

Mouse models to study genes involved in hematological malignancies

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Abstract

Hematological malignancies, including leukemia and lymphoma, consist a group of highly heterogeneous neoplasms characterized by numerous genetic lesions specific for the type of the disease. In order to understand, the role of a particular alteration in the development of a malignancy functional studies have to be carried out in vitro, in cell lines derived from primary cancer cells. Further efforts to understand the mechanisms underlying blood disorders including malignant transformation and progression relies on model organism research. Numerous transgenic mouse models, carrying human oncogenes have been generated resembling distinct types of hematological disorders. Recent technological advances revolutionized the generation of animal models making it much easier, faster, and precise. The introduction of the CRISPR-Cas9 technology allows for rapid generation of novel knockout or transgenic animals, and the development of conditional site- and time-specific Cre-Lox gene targeting technology, allows studying the function of genes which are relevant to normal hematopoiesis and development of hematological malignancies, but lethal when knocked out in embryonic cells. Besides the studies on gene function, mouse models of human leukemia allow for discovery and testing of novel antileukemic drugs. These new technologies are deepening our understanding of disease pathophysiology and treatment resistance, as well as are leading to novel therapeutic strategies for improved outcomes in patients.

Keywords: Mouse model, CRISPR/Cas9, CRE-Lox, Leukemia, Lymphoma

1. INTRODUCTION

Despite enormous progress in omics and in vitro techniques the animal models are still non-substitutable in biomedical research. After determination of genomic, transcriptomic, Proteomic, or metabolomic alterations in the patient samples and in vitro functional studies the biological meaning of the observed abnormality has to be finally studied in a whole living organism (Fig. 1). It is still the only way to determine the role of the candidate factors and the mechanism of the disease. In addition, animal tests are absolutely necessary before therapeutic agents will be used in humans. For almost a century laboratory mice have been invaluable research tools because of its short generation time, easy breeding, and low maintenance costs. Development of the inbred mouse strains with virtually identical genetic background greatly reduced the number of animals used and improved the reproducibility of experiments.

The first mouse models used to study hematological malignancies were carcinogen-induced transplantable models. Those models allowed to study the kinetics of leukemogenesis and response to therapy.^{1,2} Although artificial (carcinogens do not play an important role in pathogenesis of human leukemia) they deepened our understanding of the disease and led to the development of antimetabolite agents that were the first effective therapeutics in leukemia. Next, virally induced models have been employed in the studies on hematological malignancies.³ They further contributed to the understanding of leukemogenesis and identification of active therapeutic agents. However, their relevance to the human disease is also questionable, because viruses rarely play a role in the development of human hematological malignancies.

2. TRANSGENIC (GAIN-OF-FUNCTION, KNOCK-IN) MODELS

Leukemias and lymphomas are characterized by non-random, recurring genetic abnormalities that result either in activation of oncogenes or expression of fusion genes thereby leading to the malignant transformation of hematopoietic cells. In transgenic knocked-in models, mice are created through the manipulation of embryonic stem (ES) cells. In the classical approach, DNA is directly injected into the pro-nucleus of fertilized zygotes which are then injected into pseudopregnant females. This results in a non-targeted integration of the transgene and is reliant on expression of the transgene to generate the phenotype. In a more modern approach, DNA is electroporated into ES cells and integrants are selected for by expression of antibiotic resistance genes. Selected cells are then injected into tetraploid blastocysts, which are in turn implanted into pseudopregnant females.

