxel or Ixempra® of Pat-21 tumors, the *lower graph* indicates the response of Pat-7 tumors. On the right is a stained section of the Pat-21 xenograft grown in mice (*upper panel*) and the *lower two panels* show a western blot reacted with anti-pGp (*upper*) and anti-MRP (*lower*). Lane 2 contains proteins from Pat-21 and lane 3 from Pat-7

In the early 1990s, natural epothilones, produced by the myxobacterium *Sorangium cellulosum* and their analogs, were identified and were found to have antineoplastic activities [13]. Like the taxanes, epothilones promote tumor cell death by stabilizing microtubules and inducing apoptosis. However, as macrolide antibiotics, the epothilones are structurally unrelated to taxanes and were shown to have activity against several laboratory cell models of taxane resistance. At BMS, we initiated an epothilone analog program in order to optimize the *in vivo* antitumor efficacy and therapeutic index of this chemical class. Over 300 semisynthetic analogs were made and tested in various *in vitro* and *in vivo* systems. From these efforts, BMS-247550 (ixabepilone), a lactam analog of epothilone B, emerged as the most efficacious epothilone in a battery of *in vivo* preclinical chemotherapy studies, outperforming paclitaxel in each of the paclitaxel-resistant tumor models tested. The discovery of ixabepilone with the accompanying confidence that it has good potential to overcome clinically relevant taxane resistance was tremendously helped by the availability of two early passage human xenografts obtained from a breast and ovarian cancer patient, respectively, who either was intrinsically resistant (Pat-21) or had acquired resistance to TAXOL (Fig. 10.4). These two models recapitulate the taxol resistance observed in the patients of origin, demonstrating two differing mechanisms of taxane resistance—overexpression of beta-III tubulin in Pat-21 and Pgp in Pat-7 (Fig. 10.4). These results obtained preclinically predicted the clinical activity of ixabepilone which showed, in a pivotal phase II study of ixabepilone in patients with metastatic breast cancer refractory to an anthracycline, a taxane, and capecitabine, that it possessed significant antitumor activity as a single agent in this highly drug refractory population. These data became part of the basis for which ixabepilone was approved.

## 10.7 Imatinib Resistance in Chronic Myeloid Leukemia

BCR–ABL, a fusion oncogene generated by a reciprocal translocation between chromosomes 9 and 12, encodes the BCR–ABL fusion protein, a constitutively active cytoplasmic tyrosine kinase present in >90% of all patients with chronic myelogenous leukemia (CML) and in 15–30% of adult patients with acute lymphoblastic leukemia (ALL). Numerous studies have demonstrated that the underlying pathophysiology of CML is the kinase activity of BCR–ABL. The clinical success of the BCR–ABL kinase inhibitor imatinib (Gleevec®) has validated its use in the management of CML. Imatinib is particularly effective in the early (chronic) phase of the disease, where the complete hematologic response (CHR) rate can be in excess of 90%. However, patients with advanced disease (accelerated phase and blast crisis) and Philadelphia chromosome-positive ALL (Ph+ ALL) have been less sensitive to imatinib. Furthermore, responses are transient, generally lasting less than 6 months and, clinical resistance to imatinib both innate and acquired, has been observed in all phases of

disease, which may limit treatment benefits of imatinib in the long term. In light of these limitations of imatinib therapy in CML, and the lack of therapeutic options for patients who are refractory to, or intolerant of, imatinib treatment, a clear medical need exists for more effective therapeutic options, particularly in advanced disease and Ph+ ALL. In particular, use of agents that inhibit both BCR-ABL-dependent and BCR-ABL-independent mechanisms of imatinib resistance would be a favorable approach. The use of combinations of BCR-ABL and SRC inhibitors, or multi-targeted inhibitors of both kinases, to address these problems has been suggested in some cases assessed preclinically. However, it is necessary to gain proof-of-confidence that such an approach would result in a clinically useful agent. Here, the availability of a human CML xenograft,



**Fig. 10.5** Development of an imatinib-resistant CML cell line. By growing K562 cells in increasing imatinib concentrations (up to 1.8  $\mu$ M), an imatinib-resistant cell line was generated (*upper left*). When this was grown as a xenograft and treated (*upper right*) with imatinib (green line), the tumors were also imatinib resistant and SPRYCEL sensitive [orange lines]. Quantitative PCR of Abl and Src family kinases of the resistant versus the sensitive k562 line showed (*lower left*) overexpression of Fyn

K562/IM/R, derived from the originally sensitive CML line K562, with acquired resistance to imatinib through activation of FYN, a SRC family kinase, proved highly valuable. SRC family kinase activation is known to be a frequent finding in patients in the blast phase of CML that does not respond well to imatinib. Thus, the demonstration that dasatinib (Sprycel®) is completely effective in inhibiting the growth of the resistant human tumor xenografts in mice, causing cures in some instances (Fig. 10.5), provided high confidence of its clinical utility and set the stage for its candidacy for clinical development.

## 10.8 Summary

In cancer drug discovery, perhaps more than in any other therapeutic areas, the transition from *in vitro* to animal models and then to clinical evaluation of a candidate anticancer agent is thwarted with difficulties. Often the unavailability of animal cancer models that is predictive of the tumor types that the agent is intended to treat results in disappointing clinical outcome in human trial and a low rate of success for marketing approval. In this chapter, we argued and provided examples that human tumor xenografts in its various guises should remain the cornerstone of any *in vivo* drug evaluation program, so long as careful attention is paid to select the “right” xenograft for the drug target that the experimental agent is proposed to treat. Human tumor xenograft is particularly invaluable in settings where it is necessary to obtain *in vivo* “proof of principle” for a particular target as well as in optimizing the *in vivo* activity of pharmaceutically tractable chemotypes. Lastly, human tumor xenograft that faithfully represents the pathophysiology of its origin, when available, can provide invaluable information regarding the PK–PD relationship of the drug-target effects and therefore will assist greatly the logistics and speed of the clinical development of a candidate anticancer agent.

## References

1. [http://www.cancer.org/docroot/PRO/content/PRO\\_1\\_1\\_Cancer\\_Statistics\\_2006\\_Presentation.asp](http://www.cancer.org/docroot/PRO/content/PRO_1_1_Cancer_Statistics_2006_Presentation.asp).
2. Kola, I. and J. Landis (2004). “Can the pharmaceutical industry reduce attrition rates?” *Nature Reviews. Drug Discovery* **3**(8): 711–716.
3. Bourne, D. and L. Dittert (1995). Pharmacokinetics. *Modern Pharmaceutics*. G. Banker and C. Rhodes. New York, NY, Marcel Dekker.
4. Double, J. A. and M. C. Bibby (1989). “Therapeutic index: A vital component in selection of anticancer agents for clinical trial.” *Journal of the National Cancer Institute* **81**(13): 988–994.
5. Hann, B. and A. Balmain (2001). “Building ‘validated’ mouse models of human cancer.” *Current Opinion in Cell Biology* **13**(6): 778–784.
6. Corbett, T. H., L. Polin, et al. (2002). Transplantable syngeneic rodent tumors: solid tumors in mice. *Tumor Models in Cancer Research*, T. B. A. Totowa, NJ., Humana Press.

7. Averbook, B. J., J. L. Schuh, et al. (2002). "Antitumor Effects of Flt3 Ligand in Transplanted Murine Tumor Models." *Journal of Immunotherapy* **25**: 27–35.
8. Fiebig, H. and A. Burger (2002). Human tumor xenografts and explants. *Tumor Models in Cancer Research*. B. A. Teicher. Totowa, NY, Humana Press Inc: 113–137.
9. Kaitin, K. (Ed.) (2007). "Despite more cancer drugs in R&D, overall U.S. approval rate is 8%." *Tufts Center for the Study of Drug Development Impact Report* **9**(5).
10. Longley, D. and P. Johnston (2005). "Molecular mechanisms of drug resistance." *Journal of Pathology* **205**: 275–92.
11. Leonessa, F. and R. Clarke (2003). "ATP binding cassette transporters and drug resistance in breast cancer." *Endocrine-Related Cancer* **10**(1): 43–73.
12. Jordan, M., H. Miller, et al. (2006). "The Pat-21 breast cancer model derived from a patient with primary Taxol® resistance recapitulates the phenotype of its origin, has altered beta-tubulin expression and is sensitive to ixabepilone." *Proceedings of the American Association for Cancer Research 97th Annual Meeting*: LB-280.
13. Höfle, G., N. Bedorf, et al. (1996). "Epothilone A and B – novel 16-membered macrolides and cytotoxic activity: Isolation, crystal structure, and conformation in solution." *Angewandte Chemie (International ed. in English)* **35**: 1567–1569.
14. Sawyers, C. L. (1999). "Chronic myeloid leukemia." *N Engl J Med* **340**: 1330–1340.

# **Chapter 11**

## **DGL Global Strategies in DNA Microarray Gene Expression Analysis and Data Mining for Human Blood Cancers**

**Dongguang Li**

### **Contents**

11.1	Introduction . . . . .	259
11.2	Microarray Gene Expression and Experimental Design . . . . .	262
11.2.1	Fold Change Analysis . . . . .	262
11.2.2	Classification and Clustering – Molecular Classification of Leukaemia . . . . .	263
11.3	Genetic Algorithms . . . . .	264
11.4	DGL Global Optimisation Algorithms . . . . .	265
11.4.1	Leukeamia Data . . . . .	265
11.4.2	Overall Methodology . . . . .	265
11.4.3	Orthogonal Arrays and Sampling Procedure . . . . .	265
11.4.4	Objective Function . . . . .	266
11.4.5	Search Spaces Reduction for Global Search . . . . .	267
11.4.6	Mathematical Form of DGL Optimisation . . . . .	268
11.4.7	Multi-Subsets Class Predictor . . . . .	269
11.4.8	Validation (Predicting Through a Voting Mechanism) . . . . .	270
11.4.9	Experimental Results . . . . .	270
11.5	Discussion . . . . .	274
11.6	Conclusion . . . . .	277

### **11.1 Introduction**

Computation is required to extract meaningful information from the large amount of data generated by gene expression profiling [1, 2, 3]. Most of the algorithms commonly applied to microarray data analysis have been correlation-based approaches named cluster analysis [4]. For example, an efficient two-way clustering algorithm was applied to a colon cancer data set consisting of the expression patterns of different cell types. Gene expression in 40 tumour and 22 normal colon tissue samples was analysed across 2000 genes [4]. Cluster

---

D. Li

School of Computer and Information Science, Faculty of Computing, Health and Science, Edith Cowan University, Mount Lawley, WA 6050, Australia  
d.li@ecu.edu.au

analysis groups the genes involved in microarray data. Those clustered genes are likely to be functionally linked and need to be looked into closely. Although cluster analysis has widely been accepted in analysing the patterns of gene expression, the methods developed may not be able to fully extract the information from the microarray data corrupted by high-dimensional noise. If the noise from the genes that are irrelevant is not sufficiently reduced, incorrect classification for samples or misleading information on selecting informative genes may result. For selecting informative genes for sample classification, a neighbourhood analysis method was developed to obtain a subset of genes that discriminate between the acute lymphoblastic leukaemia (ALL) and the acute myeloid leukaemia (AML) successfully [5]. In the microarray data set containing 7129 genes, those genes whose expression levels differ significantly in ALL and AML were identified and then they were subsequently used to predict the class membership (either ALL or AML) of new leukaemia cases. Both approaches described above [4, 5] were focused on comparing samples in each single gene dimension and assumed that the relevant genes were similarly and uniformly expressed among samples of each type. To overcome these defects, a multi-variate approach that compares samples in a multi-gene dimension using the genetic algorithms (GAs) was proposed [6]. Samples were classified based on the class membership of their k-nearest neighbours (KNN) in the gene space. The dimensionality (length) of the gene subset was arbitrarily set to 50. GA was used to select hundreds and thousands of subsets of 50 genes that could potentially discriminate between two classes of samples (tumour and normal tissues). The frequency with which genes were selected was statistically analysed in the large number of 50-dimension gene subsets. The most frequently selected 50 genes were used to predict 34 new samples. Although the performance of GA predictor with 50 genes was remarkable, only 29 of 34 test samples were correctly predicted with high confidence [7]. To improve the successful rate of classification, more reliable and accurate algorithms are needed.

