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Mouse Models of Erythropoiesis and Associated Diseases

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Abstract

Animal models of erythropoiesis have been, and will continue to be, important tools for understanding molecular mechanisms underlying the development of this cell lineage and the pathophysiology associated with various human erythropoietic diseases. In this regard, the mouse is probably the most valuable animal model available to investigators. The physiology and short gestational period of mice make them ideal for studying developmental processes and modeling human diseases. These attributes, coupled with cutting-edge genetic tools such as transgenesis, gene knockouts, conditional gene knockouts, and genome editing, provide a significant resource to the research community to test a plethora of hypotheses. This review summarizes the mouse models available for studying a wide variety of erythroid-related questions, as well as the properties inherent in each one.

Keywords

Mouse models; Transgenic mice; Knockout mice; Conditional knockout mice; Cre-*loxP*; Erythropoiesis; Globin gene switching; Hemoglobin

1 Introduction

Mouse models provide valuable resources to understand the molecular mechanisms underlying normal cellular processes of development, repair, and regeneration, as well as associated genetic perturbations resulting in disease. In addition, mouse models serve as experimental vehicles in which to test pharmaceutical and gene therapies. The common house mouse, *Mus musculus*, has been utilized in this capacity for over a century, beginning with inbred strains of mice as human disease models, leading to the mouse genome project, transgenics and genome editing capabilities of present day. Mice have a physiology similar to humans and their long history of use offers many advantages over other model systems. Early studies produced several thousand spontaneous and radiation-induced mutant strains, which are still available from a number of commercial vendors. These are useful because

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mutations in mouse genes homologous to their human counterparts often result in disease etiologies similar to humans. Later, inbred and congenic strains were created; these provide a uniform genetic background on which to perform studies free from the effect of modifier genes and polymorphisms. More recently, the advent of transgenic and gene targeting methodologies makes it possible to probe the biochemical and molecular pathways of human gene expression, offering the possibility that understanding these processes might lead to points of intervention in disease etiology. Variants of these two procedures made mice the animal of choice for modeling erythropoiesis and red blood cell diseases. Transgenesis allows introduction of human gene sequences or entire loci into the mouse genome or "knock-in" of human sequences to replace their mouse counterparts, while gene targeting to create gene "knockouts" or conditional knockouts, permits genetic studies of the endogenous globin loci, which often are arranged and regulated to a large degree like the human loci. Finally, the 21-day parturition period in mice compacts developmental studies into a useful time frame.

2 Globin Gene Switching and Erythropoiesis

Much of the technology utilized in modern mouse models was developed and/or applied to studies of the hematopoietic system, largely to work on globin gene switching and erythropoiesis. Novel transgenesis technology created opportunities for studying human gene expression in vivo within an animal model. Previous work was limited to analysis of transgenes in established cell lines. Murine transgenesis was particularly attractive, since endogenous globin synthesis had been well characterized during embryogenesis [1]. Developmental studies on hematopoiesis and globin gene switching could be carried out on staged embryos and fetuses during pregnancy beginning with the onset of erythropoiesis at day seven post-conception through post-partum in adult mice. All of the hematopoietic tissues present at a particular stage of development could be assessed, including yolk sac, liver, bone marrow, spleen, and peripheral blood.

3 Early Transgenic Models

Transgenic mice were first produced in the early 1980s by a number of investigators using simple gene or cDNA sequences linked to a promoter of choice. Most of the transgenes integrated in these mice suffered from position effect variegation (PEV) and as the technique developed, various *cis*-acting regulatory elements were included in transgene constructs in an attempt to overcome PEV, including enhancers, introns, insulators, and poly-adenylation signals. Only after locus control regions (LCRs) were discovered and incorporated into transgenes was PEV ameliorated [2].

Initial experiments involved microinjection of simple trans-genes encompassing the human adult β-globin gene or the fetal γ-globin gene (Fig. 1, left side) [3, 4]. Expression was affected by position-of-integration of the transgenes within the murine genome and copy number-dependence was not observed, hallmarks of PEV. In spite of variable levels of expression associated with PEV, when the transgenes were expressed, correct regulation regarding developmental stage- and tissue-specificity was observed, demonstrating that *cis*-regulatory elements controlling spatial and temporal expression were gene-proximal.