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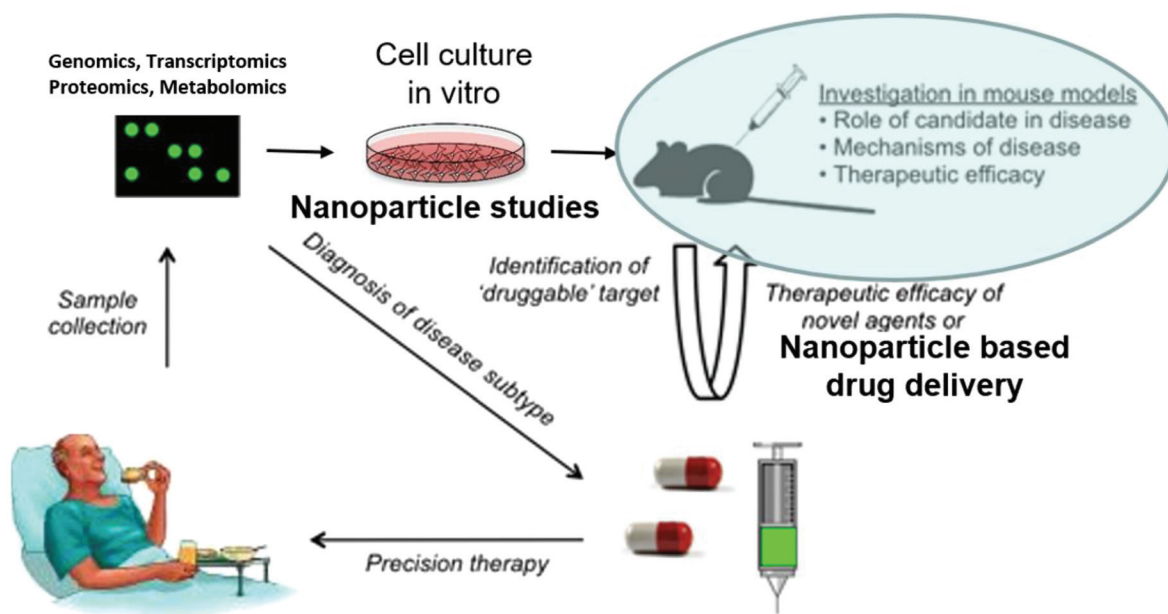


Figure 1. The place of mouse models in biomedical research.

Offspring are then backcrossed with wild type mice to generate the homozygously transgenic mice. Vectors can now be generated that target specific sites in the genome through homologous recombination.

3. LOSS-OF-FUNCTION (KNOCK-OUT) MODELS

Several growth and survival pathways can be hyper-activated by the loss of their negative regulators. Deleting or inactivating of such tumor suppressor genes is an alternative way of malignant transformation. Knockouts are accomplished through a variety of techniques.

The homologous recombination method involves creating a DNA construct containing a fragment homologous to the targeted and a drug resistance marker in place of the desired knockout gene. The construct can be delivered to stem cells either through microinjection or electroporation. This method is inefficient and relies on the cell's own repair mechanisms to recombine the DNA construct into its nuclear DNA.

More efficient are the recent methods based on site-specific nucleases precisely targeting a DNA sequence in order to introduce a double-stranded break. The break is then repaired through non-homologous end joining (NHEJ), causing insertions or deletions of base pairs, which cause frameshift mutations and thus create a knockout of that gene.

Zinc-finger nucleases consist of DNA-binding domains that can precisely target a DNA sequence.⁴ Each zinc finger can recognize codons of a desired DNA sequence, and therefore can be modularly assembled to bind to a particular sequence.⁵ These binding domains are coupled with a restriction endonuclease that can cause a double stranded break (DSB) in the DNA. Repair processes may introduce mutations that destroy functionality of the gene.

Transcription activator-like effector nucleases (TALENs) also contain a DNA-binding domain and a nuclease that can cleave DNA.⁶ The DNA-binding region consists of amino acid repeats

that each recognize a single base pair of the desired targeted DNA sequence.⁵ If this cleavage is targeted to a gene coding region, and NHEJ-mediated repair introduces insertions and deletions, a frameshift mutation often results, thus disrupting function of the gene.

Clustered regularly interspaced short palindromic repeats/Cas (CRISPR/Cas) system is a method for genome editing that contains a guide RNA complexed with a Cas protein.^{5,7} The guide RNA can be engineered to match a desired DNA sequence through simple complementary base pairing, as opposed to the time consuming assembly of constructs required by zinc-fingers or TALENs. CRISPR arrays, comprised of direct repeats (DR), and spacer tags are the first transcribed into a single large pre-CRISPR RNA pre-crRNA by a promoter located within the CRISPR leader. The resulting transcript is cleaved and processed into individual mature crRNAs by the Cas6 endonuclease (type I systems) or the ubiquitous RNase III enzyme (type II systems). Processing is mediated by characteristic secondary structures (hairpins) formed by type I pre-crRNAs or by a trans-activating RNA (tracrRNA) possessing homology to direct repeat sequences in type II systems. In the more widely used type II CRISPR/Cas9 system, crRNAs anneal to trans-activating crRNAs (tracrRNAs) and direct sequence-specific cleavage and silencing of pathogenic DNA by Cas proteins. The CRISPR/Cas9 system can thereby be easily targeted to cleave virtually any DNA sequence by designing the crRNA. The CRISPR/Cas9 system has been shown to be directly portable to human cells by co-delivery of plasmids expressing the Cas9 endonuclease and the necessary crRNA components.⁷⁻¹⁰ Cas9 endonucleases have also been converted into nickases,⁹ enabling an additional level of control over the mechanism of DNA repair.