During the past five decades, the field of the global optimisation has been growing at a rapid pace and many new theoretical, algorithmic, and computational contributions have resulted [8]. Global optimisation is concerned with the computation and characterisation of global minima (or maxima) of nonlinear functions. Global optimisation problems are widespread in the mathematical modelling of real world systems for a very broad range of applications. The majority of problems can be described as some form of global optimisation procedures. In the gene selection problem, one would need to find how to form gene subsets to obtain the optimum classification response – changing one gene element in a given subset may improve the classification performance of the subset at one testing sample, but worsen it at another.

An objective function is necessary to evaluate how close each gene subset gets to the target requirement. The gene selecting process involves finding the gene subset that corresponds to the minimum (or maximum) of the objective function. Plotting the objective function against the gene search space of each element gene in the gene subset, one axis per element gene would be needed,

plus the orthogonal axis for the objective function. The objective function plot would appear as a multi-peak, multi-variable plot. Because there are an enormous number of inter-related possible gene combinations, the best gene subset cannot be found by any simple process. It is not obvious how to select the genes analytically to find the best solution. The methods currently used in gene selection, such as the clustering, neighbourhoods analysis, and GAs, almost all depend on a starting condition either selected by the user or generated internally by the program that is sometimes not obvious. Changing the initial conditions will give a different result, and one has no way of knowing how much improvement could be effected.

Currently available multi-variable optimisation algorithms for selecting the gene subset may not give optimum solutions. Usually, those algorithms obtain their final solutions either from optimising a starting guess or by techniques, which may or may not involve a pseudo-random process that gives different answers every time, depending upon the initial conditions. A true global optimisation algorithm should always find the very best solution possible within the boundary conditions stipulated. The possibility of creating a true global optimisation algorithm for a large number of inter-dependent variables has been preposed in this study. Although many optimisation algorithms may be appropriate for the gene classification problem, DGL (DGL is the abbreviation for Dongguang Li) global optimisation was proposed and applied to the cancer classification in this study for its superb performance in theory and applications.

Some of strategies of DGL global optimisation were firstly successfully applied to the optical thin film design problem [9]. It was also a candidate for the real function test bed of the First International Contest on Evolutionary Optimisation in order to solve 10 hard mathematical multi-variable optimisation problems [10]. It is of great interest to develop techniques for extracting useful information from the microarray data sets. In this chapter, I report the application of the DGL global optimisation approach for classifying and validating two well-known data sets [4, 5] consisting of the expression patterns of different cell types.

In previous years, many clinicians have been unable to provide a clear cut classification of cancerous patients, based upon the biopsy. However, with the system proposed here, the surveying of the expression of thousands of genes is made practical.

This chapter is concerned with the challenge of mining knowledge from DNA microarray expression data. With the objective to discover unknown patterns from microarray data, methodologies are derived from machine learning, artificial intelligence, and statistics. Nowadays, microarray expression data accumulate at an alarming speed in various storage devices, and so does valuable information. However, it is difficult to understand information hidden in data without the aid of data analysis techniques. Both the machine learning and the data mining have been applied to the field in order to better understand the microarray expression data sets. A data mining system usually enables one to collect, store, access, process, and ultimately describe and visualise data sets.

The discussion of data collection and storage is not included here though it is important for mining microarray expression data. In particular, data mining has successfully provided solutions for finding information from data in many medical research fields such as bioinformatics and pharmaceuticals. Many important problems have been addressed by data mining methods, such as neural networks, fuzzy logic, decision trees, GAs, and statistical methods. Data mining tasks can be descriptive and predictive. In other words, it is an inter-disciplinary field with a general goal of predicting outcomes and uncovering relationships in data [11, 12, 13, 14].

Microarray data analysis is one of the most attractive fields of data mining. With the help of gene expressions obtained from microarray technology, heterogeneous cancers can be classified into appropriate subtypes [15]. Many different kinds of machine learning and statistical methods have recently been applied to analyse gene expression data [16, 17, 18, 19]. Data mining tasks normally include data pre-processing, data modelling, and knowledge description. One more example application to classify the publicly available data on leukaemia has also been described in detail to take the advantages of data mining.

## 11.2 Microarray Gene Expression and Experimental Design

DNA microarrays, also called gene arrays or gene chips, usually consist of thin glass or nylon substrates containing specific DNA gene samples spotted in an array by a robotic printing device [20]. Researchers spread fluorescently labelled m-RNA from an experimental condition onto the DNA gene samples in the array. This m-RNA binds strongly with some DNA gene samples and weakly with others, depending on the inherent double helical characteristics. A laser scans the array and sensors detect the fluorescence levels (using red and green dyes), indicating the strength with which the sample expresses each gene. The logarithmic ratio between the two intensities of each dye is used as the gene expression data. The relative abundance of the spotted DNA sequences in a pair of DNA or RNA samples is assessed by evaluating the differential hybridisation of the two samples to the sequences on the array. Gene expression levels can be determined for samples taken at multiple time instants of a biological process or under various conditions. Each gene corresponds to a high-dimensional row vector of its expression profile [21]. The fundamental goal of microarray experiments is to identify genes that are differentially expressed in the conditions being studied [22].

### 11.2.1 Fold Change Analysis

Fold change is defined as follows:

Take log<sub>2</sub> transformed normalised intensities from robust multi-chip averaging (RMA) for two samples;

```
Let a = log2 (intensity sample 1)
b = log2 (intensity sample 2)
If a > b: fold change = 2 (| a-b |)
If a < b: fold change = -2 (| a-b |)
If a = b: fold change = 0
```

The pre-processing of gene expression profile is often necessary to reach the goal of converting from raw data to biological significance. The following steps are common:

- normalising the hybridisation intensities within a single array experiment;
- transforming the data using a nonlinear function, like the logarithm in case of expression ratios;
- estimating and replacing missing values in expressions or adapting existing algorithms to handle missing values;
- filtering gene expression profile to eliminate those that do not satisfy some simple criteria;
- standardising or rescaling the profiles to generate vectors of length one.

Mouse 430 v2 Affymetrix GeneChip® arrays are used for all experiments. Probe intensity data as CEL files are imported into the R software environment (<http://www.R-project.org>). Probe level data quality is assessed using image reconstruction, histograms of raw signal intensities, and MA plots. Normalisation is performed for each batch separately using the robust multi-chip average (affy/RMA, <http://www.bioconductor.org>) method using all probe intensity data sets together to form one expression measure per probe set per array. Fold change analysis is conducted for each pair of comparisons as listed above.

### ***11.2.2 Classification and Clustering – Molecular Classification of Leukaemia***

Since the fold change does not address the reproducibility of the observed difference and cannot be used to determine the statistical significance, raw data are rarely of direct benefit. Its true value is predicated on the ability to extract information useful for decision support or exploration and understanding the phenomenon governing the data source. In the microarray domain, data analysis was traditionally a manual process. One or more analyst(s) would become intimately familiar with the data and, with the help of statistical techniques, provide summaries and generate reports. However, such an approach rapidly broke down as the size of data grew and the number of dimensions increased. When the scale of data manipulation and exploration goes beyond human capacities, people need the aid of computing technologies for automating the process. All these have prompted the need for intelligent data analysis methodologies, which could discover useful knowledge from data.

Classification is also described as supervised learning [23]. Classification and clustering are two data mining tasks with close relationships. A class is a set of data samples with some similarity or relationship, and all samples in this class are assigned the same class label to distinguish them from samples in other classes. A cluster is a collection of objects that are similar locally. Clusters are usually generated in order to further classify objects into relatively larger and meaningful categories. Clustering is also called unsupervised classification, where no predefined classes are assigned [23]. According to a data set with class labels, data analysis builds classifiers as predictors for future unknown objects. A classification model is formed first based on available data. Future trends are predicted using the learned model. In the following case, the data sets used are from a public microarray database and the samples are collected to build a model that can be used to classify new samples into categories of ALL or AML for leukaemia.

Classification of acute leukaemia, having highly similar appearance in gene expression data, has been made by combining a pair of classifiers trained with mutually exclusive features [24]. Gene expression profiles were constructed from 71 patients having ALL or AML, each constituting one sample of the DNA microarray. Each pattern consists of 7129 gene expressions. Feature selection was employed to generate 25 top-ranked genes for the experiment. In the following sections, a case study from theory to practice is presented in detail.

### 11.3 Genetic Algorithms

GAs is motivated by the natural evolutionary process. Most of classification with artificial intelligence uses GAs as core algorithms. Solutions of the problem at hand are encoded in chromosomes or individuals. An initial population of individuals is generated at random or heuristically. The operators in GAs include selection, crossover, and mutation. To generate a new generation, chromosomes are selected according to their fitness score. The selection operator gives preference to better individuals as parents for the next generation. The crossover operator and the mutation operator are used to generate offspring from the parents. A crossover site is randomly chosen in the parents. The mutation operator is used to prevent premature convergence to local optima [25]. The basic concept in GAs is to introduce effective parallel searching in the high-dimensional problem space.

To solve the problem of mining microarray expression data, GAs are especially useful for the following reasons:

- the problem space is large and complex;
- prior knowledge is scarce;
- it is difficult to determine a machine learning model to solve the problem due to complexities in constraints and objectives;
- traditional search methods, such as stochastic, combinatorial, and classical so-called hard optimisation-based techniques, perform badly.

## 11.4 DGL Global Optimisation Algorithms

Although GAs is popular and useful, many problems at hand cannot be resolved easily and accurately. This section combines a powerful algorithm, called DGL global optimisation [26], with the methods of cancer diagnosis through gene selection and microarray analysis. A generic approach to cancer classification based on gene expression monitoring by DNA microarrays is proposed and applied to a test leukaemia case.

By using the orthogonal arrays (OAs) for sampling and a search space reduction process, a computer program has been written that can operate on a personal laptop computer. The leukaemia microarray data can be classified 100% correctly without previous knowledge of their classes.

### 11.4.1 Leukeamia Data

The original data were downloaded from the web (<http://www.broad.mit.edu/cgi-bin/cancer/datasets.cgi>). The data contain the expression levels of 7129 genes across the 72 samples, of which 47 are the ALL samples and 25 the AML samples. These data sets contain measurements corresponding to ALL and AML samples from bone marrow and peripheral blood that is divided into a training set (38 samples) and a test set (34 samples).

### 11.4.2 Overall Methodology

The proposed DGL global optimisation method in this study includes following major steps:

- sampling within search spaces by using a suitable OA instead of conducting a random search
- constructing objective function for optimisation algorithms;
- searching spaces reduction strategies;
- searching for global optimal solutions;
- building up a multi-subsets pyramidal hierarchy class predictor for classification;
- predicting through a voting mechanism.