Discovery of the β-globin locus LCR in 1987 provided a major breakthrough in globin gene switching [2]. The LCR consists of a collection of DNA regions upstream of the β-like globin gene cluster that are hypersensitive to DNaseI. The LCR opens up the globin domain and makes it available for transcription and is required for high-level expression of all the genes within the cluster. When this element was linked to either a globin gene or a heterologous gene, PEV disappeared; that is, site-of-integration-independent, copy number-dependent expression of the linked transgene was obtained [2]. Generally, all of these transgenes used fragments that encompassed one or two human β-like globin genes coupled to derivative LCR sequences, such as mini- or micro-LCR cassettes (μLCR), individual, or multiple DNaseI-hypersensitive sites (HSs). As transgenic technology advanced, and more DNA could be successfully microinjected into oocytes in an intact state, multiple β-like genes could be linked to μLCRs or the intact LCR could be linked to single-globin genes in cosmid constructs [5].

Analysis of α-globin transgenics identified the HS-40 upstream master regulatory region, and *cis*-regulatory elements controlling ζ-globin and α-globin gene regulation [6, 7]. Particularly notable were studies of the 3′untranslated region (UTR) and its role in α-globin mRNA stability (reviewed in [8]).

4 YAC and BAC Transgenesis

The 1990s saw the advent of whole human loci transgenesis using first yeast artificial chromosomes (YACs), then bacteriophage P1 artificial chromosomes (PACs), and later bacterial artificial chromosomes (BACs). YACs and BACs allow the manipulation of very large stretches of DNA, permitting the analysis of entire gene clusters such as the β-globin locus. Transgenesis with these vectors resulted in murine models that more accurately mirrored human gene expression [9–11]. The first human β-globin locus yeast artificial chromosome (β-YAC) transgenic mice were produced in 1993 [12, 13], ushering in the era of whole locus transgene studies (Fig. 1, right side). More recently, the use of β-globin locus bacterial artificial chromosomes (β-BACs), coupled with “recombineering,” has been employed [14]. Both types of transgenes allow the introduction of alterations within the context of the entire human β-locus using homologous recombination in either yeast or bacteria, respectively, without retention of exogenous DNA sequences. Thus, the effect of the mutation may be studied in an intact locus throughout development within the mouse. Initial studies demonstrated that the human globin gene expression pattern was, by and large, recapitulated during ontogeny in the mouse. Modified transgenes were utilized to produce hereditary persistence of fetal hemoglobin (HPFH) and sickle cell mouse models (reviewed in [15, 16]).

Many of these transgenics were mated with murine knockout mutant lines to produce the mouse models of sickle cell disease and thalassemia now utilized to study the pathophysiology of these diseases.

5 Knockout Models

Coincidental and in parallel to transgenic studies, were projects analyzing the regulation of the endogenous murine β-like and α-like globin genes. The strategy in this case utilized gene targeting and chimeric mice to produce knockout mutations of genes or globin regulatory sequences (Fig. 2). In general, the deficiencies produced revealed similar mechanisms of action regarding globin gene switching in humans. One important caveat is that mice do not have a fetal globin analogous to the human γ-globin genes. A single switch from embryonic globin synthesis to definitive globin synthesis occurs, whereas two switches exist in humans, from primitive embryonic erythropoiesis to fetal definitive erythropoiesis and later from fetal definitive erythropoiesis to adult definitive erythropoiesis. However, the sites of hematopoiesis during development are conserved between mice and humans. Although γ-globin gene expression is confined to the fetal liver in humans, it is expressed mainly in the embryonic yolk sac in the mouse. Expression in the yolk sac can be attributed to the evolutionary homology of the human γ-globin and murine βh1-globin genes, both of which derive from an ancestral γ-globin gene. Mouse βh1-globin gene expression is limited to the embryonic yolk sac; the human γ-globin gene apparently “remembers” its evolutionary origin when in mice and is likewise expressed. However, unlike the murine βh1-globin gene, expression of the human γ-globin genes in the mouse continues during fetal liver definitive erythropoiesis and in a small population of adult definitive red cells [13]. Thus, studies on γ-globin gene expression can only be carried out in mice using the human transgene approach. Knockouts of adult α-globin and β-globin provided the mouse lines that were bred with human transgenic lines to produce mouse models of hemoglobinopathies, in which only human globin chains are incorporated into the hemoglobin molecule.