4. CONDITIONAL KNOCKOUT SYSTEMS

About 15% of gene knockouts are developmentally lethal when introduced into the embryos. To study the function of those

Table 1**Murine models of hematopoietic malignancies.**

Disease	Gene target	Model
Acute myeloid leukemia (AML)	PU.1 + p53	Conditional knockout in hematopoietic cells
	NRas:Bcl-2	Conditional transgenic
	TERC	Conditional knockout
	AML-ETO	Inducible transgenic
	RAR α fusion	Transgenic, variable
Chronic myeloid leukemia (CML)	BCR-ABL1	Conditional transgenic in hematopoietic cells
	BCR-ABL1	Transposon-based insertional mutagenesis
Chronic lymphocytic leukemia (CLL)	miR-16	Spontaneous in New Zealand Black
	TCL 1	Serial transfer transgenic
	BCR	NSG TM with orthotopic splenic engraftment
preB cell acute lymphoblastic leukemia (pre-B ALL)	ETV6–RUNX1	Conditional transgenic under the Sca-1 promoter
	E2A–PBX1	Conditional transgenic using Lck enhancer, TCR V β promoter
T cell acute lymphoblastic leukemia (T-ALL)	RDM14	Inducible transgenic
	K-ras	CRE-Lox inducible activation
	PTEN	CRE-Lox inducible knockout
	SCL/LMO1	Transgenic
	NOTCH1	Transgenic
	NOTCH3	Transgenic
	MYC	Conditional transgenic using Ig heavy chain
	MYC + RAS	Conditional transgenic with retrovirus transduction
Follicular lymphoma (FL)	MYC/BCR	Conditional transgenic
	BCL-2	Transgenic linked to Vav regulatory sequence
Peripheral T-cell lymphoma (PTCL)	ITK-SYK fusion	Inducible transgenic using CD4-Cre
Anaplastic large cell lymphoma (ALCL)	NPM-ALK	Inducible transgenic using CD4-Cre
Cutaneous T-cell lymphoma (CTCL)	IL-15	Transgenic

genes systems vectors that conditionally express genes in response to doxycycline (Tet on/off systems) or CRE recombinase (CRE/Lox systems) have been generated.¹¹

Tet on/off system is composed of two parts: the Tet operon promoter (TetO) that regulates the expression of the gene of interest, and either the transactivator (tTA) or the reverse transactivator (rtTA) transgenes, which are capable of binding to and regulating TetO. In the Tet-Off system, TetO is constitutively bound by tTA, which stimulates the expression of the gene of interest: when a tetracycline analogue is introduced, it binds the tTA and prevents its interaction with TetO, thus shutting off expression of the gene. In the Tet-On system, rtTA is unable to bind TetO by itself, leaving the gene switched off. In the presence of tetracycline, rtTA binds to TetO and turns on the gene. Therefore, the temporal control of gene expression is achieved by choosing when to feed the mouse tetracycline, and tissue specificity is governed by the promoter driving tTA or rtTA expression. The regulated induction of oncogene expression through doxycycline administration to mice that have undergone thymic maturation also mimics the somatic acquisition of genetic alterations that could otherwise be recognized by the immune system as foreign antigens. The Tet systems are reversible by withdrawal of tetracycline.

Cre-lox system consists of genetic elements flanked by loxP sites that are recognized by Cre recombinase.¹² When cells that have loxP sites in their genome express Cre, a recombination event occurs between the loxP sites. The double-stranded DNA is cut at both loxP sites by the Cre protein. The strands are then rejoined with DNA ligase. The result of recombination depends on the orientation of the loxP sites. For two lox sites on the same chromosome arm, inverted loxP sites will cause an inversion of the intervening DNA, while a direct repeat of loxP sites will cause a deletion event. If loxP sites are on different chromosomes it is

possible for translocation events to be catalyzed by Cre induced recombination. Cre-Lox system allows the DNA modification to be space-specific—targeted to a specific tissue or cell type or be time-specific triggered by a specific external stimulus. Unlike the Tet systems that are reversible by withdrawal tetracycline, the Cre-lox driven recombination is irreversible after the discontinuation of Tamoxifen.

Using the described above systems of gene manipulations several mouse models of leukemias and lymphomas have been developed (Table 1). Those models considerably contribute to better understanding of function of genes involved in hematological malignancies.

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