### 11.4.3 Orthogonal Arrays and Sampling Procedure

The OA used in this research is L242(1123) that is too large to be shown here. The OA L242(1123) has 242 rows (observations or tests), 23 columns (factors or variables), and 11 levels for each factor. The complete L242(1123) is available on the website <http://www.scis.ecu.edu.au/dli/>. The L242(1123) was initially

used in selecting a gene subset with 23 gene elements. The search space of 2000 genes in the colon data was divided into 11levels equally. If all the genes are assigned a unique ID number from 1 to 2000 and the initial search space ranges from 1 to 2000, then the selected gene IDs are 1, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, and 2000. As the first row of L242(1123) reads (1, 10, 2, 3, 8, 8, 2, 4, 8, 9, 5, 4, 10, 5, 7, 1, 5, 5, 8, 1, 10, 11, 2), the constructed gene subset will read (1, 1800, 200, 400, 1400, 1400, 200, 600, 1400, 1600, 800, 600, 1800, 800, 1200, 1, 800, 800, 1400, 1, 1800, 2000, 200). Since the duplicated gene IDs are not allowed in a gene subset, those repeated gene IDs are shifted forwards or backwards a little. The modified 23-gene subset now reads (1, 1800, 200, 400, 1400, 1399, 199, 600, 1401, 1600, 800, 599, 1799, 799, 1199, 2, 801, 798, 1401, 3, 1798, 2000, 201). According to the L242(1123), 242 different 23-gene subsets were created and evaluated with the defined objective function. All the 242 subsets were ranked based on their values of objective function. Ten percent of top performers in classifying the training set were kept, and those gene IDs included in the top 10% gene subsets were ranked in order to work out the minimum ID and the maximum ID. The new and reduced search space ranged from the minimum ID to the maximum ID. The above process was repeated until the search space was small enough (e.g. less than 11 genes left) or the objective function could not be improved any further. The rank no.1gene subset in the last round of optimisation was chosen as the optimal solution for the 23-gene subsets. The optimisation was run 23 times with different lengths (23, 22, ..., 2, 1) of gene subsets at each run. Total of 23 optimal solutions were obtained. All the 23 optimal solutions constructed a multi-subset cancer class predictor and then were used to classify the samples in the test data set. All the 23 gene subsets were arranged to form a pyramidal layer-by-layer hierarchy with the shortest subset (1gene) on the top and the longest subset (23 genes) in the bottom (see Table 11.2 for details).

#### **11.4.4 Objective Function**

An objective function is also called a fitness or merit function, which is a measure of the ability for a selected gene subset to classify the training set samples according to the DGL optimisation procedure. There are several ways, such as neighbourhood analysis [5], support vector machines [27, 28], and KNN [6], to construct an objective function for the optimisation and gene selection algorithms. Among them, KNN is used for the proposed DGL global optimisation because it is easy to compute. The Euclidean distance between a single sample (represented by its pattern vector  $V_m$ ) and each of the pattern vectors of the training set containing  $M$  samples is calculated:  $V_m = (g_1, g_2, \dots, g_n)$ , where  $n$  is the number of genes in the vector that can be set to from 1 to 23 in order to form the gene vectors (or subsets) with different lengths,  $g_n$  is the expression level of the  $n$ th gene in the  $m$ th sample,  $m = 1, 2, \dots, M$ . For

**Table 11.1** k-nearest neighbour (KNN) rules ( $k = 5$ )

Among the ranked 5 nearest neighbours	Classification	Class code
All 5 are ALL samples	ALL	1
All 5 are AML samples	AML	-1
4 are ALL and 1 is AML	ALL	1
4 are AML and 1 is ALL	AML	-1
3 are ALL and 2 are AML	Unknown	0

the leukaemia data set  $M = 40$ , each sample is classified according to the class membership of its KNNs as determined by the Euclidean distance in n-dimensional space. If all or majority of the KNNs of a sample belongs to the same class, the sample is classified as that class. Otherwise, the sample is considered unclassifiable. The  $k$  was arbitrarily set to 5 in this study. The detailed rules are shown in Table 11.1.

If the class membership of a training set sample and its five nearest neighbours in the particular n-dimensional space defined by a gene subset agree or four out of five nearest neighbours agree, the sample is classified and a score of 1 is assigned to that sample. These agreement scores are summed across the training set. For convenience, this sum is divided by the number of training samples as the value of the objective function for the selected gene subset. The bigger the value is, the better the selected gene subset performs in classification. A maximal objective function value is 1, which means all the samples in the training set is classified correctly by the gene subset under testing. The goal of the optimisation procedure is to discover the optimal gene subset (optimal solution) with the maximal value of the objective function. As in other methods, an objective function is calculated for each subset of genes by the sum over all classifying scores of the samples in the training data set. The optimisation process then conducts the searching for the gene subset that has the best objective function value (minima or maxima). Therefore, by finding the lowest or highest value of the objective function, one will have the best performing gene subset discovered. This procedure can be made more sophisticated by introducing weighting factors to increase the importance of user-specified samples in training sets, as well as using other forms of the distance formula between one subset and another.

#### 11.4.5 Search Spaces Reduction for Global Search

With local optimisation (a fast method for a large number of genes), the program finds the nearest minimum and stops. For some so-called global optimisation procedures, the algorithm not only finds a local minimum but can also find some neighbouring minima. The processes, however, is a hit and miss situation, because starting at a different place can result in different solutions. The global algorithm in DGL repeatedly narrows the region where the global minimum

is known to lie by using a special OA's sampling that operates simultaneously in all orthogonal dimensions, one for each gene in the gene subset to find the optimum solution. As the process runs, one can observe the range of genes for each gene variable in an n-dimensional subset being reduced.

### **11.4.6 Mathematical Form of DGL Optimisation**

Consider a multi-dimensional continuous function  $f(x)$  with multiple global minima and local minima on subset  $G$  of  $R^n$

(i) A local minima is defined as follows

For a given point  $x^* \in G$ , if there exists a  $\delta$ -neighbourhood of  $x^*$ ,  $O(x^*, \delta)$ , such that for  
 $x \in O(x^*, \delta)$ ,

$$\text{and } f(x^*) \leq f(x) \quad (1)$$

then  $x^*$  is called a local minimal point of  $f(x)$ .

(ii) Definition of global minima:

If for every  $x \in G$  the inequality (1) is correct, then  $x^*$  is called a global minimum of  $f(x)$  on  $G$ , and the global minima of  $f(x)$  on  $G$  form a global minimum set.

(iii) How to find the global minima:

Now for a given constant  $C_0$  such that the level set

$$H_0 = \{x | f(x) < C_0, x \in G\} \text{ is nonempty,}$$

if  $\mu(H_0) = 0$ , where  $\mu$  is the Lebesgue measure of  $H_0$ , then  $C_0$  is the minimum of  $f(x)$  and  $H_0$  is the global minimum set.

Otherwise, assume that  $\mu(H_0) > 0$  and  $C_1$  is the mean value of  $f(x)$  on  $H_0$ .

$$\text{Then } C_1 = 1/\mu(H_0) \int_{H_0} f(x) d\mu \quad (2)$$

and

$$C_0 \geq C_1 \geq f(x^*) \quad (3)$$

One then gradually constructs the level set  $H_k$  and mean value  $C_{k+1}$  of  $f(x)$  on  $H_k$  as follows:

$$H_k = \{x | f(x) < C_k, x \in G\} \quad (4)$$

and

$$C_{k+1} = 1/\mu(H_k) \int_{H_k} f(x) d\mu \quad (5)$$

With the assistance of OA's sampling, a decreasing sequence of mean values  $\{C_k\}$  and a sequence of level sets  $\{H_k\}$  are obtained.

$$\begin{aligned} \text{Let } & \lim_{k \rightarrow \infty} C_k = C^* \\ & \text{and} \end{aligned} \tag{6}$$

$$\lim_{k \rightarrow \infty} H_k = H^* \tag{7}$$

It can be proved that  $C^*$  is the minimum of  $f(x)$  on  $G$ , and  $H^*$  is the global minimum set. There are several strategies to avoid missing the global optimum when seeking the minimum solution. Among these, the most important step is to select or design a suitable OA with which the function within domains can be repeatedly sampled. The algorithm is automatically constrained to stay within the function domain and will not request function evaluations outside this domain.

There are two stopping criteria possible, either when the target objective function value is reached or when the maximum domain length is smaller than the user selected value. In this research, one uses the latter stop criteria, corresponding to the variation possible for each gene element in the subset – which can be as little as one gene. This means that the global minimum has been found for a particular gene selection range of each gene element, with a variation of less than one gene for each gene element. Strictly speaking then, the global optimum is not defined at a point but as lying within a region.

#### **11.4.7 Multi-Subsets Class Predictor**

Although DGL optimisation will result in an optimal gene subset with a given length, the classification performance varies. It seems that for both the colon and the leukaemia data sets, there is no guarantee to name a single gene subset that is capable of classifying all the samples in the testing set correctly. It is observed that the gene subsets with different lengths tend to misclassify or un-classify the different samples in the testing data sets. In another words, the gene subsets with the same length will always misclassify a few same samples in the testing data sets although those are all the optimal subsets identified by optimisation procedures. This fact indicates that the key factor to improve the signal to noise ratio in classifying the very noisy data, such as the microarray gene expressions, is the length of the gene subset. Based on the above observation, a multi-subsets class predictor was constructed for classification by using all the 23 optimal gene subsets with the lengths from 1 to 23 genes. The maximal number of genes involved in the predictor is 276 in total. As some of genes may appear more than one time, the actual number of the unique gene IDs is a bit less and varies from case to case.

#### ***11.4.8 Validation (Predicting Through a Voting Mechanism)***

The established multi-subsets class predictor is validated with the testing data sets for both the colon and the leukaemia data. Each gene subset in the predictor predicts the class of every sample in the testing data sets independently according to the same KNN rules ( $k = 5$ ) used in the training stage. The predicted class code (in leukaemia data: 1 for ALL, -1 for AML, and 0 for unknown) is assigned to the particular sample accordingly. Each single class code is treated as a single vote. For each sample in the testing data sets, up to 23 votes contributed by 23 gene subsets in the predictor can be obtained. The final class predicted by the predictor depends on the sign of the sum of the 23 votes of the sample under test. A positive sign indicates that there are more gene subsets in the predictor vote for class 1, and the sample is finally classified as 1 by the multi-subsets predictor. A negative sign indicates that there are more gene subsets in the predictor vote for class -1, and the sample is finally classified as -1 by the multi-subsets predictor. When the sum is zero, there are equal numbers of gene subsets among the 23 gene subsets for the class 1 and the class -1. In this case, the corresponding sample should be classified as 0 (unknown or unclassified). It is not difficult to interpret the actual values of the classification results. The absolute value of the sum of the 23 votes should indicate the predicting strength. The larger the value is, the more confident the prediction is.

#### ***11.4.9 Experimental Results***

A Microsoft Windows based computer program with a user-friendly graphic interface has been written. The entire experimental computation was carried out on a personal laptop computer (1.7GHz Intel Pentium Pro/II/III). The software can be downloaded from the supporting website of this chapter [29] and is available free to researchers. Both the colon cancer and the leukaemia samples were classified 100% correctly. The classification processes are automated after the gene expression data being inputted. It can find the global optimum solutions and construct a multi-subsets class predictor containing up to 23 gene subsets based on a given microarray gene expression data collection, such as the colon or leukaemia data, within a period of several hours.