6 Sickle Cell, Thalassemia, and Cooley’s Anemia Models

A number of mouse models for various hemoglobinopathies have been generated (Table 1). Using slightly different approaches for transgenesis and gene knockouts and knock-ins, alone or in combination, lines modeling sickle cell disease, β- or α-thalassemias, and Cooley’s anemia have been produced [17]. In many instances, the human phenotype for each of these diseases is remarkably conserved in the mouse (reviewed in [17, 18]). These mice offer the possibility for understanding the pathophysiology of these diseases in more detail, may serve as vehicles to test emerging pharmaceutical compounds and gene therapy approaches, will be useful for the isolation and proliferation of hematopoietic stem cells (HSCs), embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs), and for testing the efficacy of bone marrow transplantation and engraftment of gene-corrected stem cells.

Early models of sickle cell disease models (Table 1) include S Antilles and a modified version of this model [19, 20], S+S Antilles [21–23], and SAD [24, 25]. Although these models provided important information about sickle cell disease, they only mimicked the sickle cell trait exhibited in human β^S heterozygous carriers, and did not present the severe hemolytic anemia that is a hallmark of homozygous sickle cell patients. The phenotypes

associated with these mice were due to the inhibition of sickling by endogenous murine hemoglobin.

Newer SCD model mice (Table 1) express exclusively human globins and overcome the failure of earlier models to exhibit faithful sickle cell pathology. They include the Berkeley model, the Birmingham model, and the San Francisco model [33–36]. None of these models express murine α- and β-globin genes. Berkeley model mice express exclusively human α-, β^S-, and γ-globin genes [26]. The $^G\gamma$-and $^A\gamma$-globin genes were included because γ-globin has anti-sickling properties that decreased the likelihood of fetal death during gestation as the switch from primitive embryonic erythropoiesis to fetal definitive erythropoiesis occurred. This model also showed some characteristics of β-thalassemia, including an increased susceptibility to oxidative damage due to free-radical production. The Birmingham mouse was the first model produced that displayed the sickle trait [27]. Subsequent improvements led to the creation of an improved SCD mouse model containing human $^A\gamma$-, β^S-, and α_1-globin genes driven by the human β-globin LCR [28]. Although coinjection of human globin constructs in the Berkeley and Birmingham models led to the integration of all the genes at a single chromosomal site, normal gene order and spatial organization required for appropriate expression of the members of the β-like globin gene family was disrupted. The San Francisco model partially remedied this by creating novel sickle cell mice harboring a β^S-globin YAC containing the entire human β-globin gene cluster and the human α_2-globin gene linked to a mini-LCR [29].

One other group of SCD mouse models are the enhanced γ-globin expression models. Increasing the level of human γ-globin in transgenic mice to various levels in the SAD mouse improved life span, pathology, and hematological profile [30], thereby establishing the physiological range of HbF that would alleviate the SCD condition in mice. Another set of knockout mice expressing exclusively human globins and three levels of HbF (low, medium, and high) also showed a more faithful SCD pathology than the previous models [31]. In this model, murine knockout mice [26, 32] were mated with mice that expressed the co-integrated human mini-LCRα_2 and mini-LCRβ^S used to create the S+S-Antilles model. Lethality of these mice was rescued by breeding with transgenic mice expressing three different human γ-globin constructs (γL, γM, γH) with increasing postnatal HbF levels. These mice exhibited more balanced α- and β-globin chain synthesis and thus present a closer approximation of human SCD.

Thalassemia model mice were generated with deletions of adult murine β-like globin genes, β^{major} and β^{minor} [32, 33] and both adult α-globin genes [34]. The disease phenotypes of these mice were rescued by the introduction of human globin transgenes. Mouse models with deletions in β^{major}- and α-globin chains show varying severity of disease [33–35]. The βIVS-2–654 C→T mutation, which causes aberrant RNA splicing and leads to β^0 thalassemia, has also been modeled in mice [36]. Two models of β thalassemia major or Cooley's anemia were produced. Both are totally humanized; that is, they carry the human α2/α1 genes and a human $\gamma\beta^0$ gene cassette [37] or $\gamma^{HPFH}\delta\beta^0$ gene cassette [38]. In the homozygous state, both the mouse strains expire shortly after birth due to severe anemia following the completion of the γ-globin to β^0-globin switch. However, the later mouse model can be rescued from lethal anemia by regular blood transfusions.