For the convenience of computation, every gene was assigned a unique integer ID number, from 1 to 7129, according to the order in their original data sets. The aim was to study how changes in the choices of various gene element variables for a gene subset with a given length affect a response variable (success rate in classifying training samples). For each of the gene elements that are used to form a gene subset, 11 choices (levels) were selected for inclusion in the OA's sampling based on L242(1123). Those eleven choices of gene IDs were generated by the formula (the length of the current search space divided by 10) at an equal distance. Some shifting on the selected gene IDs was necessary to

Table 11.2 Optimal gene subsets and selected genes for the leukaemia data class predictor

Gene subsets	Gene IDs
1-gene-subset	5501
2-gene-subset	3320 1068
3-gene-subset	2020 4782 2348
4-gene-subset	4270 2039 4050 2642
5-gene-subset	2642 1837 4050 1488 5605
6-gene-subset	3137 2642 3336 2368 2852 4050
7-gene-subset	2020 1725 2531 2096 4991 2348 2120
8-gene-subset	2642 4492 307 6368 3753 4708 5655 4050
9-gene-subset	1481 4991 2224 2642 109 4050 5094 3565 6441
10-gene-subset	2619 3119 3056 2971 4339 5297 2861 2020 5247 2001
11-gene-subset	1584 4023 2020 1506 2852 4459 1060 6467 2295 2348 2483
12-gene-subset	6910 4669 2642 6939 1891 4050 2020 4916 6487 1442 4950 2128
13-gene-subset	1934 3906 3010 3392 5906 7129 4453 4744 4961 2280 2642 1 4050
14-gene-subset	1362 2642 6771 4050 2090 6681 2811 988 4574 4727 5673 3191 1427 3565
15-gene-subset	2020 1853 501 1387 4414 3565 3056 1630 6243 1143 2342 5251 4139 4720 1834
16-gene-subset	4751 6438 3414 4224 5949 4889 1056 6559 2642 2648 5210 5166 1 4050 3888 5134
17-gene-subset	7129 1834 4640 3189 6872 3118 2433 4050 1740 5326 4768 2469 6042 1 4444 2642 4252
18-gene-subset	5828 442 3299 6548 2400 2378 3525 5452 4127 2642 5770 5342 6319 1945 4050 2780 6136 4464
19-gene-subset	714 1714 5912 4711 3839 3215 2506 2642 3804 1900 5299 5609 4050 1 6655 6372 2791 1211 3068
20-gene-subset	3515 7129 3854 6762 5826 4050 1250 2416 1021 3322 5451 5508 4410 2642 2327 4037 6639 4278 4334 5745
21-gene-subset	92 6833 2642 1385 1801 3102 4251 4050 6832 5651 2449 4189 1925 5826 301 1126 3034 6940 1594 3342 5384
22-gene-subset	5064 5692 6034 4050 6435 2642 628 501 4960 4908 5882 2227 3565 3998 2004 4723 7021 1 2829 1513 3423 3642
23-gene-subset	7129 5477 714 4534 4572 643 3066 4991 2327 1229 4050 1425 1 4634 3565 6416 4452 3149 1250 4063 3026 2642 3780

Table 11.3 Validation of the leukaemia data with the multi-subset class predictor

Gene subsets	Prediction of 34 test samples (no. 39–72)															Results of Classification																					
	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Correct	Incorrect	Unknown
1-gene subset	+1	0	+1	0	+1	+1	+1	+1	+1	+1	+1	0	-1	0	0	+1	+1	0	0	+1	-1	0	+1	+1	0	0	+1	+1	0	0	+1	18	2	14	52.9		
2-gene subset	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	+1	-1	0	+1	+1	+1	-1	-1	-1	-1	+1	+1	-1	+1	+1	+1	+1	+1	+1	27	5	2	79.4	
3-gene subset	+1	+1	+1	-1	+1	+1	+1	+1	+1	+1	+1	-1	-1	+1	-1	-1	+1	+1	0	-1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	25	5	4	73.5	
4-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	+1	-1	-1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	+1	+1	31	0	3	91.2	
5-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	+1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	31	0	3	91.2	
6-gene subset	+1	-1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	30	1	3	88.2	
7-gene subset	+1	+1	+1	0	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	+1	+1	0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	27	4	3	79.4	
8-gene subset	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	+1	+1	0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	29	3	2	85.3	
9-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	+1	+1	+1	0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	31	0	3	91.2	
10-gene subset	+1	+1	+1	0	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	+1	+1	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	24	8	2	70.6	
11-gene subset	+1	+1	+1	-1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	+1	+1	+1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	26	6	2	76.4	
12-gene subset	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	+1	+1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	32	0	2	94.1	
13-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	+1	+1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	31	0	3	91.2	
14-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	+1	+1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	31	0	3	91.2	
15-gene subset	+1	+1	+1	0	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	-1	+1	+1	+1	0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	27	4	3	79.4	

Table 11.3 (continued)

		Prediction of 34 test samples (no. 39–72)																						Results of Classification															
Gene subsets		39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Correct	Incorrect	Unknown	Success rate %
16-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	0	+1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	+1	+1	+1	31	0	3	91.2		
17-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	0	+1	-1	-1	-1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	32	0	2	94.1			
18-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	0	+1	-1	-1	-1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	32	0	2	94.1			
19-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	0	+1	-1	-1	-1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	31	0	3	91.2			
20-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	0	+1	-1	-1	-1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	31	0	3	91.2			
21-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	0	+1	-1	-1	-1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	31	0	3	91.2			
22-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	0	+1	-1	-1	-1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	31	0	3	91.2			
23-gene subset	+1	0	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	0	+1	-1	-1	-1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	31	0	3	91.2			
Sum of votes:		+23	+7	+23	+15	+23	+23	+23	+23	+23	+23	+23	-22	-22	-10	-22	-17	+7	+23	-15	-23	-13	-8	-15	-4	-19	-21	-13	-8	+20	+22	+23	+22	+22	+23				
Classified as:		+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	-1	-1	-1	-1	-1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	34	0	0	100			

Every gene subset is used to classify the 34 test samples. The predicted class for every sample is represented by a vote value [+1 for acute lymphoblastic leukaemia (ALL), -1 for acute myeloid leukaemia (AML), and 0 for unknown]. For the sum of votes, one adds up the vote values across all 23 gene subsets for every sample. If the sum of the 23 prediction votes is positive value (which indicates that there are more gene subsets favouring the ALL class than the AML class), the corresponding sample is classified as an ALL sample with the code of 1. If the sum of the 23 prediction votes is negative value (which indicates there are more gene subsets favouring the AML class than the ALL class), the corresponding sample is classified as an AML sample with the code of -1. In case of the sum of the 23 prediction votes is equal to zero, the corresponding sample is unclassified with the code of 0.

avoid having any repeating genes in a single gene subset. 242 subsets were evaluated with the objective function in the current iteration, and 10% of top performing subsets were used to reduce the search space. Only two top performing gene subsets were passed to the next iteration. Within the search space of 7129 genes for the leukaemia data, DGL global optimisation found 23 optimal gene subsets with different lengths from 1 to 23 genes, which formed two pyramidal hierarchy class predictors (shown in Table 11.2). Those gene subsets were assumed to be the best performing gene combinations for classifying the gene data sets used in this study. The selected gene subsets were then used in classifying the test samples in the leukaemia data sets. Table 11.3 summarises the classification results. Once the validation of all the 23 optimal gene subsets was completed, the proposed multi-subsets voting mechanism was adopted. One of the classification results (1 for class 1, -1 for class 2, and 0 for unclassified) was obtained by balancing the votes from the 23 gene subsets for the particular testing sample of interest. It is a process of counting votes to make a final decision on the class of the sample under test.

## 11.5 Discussion

It is worth observing that the established multi-subsets class predictor could be reduced in size through removing the first five or more unstable short gene subsets. The remaining subsets would still perform well, which is shown on the supporting website [29]. In general, the predicting strength may be improved. However, having those genes selected in the short subsets included may be significant to biologists as they could well be informative. Another interesting observation is that there are not many genes that play a more important role than any other gene. The genes 2642 and 4050 were the most frequently used genes being included 16 times. The gene IDs assigned by this study and real gene accession numbers from the original data sets are listed in the Table 11.4. Some previous research works proposed to find out many near optimal gene subsets through a well-tuned GA procedure and pick up top 50–200 most frequently appeared genes to construct a long gene subset as a predictor [7]. Although the performance of such a predictor was reasonably good, the large amount of computation might not be affordable or cost effective and might not be necessary.

For the leukaemia data, 219 (shown in Table 11.4) out of 7129 genes in the data set were selected by DGL for constructing the class predictor. Table 11.5 lists the genes appearing more than once in the leukaemia class predictor based on frequency rank. It is worthwhile to note that the gene 2642 (U05259\_ma1) and the gene 4050 (X03934) both appear 16 times (their frequency is much higher than other's). A subset with only these two genes is able to classify 31 out of 34 samples in the leukaemia test data set and three samples remain as unclassified. When a subset of top four genes (2642, 4050, 2020, and 1) is

**Table 11.4** The 219 genes selected by the leukaemia class predictor

IDs	Gene accession number	IDs	Gene accession number	IDs	Gene accession number	IDs	Gene accession number	IDs	Gene accession number
5501	Z15115	1506	L36051	2342	M90696	6136	U28749_s	1925	M31165
33320	U50136_rna1	4459	X67683	5251	D28791	4464	X68149_s	301	D25303
1068	J03040	1060	J02883	4139	X13956	714	D87443	1126	J04809_rna1
2020	M55150	6467	U29463_s	4720	X85134_rna1	1714	M14123_xpt2	3034	U31449
4782	X90908	2295	M85169	1834	M23197	5912	HG880-HT880	6940	Z30644
2348	M91432	2483	S73813	4751	X877342	4711	X84195	1594	L41147
4270	X54936	6910	U84388	6438	S77154_s	3839	U82320	3342	U51166
2039	M57471	4669	X81889	3414	U56814	3215	U43522	5384	U13022
4050	X03934	6939	Z30643	4224	X52001	2506	S77576	5064	Z15108
2642	U05259_rna1	1891	M28713	5949	M29610	3804	U80017_rna2	5692	D89377_s
1837	M23379	4916	X99657	4889	X98263	1900	M29273	6034	U50360_s
1488	L34357	6487	X75346_s	1056	J02843	5299	L07919	6435	U05012_s
5605	D29675	1442	L27479	6559	U41315_rna1_s	5609	X14085_s	628	D83784
3137	U38846	4950	Y07596	2648	U05875	6655	Z11518_s	4960	Y07846
3336	U50939	2128	M63379	5210	Z79581	6372	M81182_s	4908	X99268
2368	M93284	1934	M31642	5166	Z48804	2791	U14550	5882	HG417-HT417_s
2852	U18004	3906	U89278	3888	U86782	1211	L05512	2227	M76558
1725	M14636	3010	U30245	5134	Z25491	3068	U33818	3998	U96629_rna2
2531	S81221	3392	U53476	1834	M23197	3515	U62437	2004	M37763
2096	M61156	5906	X07618_s	4640	X80062	3854	U83303_cds2	4723	X85372
4991	Y09615	7129	Z78285_f	3189	U41813	6762	M21388	7021	M33318_r
2120	M62994	4453	X67155	6872	M92642	5826	HG3125-	2829	U16296
							HT3301_s		
4492	X69908_rna1	4724	X85373	3118	U37283	1250	L08424	1513	L36645
307	D26067	4961	Y07847	2433	S34389	2416	M97639	3423	U57099
6368	M80397_s	2280	M83651	1740	M15841	1021	HG511-HT511	3642	U70732_rna1
3753	U79249	1	AFFX-BioB_5	5326	M13577	3322	U50315	5477	X71661

Table 11.4 (continued)

IDs	Gene accession number								
4708	X84002	1362	L19067	4768	X89750	5451	X14766	4534	X74104
5655	U58046_s	6771	X87344_cds10_r	2469	S70348	5508	HG2157-HT2227	4572	X76105
1481	L33881	2090	M60749	6042	L10333_s	4410	X64643	643	D85376
2224	M7624	6681	X74874_rna1_s	4444	X66534	2327	M88282	3066	U33447
109	AC002115_cds4	2811	U15177	4252	X53742	4037	X02751	1229	L07077
5094	Z24727	988	HG4245-HT4515	5828	HG3187-HT3366_s	6639	U83598	1425	L25270
3565	U66048	4574	X76180	442	D45370	4278	X55666	4634	X79865
6441	S78873_s	4727	X85750	3299	U49187	4334	X59711	6416	S57153_s
2619	U03644	5673	D85425_s	6548	Z69030_s	5745	HG2261-HT2351_s	4452	X67098
3119	U37352	3191	U41816	2400	M95925	92	AB003698	3149	U39412
3056	U32944	1427	L25444	2378	M94167	6823	J00220_cds5	4063	X04434
2971	U227185	3565	U66048	3525	U63289	1385	L20348	3026	U31120_rna1
4339	X59812	1853	M25077	5452	X15422	1801	M21154	3780	U79287
5297	L07615	501	D50931	4127	X12901	3102	U36501		
2861	U18288	1387	L20773	5770	X52009_s	4251	X53587		
5247	D17532	4414	X64838	5342	M37712	6832	J00210_rna1		
2001	M37435	1630	L47738	6319	M60450_s	5651	D50477_s		
1584	L40410	6243	M24486_s	1945	M32315	2449	S76992		
4023	X01059	1143	J05213	2780	U13737	4189	X16667		

**Table 11.5** The genes appear more than once in the leukaemia class predictor

Rank	Gene IDs	Frequency	Gene accession number	Gene description
1	2642	16	U05259_rna1	MB-1 gene
2	4050	16	X03934	GB DEF = T-cell antigen receptor gene T3 delta
3	2020	6	M55150	FAH fumarylacetoacetate
4	1	6	AFFX-BioB-5	AFFX-BioB-5_at (endogenous control)
5	3565	4	U66048	Clone 161455 breast expressed mRNA from chromosome X
6	7129	4	Z78285_f	GB DEF = mRNA (clone 1A7)
7	2348	3	M91432	ACADM acyl-coenzyme A dehydrogenase, C-4 to C-12 straight chain
8	4991	3	Y09615	GB DEF = mitochondrial transcription termination factor
9	2852	2	U18004	HSU18004 Homo sapiens cDNA
10	3056	2	U32944	Cytoplasmic dynein light chain 1 (hdlc1) mRNA
11	5826	2	HG3125-HT3301_s	Estrogen receptor (Gb:S67777)
12	501	2	D50931	KIAA0141 gene
13	714	2	D87443	KIAA0254 gene
14	2327	2	M88282	T-cell surface protein tactile precursor
15	1250	2	L08424	Achaete scute homologous protein (ASH1) mRNA

used, 32 out of 34 samples can be predicted correctly with two remaining as unclassified. There are four genes (2642, 2020, 2348, and 3056) in the Table 11.5, which were identified by the previous researchers in their 50genes most highly correlated with the ALL–AML class distinction [5]. With the method of Golub et al., 29 out of 34 test samples could be classified correctly, while the DGL classified all of them correctly. Moreover, the DGL method, by selecting sets of genes based on their joint ability to discriminate, can identify genes that are important jointly, but do not discriminate individually. This indicates that the DGL method has potential in identifying genes that not only discriminate between the ALL and the AML but also distinguish existing subtypes without applying any prior knowledge.

## 11.6 Conclusion

A gene is a fundamental constituent of any living organism. The machinery of each human body is built and run with 50,000–100,000 different kinds of genes or protein molecules. With the completion of the Human Genome Project, one

has access to large databases of biological information. Proper analysis of such huge data holds immense promise in Bioinformatics. The applicability of data mining in this domain cannot be denied, given the lifesaving prospects of effective drug design. This is also of practical interest to the pharmaceutical industry [21]. The success of DNA technologies and the digital revolution with the growth of the Internet have ensured that huge volumes of high-dimensional microarray expression data are available all around us. Data mining is an evolving and growing area of research and development. The problem is to mine useful information or patterns from the huge data sets. Microarrays provide a powerful basis to monitor the expression of tens of thousands of genes, in order to identify mechanisms that govern the activation of genes in an organism. Microarray experiments are done to produce gene expression patterns, which provide dynamic information about cell function. The huge volume of such data, and their high dimensions, make gene expression data to be suitable candidates for the application of data mining functions.

In this chapter, I have provided an introduction to knowledge discovery from microarray experimental data sets. The major functions of data mining have been discussed from the perspectives of machine learning, pattern recognition, and artificial intelligence. Soft computing methodologies, involving fuzzy sets, neural networks, GAs, rough sets, wavelets, and their hybridisations, have recently been used to solve data mining problems. They strive to provide approximate solutions at low cost, thereby speeding up the process. For the future research and development in microarray data analysis and mining, all the methods are useful tools. The role of soft computing in microarray gene expression study is very promising with the learning ability of neural networks to predict the searching potential of GAs and DGL and the uncertainty handling capacity of fuzzy sets.

DNA microarrays make it practical, for the first time, to survey the expression of thousands of genes under thousands of conditions. This technology makes it possible to study the expression of all of the genes at once. Large-scale expression profiling has emerged as a leading technology in the systematic analysis of cellular physiology. However, method development for analysing gene expression data is still in its infancy. The DGL optimisation uses a mathematical method based on orthogonal sets of numbers. By slicing the multi-dimensional parameter space with a horizontal plane of the objective function, with each parameter independent of the others, a peak is always surrounded by a slope. By finding all regions in which the objective function has values above that of the plane, one can narrow the search region. After finding the boundary of all the isolated regions where this occurs, the plane is raised again and the process repeated. OAs are immensely important in all areas of human investigation. In statistics, they are primarily used in designing experiments. An OA is an array of numbers constructed by utilising orthogonal Latin Squares, one can form an array of several dimensions that are orthogonal to each other and therefore allow the calculation of a resultant using many interdependent variables. Combining OA's sampling with

function domain contraction techniques results in an optimisation with two desirable properties. Firstly, the number of function evaluations can be greatly reduced, and secondly, there is a guarantee of finding the global optimum solution. In this study, a carefully selected OA was successfully used for conducting an orthogonal search space sampling. By using an OA and other mathematical techniques, it is practical to develop a global optimisation program for cancer classification and validation on a desktop computer. The primary advantages of this technique are that the global optimum is always found, excellent solutions can be found with little prior knowledge, and the new objective functions can be created according to whatever combination of parameters are required.

The mathematical procedures used in this form of global optimisation are possible to apply to a variety of other previously unsolved problems relating to the resultant of dependent variables, including experimental design and manufacturing variations. There are many other approaches people have adopted, but until now (with the exception of scanning), they all depend either on a starting design, some form of local optimisation, or some random variation. Each method will usually give rise to different solutions. For gene subsets using a large number of genes, these are still the only methods possible. In contrast, the DGL optimisation described here is a methodical global method. The proposed pyramidal hierarchy of the predictor for classification can effectively improve the signal to noise ratio in mining the high-dimensional microarray data sets. While the research in cancer classification with microarray expression data is the first to benefit from this method, the mathematical procedures, DGL global optimisation, used in this study are also applicable to a variety of other unsolved problems related to linked multi-variable problems. The application of this technique will undoubtedly have implications well beyond cancer classification application.

It is still too early to predict what the ultimate impact of microarray will be on our understanding of cancer although the possibility of the accurate diagnosis of cancers based on microarray expressions has emerged. This innovative research truly brings to light one of the hardest problems yet, the ability to accurately classify medical neoplasm. The DGL method provides a precise diagnostic tool that can find the true global optima with questions relating to gene malignancy. Furthermore, genetic screening for diseases are playing an increasingly important role in preventative medicine; if we can detect the presence of disease or predict the malignancy through microarray expression data with a desktop computer, before clinical diagnosis, a more efficient and clear cut treatment plan can be formulated, eliminating the possibility of clinician bias. More importantly, an unbiased and digital data-based approach can be easily applied to distinctions relating to future clinical outcome, such as drug response or survival. In cancer research, fundamental mechanisms that cut across distinct types of cancers could also be discovered through mining microarray data by the DGL global strategies.

## References

1. Bassett Jr, D.E.B., Eisen, M.B., and Boguski, M.S., (1999) Gene expression informatics—it's all in your mine, 21 (suppl.), *Nature Genetics*, 51–55.
2. Aittokallio, T., Kurki, M., Nevalainen, O., Nikula, T., West, A., and Lahesmaa, R., (2003) Computational strategies for analyzing data in gene expression microarray experiments, *Journal of Bioinformatics and Computational Biology*, 1(3), 541–586.
3. Zhang, S. and Gant, T.W., (2004) A statistical framework for the design of microarray experiments and effective detection of differential gene expression, *Bioinformatics*, 20(16), 2821–2828.
4. Alon, U., Barkai, N., Notterman, D.A., Gish, K., Ybarra, S. Mack, D., and Levine, A.J., (1999) Broad patterns of gene expression revealed by clustering analysis of tumor and normal colon tissues probed by oligonucleotide arrays, Proc. Natl Acad. Sci. USA, 96, 6745–6750.
5. Golub, T.R., Slonim, D.K., Tamayo, P., Huard, C., Gaasenbeek, M., Mesirov, J.P., Coller, H., Loh, M.L., Downing, J.R., Caligiuri, M.A., Bloomfield, C.D., and Lander, E.S., (1999) Molecular classification of cancer: Class discovery and class prediction by gene expression monitoring, *Science*, 286, 531–537.
6. Li, L., Darden, T.A., Weinberg, C.R., Levine, A.J., and Pedersen, L.G., (2001) Gene assessment and sample classification for gene expression data using a genetic algorithm/k-nearest neighbour method, *Combinatorial Chemistry & High Throughput Screening*, 4, No. 8, 727–739.
7. Li, L., Weinberg, C.R., Darden, T.A., and Pedersen, L.G., (2001) Gene selection for sample classification based on gene expression data: study of sensitivity to choice of parameters of the GA/KNN method, *Bioinformatics*, 17, No. 12, 1131–1142.
8. Horst, R. and Pardalos, P.M., (1995) *Handbook of Global Optimization*, Kluwer Academic Publishers, Netherlands.
9. Li, D. and Nathan, B., (1996) Global optimization advances multivariable thin-film design, *Laser Focus World*, No. 5, 135–136.
10. Li, D. and Smith, C., (1996) A new global optimization algorithm based on Latin Square theory, *Proceedings of 1996 IEEE International Conference on Evolutionary Computation*, ISBN: 0-7803-2902-3, 628–630.
11. Han J. and Kamber, M., (2001) *Data Mining: Concepts and Techniques*. San Diego: Academic Press.
12. Mitra, S., Pal, S.K., and Mitra, P., (2002) “Data mining in soft computing framework: A survey,” *IEEE Transactions on Neural Networks*, vol. 13, pp. 3–14.
13. Hand, D., Mannila, H., and P. Smyth, (2001) *Principles of Data Mining*. London: MIT Press.
14. Kantardzic, M., (2002) *Data Mining: Models, Methods, and Algorithms*. Hoboken, NJ: Wiley Interscience, IEEE Press.
15. Schena, M., Shalon, D., Davis, R.W., and Brown, P.O., (1995) “Quantitative monitoring of gene expression patterns with a complementary DNA microarray”, *Science*, 270, 467–470.
16. Alizadeh, A.A., et al., (2000) “Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling”, *Nature*, 403, 503–511.
17. Brown, M.P.S., Grundy, W.N., Lin, D., Critianini, N., Sungnet, C., Furey, T.S., Ares, M., Haussler, D., (2000) “Knowledge-Based analysis of microarray gene expression data using support vector machines”, *Proceedings of National Academy of Sciences*, 97, 262–267.
18. Deutsch, J.M., (2003) “Evolutionary algorithms for finding optimal gene sets in microarray prediction”, *Bioinformatics*, 19, 45–52.
19. Khan, J., Wei J.S., Ringner, M., Saal, L.H., Ladanyi, M., Westermann, F., Berthold, F., Schwab, M., Antonescu, C.R., Peterson, C., Meltzer, P.S., (2001) “Classification and