7 Conditional Knockout Models

Manipulation of endogenous murine genes by gene targeting has been performed primarily in embryonic stem (ES) cells. Gene targeting uses homologous recombination to generate null alleles or gene “knockouts” or to substitute an altered transgene for the native chromosomal allele (“knock-ins”). The technique is a powerful tool for studying gene function in hematopoiesis. However, this classical method for producing null mutations results in the loss of gene expression in all tissues and at all developmental stages. The mutation may be lethal, or affect other tissues and developmental pathways not under analysis, consequently complicating interpretation of data. Thus, it is important that the inactivation of a particular gene occurs in a conditional manner, such as in a predefined tissue or at a certain stage of development. The gene-targeting approach to study gene function in mice has been refined with the advent of the bacteriophage P1 Cre-*loxP* site-specific recombination system. Using this technology, tissue- or developmental stage-specific gene knockouts may be produced. Genes are expressed normally in untargeted tissues and the effect of the null mutation is confined to the tissue of interest.

Cre is a 38 kDa protein that catalyzes reciprocal site-specific recombination between 34 bp *loxP* sequences on DNA (Fig. 3). If two *loxP*Cre/loxP sites are in the same orientation in a genomic locus, expression of Cre results in the deletion of *loxP*-flanked DNA sequence in both the prokaryotic and eukaryotic cells. Generally, the two *loxP* sites are introduced into the genome such that they flank an essential portion of the target gene without affecting its function. Following Cre recombinase expression the gene is conditionally inactivated, depending upon the cell type-specific or inducible promoter that defines the spatial and temporal synthesis of Cre protein.

A number of Cre drivers have been utilized to produce conditional knockouts in the hematopoietic/erythropoietic lineages. These are summarized in Table 2. The range of lineage specificity is wide; several are not strictly specific to erythroid, or more broadly, hematopoietic lineages. However even those with broad tissue/developmental range have been useful in the production of conditional knockouts, or activation of mutant versions of genes affecting erythropoiesis. Properties of these Cre transgenes, including name, tissue-specificity, developmental stage-specificity, and compound inducibility, are summarized in Table 2. The efficiency of recombination between *loxP* sites resulting in excision and production of null mutants varies between the available Cre drivers and in some instances efficiency is influenced by the *floxed* target. Many of the Cre driver mice are available commercially from vendors such as Jackson Laboratories. Wherever possible the reference describing the original synthesis of the Cre construct and generation of transgenic mice is included, so that an investigator desiring to obtain these mice will be able to contact the laboratory of the principal investigator where they were created.

Mice bearing *floxed* target genes important to erythropoiesis or some aspect of red blood cell biology have been culled from the literature and the list is summarized in Table 3. The name of each gene is listed, along with basic properties of the encoded protein, the major red cell property or process in which the gene product functions (and consequently that the null mutation affects), diseases modeled, and the earliest reference describing creation of the

mice bearing the erythroid-specific null mutation (or activation of a mutated version of the gene in the erythroid compartment). Processes modeled by these Cre-*lox* mice include globin gene switching, hematopoietic stem cell function, erythropoiesis, and iron metabolism. Diseases modeled include paroxysmal nocturnal hemoglobinuria (PNH), polycythemia-like disease, erythrocytosis, chronic normocytic, normochromic anemia (preclinical model for the anemia of Epo deficiency), and human myelodysplastic syndromes (defects in cell cycle regulation and mitochondrial function).

A broad-coverage internet resource that may be used in conjunction with the mouse sources listed in this chapter is the International Mouse Strain Resource (IMSR, www.findmice.org), which offers users a catalog of worldwide mouse resources (live, cryopre-served, embryonic stem cells). A search engine is available on the home page that will perform a user-defined search of all or select resources. In addition, links are provided for direct access to the web sites of repositories, which describe holding resources of interest, descriptions of the holdings, and order forms. A link to the Mouse Genome Database (MGD, www.informatics.jax.org) is also provided for detailed information about the gene(s) involved, as well as the relationship of the mouse model(s) to human disease(s).

8 Reporter Mice

Mice with robust gene expression readouts in response to activation have utility in the screening or testing of potential compounds with therapeutic ramification in an in vivo setting. Reactivation of silenced γ-globin gene expression is of substantial clinical interest. A dual luciferase β-YAC transgenic mouse in which the $^{A}\gamma$-globin promoter was fused to firefly luciferase and the β-globin promoter was fused to Renilla luciferase was utilized to derive immortalized bone marrow cells that were subsequently used in a high-throughput screen (HTS) for γ-globin inducers [39]. Two other mouse models that serve a similar purpose is the so-called GG mouse, which carries a 183 kb human β-globin locus with eGFP fused to the $^{G}\gamma$-globin promoter [40] and a human β-globin locus $^{A}\gamma$-GFP (or GPA/GFP) β-DsRed PAC transgenic mouse [41].

9 Future Directions

Transgenesis via classic microinjection of oocytes or knock-in replacement of murine genes with their human counterparts in mouse ES cells remains cutting-edge and are important techniques for producing humanized mouse models. However, with the advent of genome editing via CRISPR/Cas9, TALENS, and zinc-finger nucleases, existing humanized mouse lines can be modified without the arduous process of generating an entire new mouse line. iPSCs or ESCs can be derived from existing mice, the cells subjected to genome editing, and new mice created from the engineered cells. The human transgenes themselves could be modified or endogenous mouse genes that play a role in transgene expression or gene product function could be modified. All of this would dramatically reduce the time required for mouse production, in many instances eliminating transgene introduction, gene targeting in ES cells, and many generations of breeding to produce mice with a desired combination of human transgenes and endogenous gene knockouts or mutants. Part of the power of this approach would be the ability to introduce subtle alterations affecting phenotype via a

variety of hypomorphic mutations or changes that affect protein function including those affecting posttranslational modification, allostery, protein-protein interaction, and protein folding.

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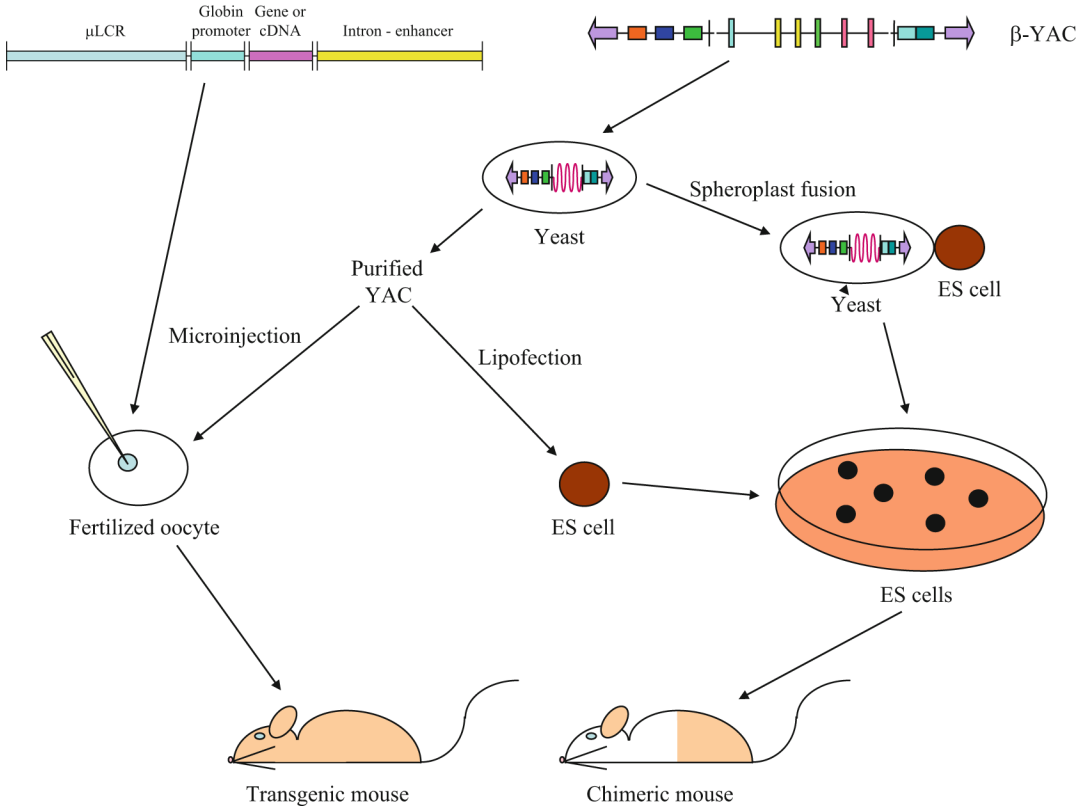

Fig. 1.