- diagnostic prediction of cancers using gene expression profiling and artificial neural networks”, *Nature Medicine*, 7, 673–679.
- 20. “Special Issue on Bioinformatics”, *IEEE Computer*, vol. 35, July 2002.
  - 21. Mitra, S. and Acharya, T., (2005) *Data mining: Multimedia, Soft Computing, and Bioinformatics*, John Wiley & Sons Inc., Newark, ISBN:0471474886.
  - 22. Draghici, S., (2002) Statistical intelligence: effective analysis of high-density microarray data. *Drug Discov Today*, 7(11 Suppl.): S55–S63.
  - 23. Tou, J.T. and Gonzalez, R.C. (1974) *Pattern Recognition Principles*. London: Addison-Wesley.
  - 24. Cho, S.B. and Ryu, J. (2002) “Classifying gene expression data of cancer using classifier ensemble with mutually exclusive features”, *Proceedings of the IEEE*, vol. 90, pp. 1744–1753.
  - 25. Wang, L. and Fu, X., (2005) *Data mining with computational intelligence*, Springer, Germany.
  - 26. Li D., (2004) “Global Optimisation for Optical Coating Design”, *Proceedings of 2004 Conferences in Internet Technologies and Applications*, ISBN 86-7466-117-3, Purdue, Indiana, USA, July 8–11.
  - 27. Peng, S., Xu, Q., Ling, X.B., Peng, X., Du, W., and Chen, L., (2003) Molecular classification of cancer types from microarray data using the combination of genetic algorithms and support vector machines, *FEBS Letters*, 555, 358–362.
  - 28. Liu, J.J., Cutler, G., Li, W., Pan, Z., Peng, S., Hoey T., Chen, L., and Ling., X.B., (2005) Multiclass cancer classification and biomarker discovery using GA-based algorithms, *Bioinformatics*, 21, No. 11, 2691–2697.
  - 29. Li, D., (2006) <http://www.scis.ecu.edu.au/Staff/staffinfo.aspx?staffid=donggual>

# Index

## A

- Abl kinase inhibitor  
  effective against imatinib-resistant BCR–ABL mutants, 158  
  ineffective against BCR–ABL–T315I mutant, 158
- Abl tyrosine kinase inhibitor imatinib mesylate (drug for leukemia therapy), 157
- Activation induced cytidine deaminase (AID)  
  activity of, 104
- Acute leukemia, 21
- Acute lymphoblastic leukemia (ALL), 21, 83
- Acute lymphocytic leukemia (ALL), 124  
  chemosensitivity and chemoresistance of engrafted, 124  
  *in vivo* models of human, 124–125
- Acute myelogenous leukemia (AML), 123  
  engrafted NOD/SCID mice, using, 123  
  engraftment, 123–124  
  higher levels, approaches, 124  
  stem cells, 127–128
- Acute myeloid leukemia (AML), 22, 68–69, 87  
  cytarabine, general response to, 87  
  cytogenetic abnormalities, patients with other, 87  
  modeling  
    acceleration of AML development with chemical mutagen, 25–27  
    collaborative induction of AML with multiple oncogenes, 24–25  
    deletion of tumor suppressor gene causes AML, 27  
    transgenic AML mouse model, 24
- AKXD recombinant inbred (RI) strains, 228
- AML associated with recurrent genetic abnormalities
- CDX2 and CDX4, 72
- C/EBP $\alpha$ , 71–72
- CEBP $\alpha$  knock-out model of acute erythroleukemia, 72
- model of acute myeloid leukemia, 71
- MOZ–TIF2, 70
- PU.1., 70–71
- AML1–ETO*, 26
- Animal cancer models in anticancer drug discovery and development  
  identifying main causes of failure of anticancer drug candidates, 246–247
- imatinib resistance in chronic myeloid leukemia, 255–257
- modern paradigm for anticancer drug discovery, 247–248
- taxane resistance in breast cancer, 253–255
- typical protocol for drug evaluation utilizing tumor xenografts—commonality and variables
- origin of model, 249
- site of implant, 248–249
- study endpoints, 250–252
- value of xenograft models in contemporary cancer drug discovery, 252–253
- Anticancer drug candidates, identifying the main causes of failure of
- main reasons for failure, 246–247
- rates and causes for attrition of clinical candidates, 246
- Anticancer drug discovery, modern paradigm for
- concept of therapeutic index, 247
- example of flow of compounds in decision network for drug discovery, 248
- APRIL, 189
- Ataxia telangiectasia, 98–99

- Ataxia telangiectasia-mutated (ATM) protein, 94  
coordination of downstream pathway, 94  
early DNA damage response, 94  
role in stabilizing DNA DSBs obligate predecessor of V(D)J recombination, 95
- ATM and sporadic cancers  
Fanconi anemia, 99
- ATR, 94  
with ATRIP, 95
- B**
- B-cell acute lymphoblastic leukemia (B-ALL), 84, 157
- B-cell lymphomas  
derived from GC or post-GC B cells, 180
- BCR-ABL* oncogene, 125
- BCR-ABL* protein, 84, 125  
molecular analysis of, 84
- BCR-ABL* signaling  
different pathways, 159–160  
Src kinases in, 160–161
- BCR-ABL-T315I* mutant, 158  
resistant to imatinib therapy and dasatinib, 158
- BLM helicase, 97
- Bone marrow pre-B-cell expansion  
BM pre-B cells in prelymphoma stage  
clonal, determining, 239  
genetic abnormality hypothesis, 238–239  
genetically determined pre-B-cell expansion, 237–238  
genetic property of BM stem cells or – induced by BM microenvironments, determining, 238  
induced by ecotropic MuLV for lymphomagenesis, determining, 238  
and lymphomagenesis, 238–239  
in NFS, SL/Kh, and SL/Kh lymphoma, 237
- Bone marrow transplantation (BMT)  
curative therapy for Ph+ leukemia, 169
- Breakpoint cluster region (BCR) gene, 83
- Breast cancer, taxane resistance in, 253–255  
examples of xenografts used for development of Ixempra, 254  
ixabepilone (Ixempra) for treatment of, 253  
mechanisms of drug resistance, 253–254
- Burkitt's lymphoma, 84, 110  
sporadic, 111
- C**
- Cancer  
and chromosomal instability, 82  
reactions to chemotherapy regimen by patients, 84, 87
- Cancer, senescence, and evolution, 136–138
- CBF $\beta$ -SMMHC* gene, 24
- CB17-scid mice  
targeted mutations in recombination activating genes (Rag) 1 and Rag2, 122
- CDX2, 72
- CDX4, 72
- C/EBP $\alpha$ , 71–72
- Cell senescence *versus* cancer, 134, 138
- Cellular damage, 134
- Chromosomal aberration, 84  
diagnostic and prognostic tests after interpreting risks, 87
- Chromosomal instability, 82
- Chromosomal lesions, 83
- Chromosomal translocations, 22–23, 24, 28, 30–33  
characterizing tumors, 83  
and myeloid malignancies, 84  
results, 84
- Chronic eosinophilic leukemia (CEL), 2
- Chronic leukemia, 21
- Chronic lymphocytic leukemia (CLL), 21, 183
- Chronic lymphocytic leukemia/small lymphocytic lymphoma  
features of indolent and aggressive CLL, 184  
mouse models of, 184–191
- Chronic myelogenous leukemia (CML), 21, 83
- Chronic myeloid leukemia, imatinib resistance in, 255–257  
development of imatinib-resistant CML cell line, 256
- Ph+ ALL insensitive to imatinib, 255  
use of combinations of BCR-ABL and SRC inhibitors to overcome resistance, 256
- Chronic myeloid leukemia (CML), 1, 27, 125, 157  
*in vivo* models of human, 125–126
- Class switch recombination and somatic hypermutation, 103–104
- CLL, *see* Chronic lymphocytic leukemia (CLL)
- Clonality, 57–58

- Cluster analysis  
analysing patterns of gene expression, 260
- Comparisons between strains, problems with, 149–150
- Competitive repopulation, 143–144
- Cytochemistry  
cytochemical stains, 56
- D**
- Data mining system, 261, 278
- DGL global optimisation algorithms  
experimental results, 270–274  
results of classification, 273  
validation of leukaemia data with multi-subset class predictor, 272
- leukemia data, 265
- mathematical form of, 268–269
- multi-subsets class predictor, 269
- objective function, 266–267
- k-nearest neighbour (KNN) rules ( $k = 5$ ), 267
- orthogonal arrays and sampling procedure, 265–266
- optimal gene subsets and selected genes for leukaemia data class predictor, 271
- overall methodology, 265
- search spaces reduction for global search, 267–268
- validation (predicting through voting mechanism), 270
- DGL global strategies and data mining for human blood cancers
- DGL global optimisation algorithms  
experimental results, 270–274  
leukemia data, 265
- mathematical form of, 268–269
- multi-subsets class predictor, 269
- objective function, 266–267
- orthogonal arrays and sampling procedure, 265–266
- overall methodology, 265
- search spaces reduction for global search, 267–268
- validation (predicting through voting mechanism), 270
- discussion, 274–277
- genetic algorithms, 264
- microarray gene expression and experimental design  
classification and clustering – molecular classification of leukaemia, 263–264
- fold change analysis, 262–263
- Dietary restriction  
break paradigm, 142
- cancer, senescence, and evolution, 136–138
- competitive repopulation, 143–144
- current paradigm, 135–136
- DR in BALB mice, 144–148
- DR in B6 mice, 148
- future research, 151–152
- model systems to examine effects of DR, 142–143
- need to examine multiple strains, 148–149
- problems with comparisons between strains, 149–150
- proposed model, 150–151
- role of p16 in mice, 138–140
- role of p53 in mice, 140–142
- Diffuse high-grade blastic B-cell lymphoma/ leukemia (DBLL) in mice  
in genetically engineered mice, 204
- Diffuse Large B-Cell Lymphoma (DLBCL), 197  
in humans, 197
- mouse models of, 197–203
- in genetically engineered mice, 199–203
- spontaneous DLBCL in mice, 197
- spontaneous variants of DLBCL in mice, 197–199
- DNA double-strand breaks (DSBs), 84  
misrepair of, 87  
repair pathways, 87
- homologous recombination, 89–100
- nonhomologous end joining, 100–111
- sources of
- endogenous, 88
- exogenous, 88–89
- DNA double-strand break repair,  
mechanisms of perspective, 111–112
- sources of DNA double-strand breaks
- endogenous, 88
- exogenous, 88–89
- DNA double-strand break repair pathways,  
mechanisms of
- homologous recombination, 89–100
- DNA microarrays, 262, 278
- DNA–PK holoenzyme, 105
- DNA repair, 134
- DR in BALB mice, 144–148
- clonal stability, 147–148
- effects of age and diet restriction (DR) on hematopoietic stem cell (HSC) and clonal stability in, 147