Transgenesis with small recombinant or whole loci globin constructs. A prototypical small recombinant globin transgene is illustrated at the *upper left*. Most early transgenes consisted of LCR sequences (mini- or micro-LCRs, one or more 5′HSs, or the entire LCR), a globin promoter linked to its gene sequences, hybrid globin gene sequences, or a cDNA reporter (occasionally a non-globin promoter was linked to a globin gene or cDNA cassette), and a globin or heterologous intron and/or enhancer (or not). These purified transgenes were microinjected into the pronucleus of fertilized mouse oocytes to produce transgenic mice as shown schematically. Cosmid and bacterial artificial chromosomes (BACs) containing larger β-like globin locus inserts were similarly microinjected (not shown). Methods used to generate transgenic mice with yeast artificial chromosomes (YACs) are illustrated beginning with the human β-globin locus YAC (β-YAC) shown at the *upper right*. The β-YAC (155 or 215 kb) and other YACs ranging up to 650 kb in size may be purified from the yeast host and microinjected similar to small recombination DNA constructs or lipofected into embryonic stem (ES) cells, which are then utilized to produce chimeric mice and establish transgenic lines. Alternately, the YAC may be transferred to ES cells by yeast spheroplast-ES cell protoplast fusion. Lipofection or fusion must be employed when the YAC exceeds 650–800 kb in size