- DR in BALB mice (cont.)**
- hematopoietic stem cell (HSC) frequency and function in, 145
  - HSC markers, 144–145, 144–146
  - proposed model, 146
- DR in B6 mice, 148**
- DR, model systems to examine effects of, 142–143**
- Dysregulated tyrosine kinases (TKs) in myeloproliferative syndromes, 2**
- E**
- E $\mu$ -myc transgenic mice, 228**
  - Essential thrombocythemia (ET), 1**
  - ETV6/RUNX1, 28**
    - mechanism of lymphoid transformation, 28–29
  - Eukaryotic cells, 108**
- F**
- Fanconi anemia, 99**
  - FIP1L1-PDGFR $\alpha$ , 65–66**
  - FISH analyses, 128**
  - Flow cytometry, 57**
  - FLT3-ITDs gene, 25**
    - disease frequency, 25
  - FLT3 mutations, 66–68**
  - Fold change analysis, 262–263**
  - Follicular B-cell lymphoma**
    - in humans, 194–195
    - mouse models of, 195–196
      - in genetically engineered mice, 195–196
      - spontaneous FBL in mice, 195
  - Fusion genes, 22–23, 26, 28–29, 30–33**
    - AML1-ETO, 26*
    - ETV6/RUNX1 related, 28*
    - examples of, 22–23
    - induces CML-like diseases, 28
- G**
- Gene selecting process, 260**
    - methods, 261
  - Genetic and virological predisposition to pre-B**
    - bone marrow pre-B-cell expansion
    - genetically determined pre-B-cell expansion, 237–238
    - insufficient for lymphomagenesis, 238–239
    - in NFS (4-week-old), SL/Kh (4-week-old), and SL/Kh lymphoma, 237
- H**
- Heat shock protein 90 (Hsp90)**
    - inhibition by IPI-504 reduces growth of myeloid leukemic cells harboring BCR-ABL-T315I mutant, 166
    - prolongs survival of CML mice, treating with, 164
    - therapeutic target for CML induced by either BCR-ABL-WT or BCR-ABL-T315I, 167
  - Hematologic malignancies**
    - exposure to environmental clastogens, 87

- induced by dysregulated TKs, mouse models of, 4–5  
retroviral BM transduction/transplantation model, 5  
transgenic mice strategy, 4–5  
leading to deregulation of cellular proto-oncogene/oncogene, 83  
molecular characterization of recurrent chromosomal abnormalities, 84  
mouse models of, 121  
Hematopoietic disease models and molecularly targeted therapy  
  murine models of, 73  
Hematopoietic malignant transformations, 110  
Homologous recombination (HR), 87, 89  
  and cancer  
    ataxia telangiectasia, 98–99  
    ATM and sporadic cancers, 99  
    Fanconi anemia, 99  
    variant alleles of Rad51-family genes, 99–100  
    Xrec2 deletions, 100  
  gene conversion, 89–91  
  schematic of, 90  
genes and proteins, 94  
  BLM, 97  
  damage sensing, 94–95  
  Rad52 epistasis group, 96–97  
  Rad51 family, 95–96  
  RecQ helicase family, 97  
  WRN, 97, 98  
and genomic instability, 93–94  
homologous sequences on  
  nonhomologous chromosomes, 93–94  
nonhomologous end joining  
  and cancer, 109–111  
  class switch recombination and somatic hypermutation, 103–104  
  deficient mouse models, 107–108  
  genes and proteins, 105–107  
  mechanics of, 104–105  
  RAG endonuclease, 102–103  
  and telomere maintenance, 108–109  
V(D)J recombination, 101–102  
single-strand annealing, 91–93  
  schematic of, 93  
synthesis-dependent strand annealing, 91  
  schematic of, 92  
Host genetic factors affecting types of lymphomas  
other host loci affecting  
  lymphomagenesis, 236  
genetic analysis, 231–232  
lymphoma resistance of SL/Ni strain, 236–237  
two dominant resistance loci in MSM/Ms mice, 236  
virus expression, 236  
Hsp90 inhibition  
  suppresses BCR–ABL–T315I-expressing leukemia, 164  
Human germinal center (GC) tumors, mouse models of, 200–202  
Human hematopoietic cells  
  engraftment levels of, 122  
Human leukemia using immune-compromised mice, modeling  
  AML stem cells, 127–128  
  development of immune-compromised mice and humanized mice  
  *in vivo* models of human acute lymphocytic leukemia, 124–125  
  *in vivo* models of human acute myelogenous leukemia, 123–124  
  *in vivo* models of human chronic myeloid leukemia, 125–126  
  *in vivo* models of multiple myeloma, 126–127  
  leukemia stem cells, 127  
  LSCs in B-ALL, 128–129  
Human lymphomas, 180  
Human mature B-cell and plasma cell neoplasms, mouse models of  
  cellular origins of mouse and, 181  
  chronic lymphocytic leukemia/small lymphocytic lymphoma, 183–184  
  mouse models of, 184–191  
diffuse high-grade blastic B-cell lymphoma/leukemia (DBLL)  
  in mice  
  in genetically engineered mice, 204  
diffuse large B-cell lymphoma  
  in humans, 197  
  mouse models of, 197–203  
follicular B-cell lymphoma  
  in humans, 194–195  
  mouse models of, 195–196  
mantle cell lymphoma  
  in humans, 193–194  
  mouse models of, 194  
marginal zone lymphomas  
  in humans, 191  
  mouse models of, 192–193

- H**
- Human mature B-cell (*cont.*)
- plasma cell neoplasms
    - conclusions, 217
    - established and newly emerging mouse models of human, 208–215
    - human PCN including multiple myeloma, 204–208
    - xenograft models of human myeloma in mice, 215–216
  - Human MCL, 193–194
  - Human Philadelphia chromosome-positive leukemia in mice, modeling
    - BCR–ABL signaling, 159–161
    - future directions, 170
    - molecular mechanisms of Ph+ leukemia, 158–159
    - mouse models of Ph+ leukemia, 161–164
    - translational research using Ph+ leukemia mouse models, 164–170  - Human Philadelphia chromosome-positive (Ph+) leukemias, 157
  - Hypereosinophilic syndrome (HES)/CEL, 62
- I**
- Imatinib, effectiveness of
- imatinib resistance
    - to overcome, 169
  - prolonging survival of CML or ALL, 164
  - in treating CML or B-ALL induced by BCR–ABL, 164
- Immunohistochemistry, 56–57
- Inducible tet-off model, 161–162
- Interleukin-2 receptor (IL-2R) gamma-chain locus (Il2rg)
- J**
- JAK2, 3
- JAK2-V617F, 62–63
- induced MPD, emerging transgenic mouse models of, 16
  - induced polycythemia in mice, studying signaling mechanisms of, 11–13
  - independent of Src family kinases, 12
  - induced polycythemia to kinase inhibitor therapy, testing response of, 13–15
  - induces polycythemia through autonomous overproduction of erythrocytes, 9
- JAK2-V617F mutation, 3
- L**
- Lentivirus-mediated transgenesis, 182
- Leukaemia, 21
- acute, 21
  - causes, 22
  - chronic, 21
  - mutations in DNA, 22
- Leukemia class predictor
- genes appear more than once in, 275–277
  - 219 genes selected by, 275–276
- Leukemia/lymphomas
- recurrent translocations in, 84
- Leukemias induced by BCR-ABL, pathological characteristics of, 163
- Leukemia stem cells (LSCs), 127
- in B-ALL, 128–129
  - heterogeneity within genetic subtypes of B-ALL, 128–129
- Leukemic cells, transplantation of, 123
- Leukemogenesis, 121
- in vivo* animal models for human AML, 123
- Leukemogenesis by dysregulated tyrosine kinases, strategies to study mechanisms of, 7
- analyzing molecular pathogenesis of disease, 7
  - in retroviral transduction model, 7
- LIG4 syndrome, 109
- Lymphocyte development, somatic alterations during, 102
- Lymphoid
- malignancies, 84
  - recurrent translocations, 85–86
- M**
- Mantle cell lymphomas (MCL), 181
- in humans, 193–194
  - mouse models of, 194
- Marginal zone lymphomas (MZL)
- in humans, 191
  - mouse models of, 192–193
- MGUS, 207
- Microarray data analysis, 262
- Microarray expression data, 261
- Microarray gene expression and experimental design
- classification and clustering, 263–264
- MM, 205
- 5T2 and 5T33, strategies for treatment of MM in neoplastic plasma cell, 212

- Mouse leukemia models  
based on mechanisms, 22  
modeling acute lymphoblastic leukemia,  
28–29  
  **ETV6/RUNX1**, 28  
modeling acute myeloid leukemia, 22–27  
  acceleration of AML development  
    with chemical mutagen, 25  
  **AML1-ETO**, 25–27  
  collaborative induction of AML with  
    multiple oncogenes, 24–25  
  deletion of tumor suppressor gene  
    causes AML, 27  
  examples of fusion genes (MLL/CBP,  
    MLL/p300, MOZ/CBP, MOZ/  
    p300, MORF/CBP), 22–23  
  inducing gene in mouse bone marrow  
    cells using retrovirus, 22–23  
  transgenic AML mouse model, 24  
modeling chronic lymphocytic leukemia,  
29–34  
  role of **TCL1** in B-cell development  
    and in B-cell leukemia  
    pathogenesis, 29  
  **TNF receptor-associated factors**  
    (TRAFs), 29  
modeling chronic myeloid leukemia-like  
diseases, 27–28  
  induced by the fusion genes, 28
- Mouse models  
of CLL/SLL  
  **APRIL** and CLL, 189–190  
  **BLC2/TRAF** model of CLL–NFKB  
    connection, 188–189  
  conclusions, 190–191  
  models of CLL based on studies of  
    New Zealand mice, 184–187  
  spontaneous small B-Cell lymphoma/  
    leukemia in mice, 184  
  **TCL1 TG** mouse model of CLL,  
    187–188
- of DLBCL  
  conclusions, 203  
  in genetically engineered mice, 199–203  
  mouse models of human germinal  
    center (GC) tumors, 200–202  
  spontaneous DLBCL in mice, 197  
  spontaneous variants of DLBCL in  
    mice, 197–198
- of FBL  
  conclusions, 196  
  in genetically engineered mice, 195–196  
  spontaneous FBL in mice, 195
- of human blood cancers, examples of,  
30–33
- of MCL  
  in genetically engineered mice, 194
- of MZL  
  conclusions, 193  
  **MALT**, 192  
  splenic, 192–193  
  spontaneous MZL in mice, 192
- MOZ** gene, 23
- MOZ-TIF2** fusion protein, 23, 70
- MPDs associated with other mutant TKs,  
modeling, 15–16
- MPDs, *see* Myeloproliferative diseases  
(MPDs)
- MPLW515L/K**, 63–65
- MRE11-RAD50-NBS1** (MRN)  
complex, 94
- MSM/Ms** strain, 236
- Mucosa-associated lymphoid tissue  
(MALT), 181
- Multiple myeloma, 126  
  *in vivo* models of, 126–127
- Multiple strains, need to examine, 148–149
- Murine hematopoietic cells, cytology of  
  eosinophils and basophils, 54  
  erythroid series, 52  
  lymphocytes, 54  
  monocytes, 54  
  neutrophil series, 52–53  
  peripheral blood cells, 53
- Murine hematopoietic disease  
  ancillary techniques for evaluation of  
    clonality, 57–58  
    commonly used markers for  
      immunophenotypic analysis, 58  
      cytochemistry, 56  
      flow cytometry, 57  
      immunohistochemistry and special  
        stains, 56–57
- Bethesda classification systems of  
  in diagnosis of disease in both mice  
    and humans, 54
- Mouse Models of Human Cancers  
  Consortium (MMHCC), 54
- nonlymphoid and lymphoid  
  hematopoietic neoplasms in  
    mice, 55
- Murine hematopoietic tissue, histology  
  of normal  
    bone marrow, 49, 50  
    lymph nodes, 51  
    murine spleen, 51