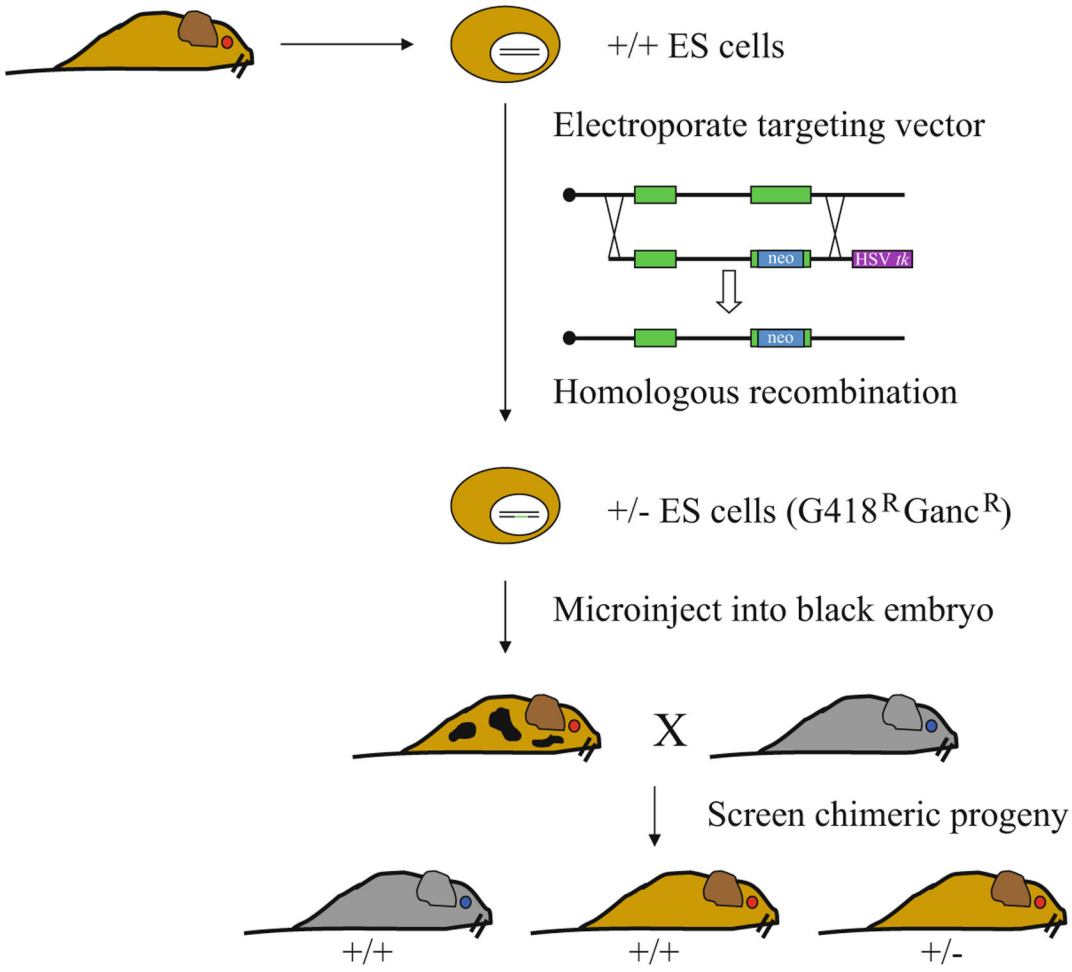

Fig. 2.

Generation of targeted mutations in endogenous murine globin loci. ES cells usually derived from 129 mice (*brown coat color*) are electroporated with a gene-targeting construct, a generalized version of which is shown. Following drug selection and screening for proper integration of the targeting vector, the ES cells are microinjected into blastocysts normally derived from C57Bl6 mice (*black coat color*). Chimeric mice are identified initially based on coat color, which is a varying mixture of brown and black. If the ES cells contributed to the germ line, the targeted mutation will be inherited by the next generation of mice as indicated at the *bottom* of the figure. Following homologous recombination in the target ES cells, the neo cassette confers resistance to the drug G418; loss of the HSV *tk* cassette following homologous recombination results in ganciclovir (GANC) resistance