- Murine hematopoietic tissue (*cont.*)  
 Peyer's patch, 52  
 spleen, 49–51  
 thymus, 52
- Murine models of hematopoietic disease  
 ancillary techniques for evaluation of, 56–58  
 Bethesda classification systems of, 54–55  
 cytology of normal murine hematopoietic cells, 52–54  
 general approach to analysis of mouse laboratory findings, 47–48  
 necropsy findings, 48–49  
 physical findings, 46–47  
 histology of normal murine hematopoietic tissue, 49–52  
 laboratory findings  
   EDTA spray-coated tubes recommended, 48  
   Mouse Phenome Database, 48  
   Wright–Giemsa staining preferred, 48  
 and molecularly targeted therapy, 73  
   murine models of, 73  
 nonlymphoid hematopoietic neoplasms in mice  
   acute myeloid leukemia, 68–69  
   AML associated with recurrent genetic abnormalities, 69–72  
   myeloproliferative disorders, 59–68
- Murine models of hematopoietic disease and molecularly targeted therapy, 73
- Myelofibrosis in JAK2-V617F recipient mice, development of, 11
- Myeloid leukemias  
 recurrent translocations, 85–86
- Myeloid neoplasias, 84
- Myeloproliferative diseases (MPDs), 1, 2  
 chronic eosinophilic leukemia (CEL), 2  
 chronic myeloid leukemia (CML), 1  
 dysregulated tyrosine kinases (TKs) in, 2  
 essential thrombocythemia (ET), 1  
 pathogenesis of, 2  
 polycythemia vera (PV), 1  
 primary myelofibrosis (PMF), 1
- Myeloproliferative disorders  
 BCR–ABL, 60–61  
 conditional activation of oncogenic K-ras allele (K-RasG12D), 66  
 FIP1L1–PDGF $\alpha$ , 65–66  
 FLT3 model of disease, 68  
 FLT3 mutations, 66–67, 66–68
- JAK2V617F, 62–63  
 models of mutant SHP2 disease, 67  
 MPLW515L/K, 63–65  
 RAS mutations, 65  
 retroviral transduction of BCR–ABL in bone marrow transplantation model, 61  
 retroviral transduction of JAK2V617F in bone marrow transplantation model, 64  
 SHP2 mutations, 65–66  
 TEL–PDGFR $\beta$ , 65–66
- N**
- NOD-scid (NOD/SCID) mice  
 advantage over CB17-scid mice, 122
- Nonhomologous end joining (NHEJ), 87, 100–111  
 and cancer, 109–110  
 susceptibility of tumorigenesis during V(D)J recombination and CSR, 110–111  
 chromosome ends sans proper telomeres – substrate for repair, 87  
 class switch recombination and somatic hypermutation, 103–104  
 deficient mouse models, 107–108  
 genes and proteins, 105–107  
 mechanics of, 104–105  
 RAG endonuclease, 102–103  
 roles in repair of programmed and unprogrammed exogenously induced DNA double-strand breaks (DSBs), 106  
 and telomere maintenance, 108–109  
 V(D)J recombination, 101
- Nonlymphoid hematopoietic neoplasms in mice  
 acute myeloid leukemia, 68–69  
 AML associated with recurrent genetic abnormalities, 69–72  
 myeloproliferative disorders, 59–68
- Nonlymphoid leukemias, 59
- Non-obese diabetic (NOD) strain, 122
- O**
- Objective function plot, 261
- P**
- Pathophysiology and therapy, insights into dysregulated tyrosine kinases, 1–4

- emerging transgenic mouse models of JAK2-V617F-induced MPD, 16  
modeling MPDs associated with other mutant TKs, 15–16  
mouse models of hematologic malignancies, 4–5  
retroviral BM transduction/transplantation model of CML, 5–7  
studies of MPD induced by JAK2-V617F in mouse retroviral transduction/transplantation model histopathology of MPD, 10–11  
JAK2-V617F induces polycythemia in mice by overproduction of erythrocytes – independent of Epo, 8–9  
JAK2-V617F induces strain-dependent leukocytosis in mice, 9–10  
studying signaling mechanisms of JAK2-V617F-induced polycythemia in mice, 11–13  
study mechanisms strategies of leukemogenesis in mice, 7–8  
testing response of JAK2-V617F-induced polycythemia to kinase inhibitor therapy, 13–15
- Ph+ CML  
response to imatinib, 87
- Philadelphia (Ph) chromosome, 83  
encoding for chimeric protein, 83–84
- Ph+ leukemia  
molecular mechanisms of, 158–159  
BCR-ABL oncogene, 158  
P190, P210, or P230, 158
- mouse models  
inducible tet-off model, 161–162  
pathological characteristics of leukemias induced by BCR-ABL, 163  
retroviral transduction/transplantation model, 162–164  
transgenic model, 161  
xenotransplantation model, 162
- mouse models, translational research using  
heat shock protein 90 (Hsp90)-therapeutic target for chronic myeloid leukemia (CML), 167  
identification of bone marrow cell populations as chronic myeloid leukemia (CML) stem cells, 168
- identification of crucial signaling pathways, 164  
identification of leukemic stem cells, 165–169  
inhibition of heat shock protein 90 (Hsp90) by IPI-504, 166  
sensitivity of leukemic stem cells to kinase inhibitors, 169  
Src kinases as therapeutic targets for Ph + B-ALL, 169  
testing of new therapeutic targets and strategies, 164–165
- treatment of  
bone marrow transplantation (BMT), 169  
combination treatment with IPI-504 and imatinib effective, 164  
with Hsp90 inhibitor (IPI-504), 164  
imatinib, effectiveness of, 164
- P16 in mice, role of, 138–140
- P53 in mice, role of, 140–142  
relationships among p53 dosage, longevity, cancer, and hematopoietic stem cell (HSC) function, 141
- Plasmas cell neoplasms (PCN), 180  
classification of PCN in WHO nomenclature, 205  
conclusions, 217  
established and newly emerging mouse models of human, 208–215  
de novo and transplantation models, 208  
extraosseous plasmacytoma (PCT) and multiple myeloma (MM), 209–211  
H2-L<sup>d</sup>- hu-IL-6 TG, 213  
VK\*MYC TG model, 215  
X-box-binding protein-1 (XBP1), 214
- genetic risk and pathogenic factors of MM, 206
- human PCN including multiple myeloma, 204–208  
subgroups of MM, 206–207  
5T myelomas, 212  
xenograft models of human myeloma in mice, 215–216  
SCID-hu model and modifications, 216
- PML-RAP $\alpha$  fusion oncogene roles in APL phathogenesis, 24
- Polycythemia and reticulocytosis induced by JAK2-V617F responds to kinase inhibitor therapy, 14

Polycythemia induced by JAK2-V617F – independent of Src family kinases, 12  
 Polycythemia vera (PV), 1, 3  
 Pre-T lymphoblastic leukemia (pre-T LBL), 84  
 Primary myelofibrosis (PMF), 1  
 Protein kinase, 122  
 Protocol for drug evaluation utilizing tumor xenografts-commonality and variables  
 examples of xenograft tumor response to cytotoxic drug and calculations to determine log cell kill, 251  
 origin of model, 249  
 site of implant, 248–249  
 study endpoints, 250–252  
     tumor cell kill, estimating, 251–252  
     tumor growth inhibition, 250  
     tumor size, estimating, 250  
 PTEN gene, 27  
 PU.1., 70–71

**R**

Rad52, 96  
 epistasis group, 96–97  
 Rad54, 96  
 Rad51 family, 95–96  
     variant alleles of, 99–100  
 RAG endonuclease, 102–103  
     RAG1/2, 102–103  
 RAS mutations, 65  
 Ras oncogene, 25  
 Recombination signal (RS) sequences, 101  
 RecQ helicase family, 97  
 Recurrent translocations  
     associated with lymphoid and myeloid leukemias, 85–86  
 Repopulating ability (RA), 133  
     loss of, 133, 144, 146  
     treatments increase RA and delay senescence, 133, 139  
     treatments increase risk of cancer, 134  
 Retroviral BM transduction/transplantation model of CML, 5–7  
 of BCR–ABL-induced chronic myeloid leukemia (CML)-like myeloproliferative disease (MPD), 6  
     drawbacks and limitations, 7  
     role of BCR–ABL in CML, 5  
 Retroviral transduction/transplantation model, 162–164  
     improving systems to develop efficient and accurate mouse model of human CML and B-ALL, 163

pathological characteristics of leukemias induced by BCR–ABL, 163  
 Retrovirus integrations in lymphoma (molecular pathogenesis)  
     kinetics of colony formation by pre-B cells, 240

**S**

SHP2 mutations, 65–66  
 SL/Kh pre-B lymphomas, 228–229  
     immunopathology of, 229–231  
     cumulative percent incidence of spontaneous pre-B lymphomas, 230  
     histopathology of major type, 230  
     type of lymphomas, 230  
     and related strains of mice, origin of parameters for lymphomagenesis in AKR and SL family mice, 229  
 SL/Ni strain, 236  
 Small lymphocytic lymphoma (SLL), 183  
 Somatic alterations during lymphocyte development, 102  
 Somatic hypermutation (SHM), 84  
     single mutations leading to changes in antibody affinity/specifity, 104  
 Splenic MZL, 191  
 Spontaneous pre-B lymphoma, 228  
 Spontaneous variants of DLBCL in mice  
     DLBCL–CBL, 197–198  
     DLBCL–HA, 198–199  
     DLBCL–IBL, 198  
 Strand annealing, 91–93  
     schematic of, 93  
     synthesis-dependent, 91  
     schematic of, 92  
 Studies of MPD induced by JAK2-V617F in mouse retroviral transduction/transplantation model  
     histopathology of MPD, 10–11  
     development of myelofibrosis in JAK2-V617F recipient mice, 11  
 JAK2-V617F induces polycythemia in mice by overproduction of erythrocytes – independent of Epo, 8–9  
 JAK2-V617F induces strain-dependent leukocytosis in mice, 9–10

**T**

TCL1 gene, 29  
 Telomeres, 108  
 TEL-PDGFR $\beta$ , 65–66

- TG mouse models of human mature B-cell lymphomas and PCN, barriers to using, 182–183
- T lymphoma *vs.* B lymphoma AKXD RI strains, 234 determining length of latent period, 235 genetic and epigenetic determination of types of lymphomas in cross of SL/Kh × AKR, 234 genetic determination of lymphomagenesis in a cross of SL/Kh × NFS, 232 genetic factor determining types of lymphomas, 234 role of thymus for T lymphomagenesis, 233 stromal cells, 234 symbiotic complexes isolated from primary lymphoma thymus of AKR mouse, 233
- Transgenic model, 161
- Tumorigenesis genomic instability and translocations leading to, 87 from Ph chromosome encoding, 83–84 structural and numerical chromosomal abnormalities, role of, 82–83 susceptibility during V(D)J recombination and CSR, 110
- Tumor suppressors, 134 cell fate decision imposed by damage, 134 inverse relationship between tissue's RA and gene expression, 134–135
- U**
- Unusual mixed-type lymphoma in thymectomized (SL/Kh × AKR)F1 mice large granular-cell lymphomas with unusual NK-B phenotype, 235 role of thymus in T lymphomagenesis, 235
- V**
- V(D)J recombination, 101
- W**
- WRN, 98
- X**
- Xenogeneic transplantation, 121–122, 124 Xenograft models in contemporary cancer drug discovery, value of, 252–253 pathophysiology of origin, 252 Xenotransplantation model, 162 Xrcc2 Deletions, 100