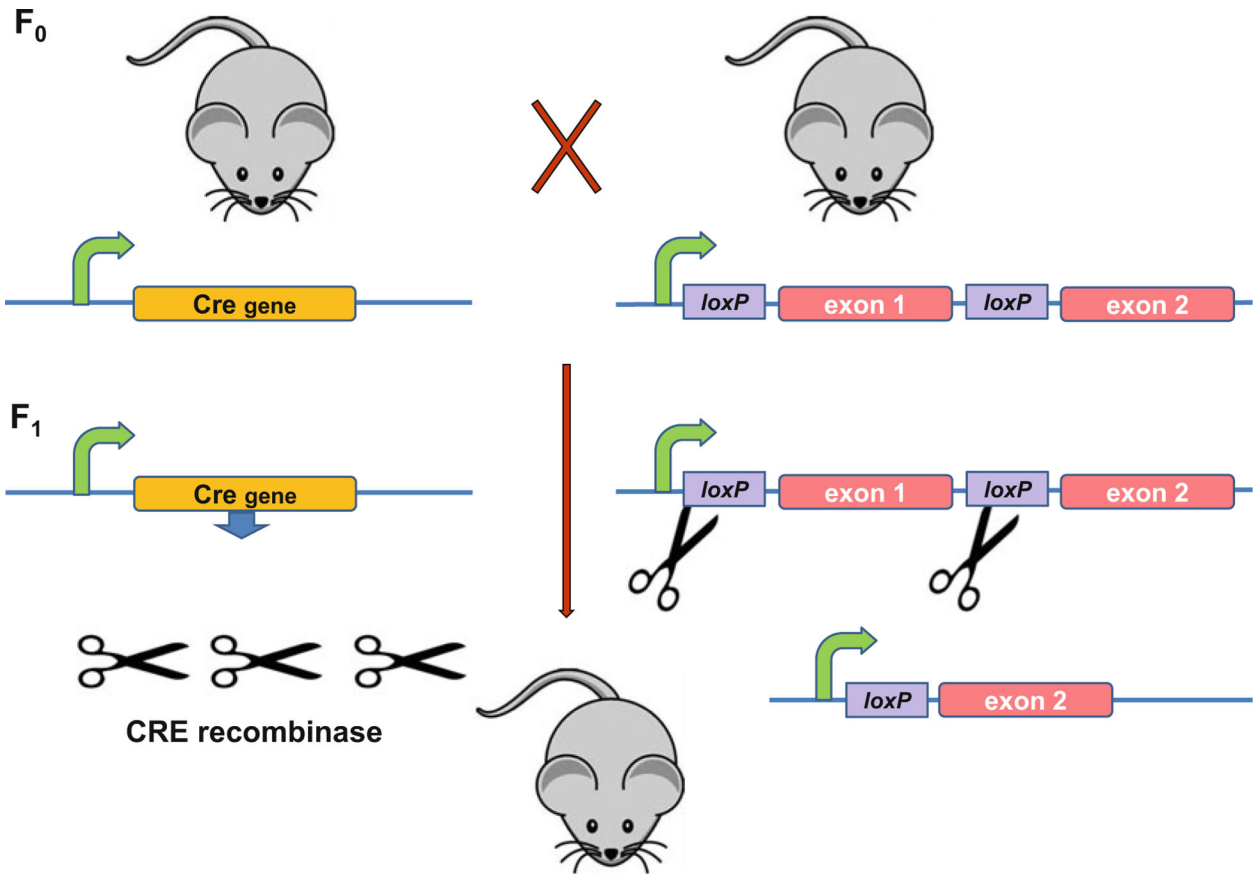

Fig. 3.

Conditional gene knockouts in mice using the Cre recombinase-*loxP* recombination system. A conditional knockout allele is produced by gene targeting as shown in Fig. 2. In this instance, the targeting construct contains two 34 bp *loxP* sites inserted in intergenic DNA sequence, usually in introns flanking an exon, multiple exons, or an exon plus the promoter of the targeted gene (*right side of figure*). The *loxP* sites do not interfere with gene expression. The resultant mice are bred with Cre expression mice (*left side of figure*), in which Cre recombinase gene expression is driven by a tissue-specific, developmental stage-specific, or inducible promoter, or a combination of these. Cre recombinase catalyzes excision of the *loxP*-flanked (*floxed*) DNA sequences, resulting in a targeted deletion and leaving behind a single *loxP* site. Intergenic sequences are shown in blue; promoters are indicated as *curved green lines*

Table 1

Murine models of sickle cell disease, thalassemias and Cooley's anemia

	Genotype	Phenotype	Severity of disease
SCD mouse model			
S Antilles	$\alpha^{H}\ \beta^{S\ Antilles}$	Mild adult anemia Low irreversible sickling	Mild
S+S Antilles	$\alpha^{H}\ \beta^{S}\ \beta^{S\ Antilles}$	Neonatal anemia Significant sickling	Mild—moderate
SAD	$\alpha^{H}\ \beta^{SAD}$	Neonatal anemia Low irreversible sickling Multi-organ pathology	Moderate
Berkeley model (murine knockout)	$\alpha^{H}\ \beta^{S}\ \gamma^{H}\ \delta^{H}$	Significant adult anemia High irreversible sickling Chronic multi-organ damage	Moderate—severe
Birmingham model (murine knockout)	$\alpha^{H}\ \beta^{S}\ \gamma^{H}$	Severe anemia High irreversible sickling Multi-organ pathology	Moderate—severe
San Francisco model (murine knockout)	$\alpha^{H}\ \beta^{S}\ \gamma^{H}\ \delta^{H}$ (YAC)	Anemia Irreversible sickling	Moderate—severe
Enhanced γ-globin expression models	$\alpha^{H}\ \beta^{S}\ (\gamma^{Ha}/\gamma^{Hb}/\gamma^{Hc})$ and $\alpha^{H}\ \beta^{SAD}\ \gamma^{H}$	Decreased severity of disease with increasing HbF levels	Moderate—severe
Thalassemia mouse model			
Carolina β^{0} (murine knockout)	Δb1/b2	Heterozygous mice dramatically decreased hematocrit, hemoglobin, red blood cell counts, MCV, MCH, and MCHC, dramatically increased reticulocyte counts, serum bilirubin concentrations, and red cell distribution widths, display tissue and organ damage, spontaneous iron overload in spleen, liver, kidneys Homozygous mice: die in utero	Severe
Birmingham β^{0} (murine knockout)	$\Delta\beta^{maj}/\beta^{min}$	Heterozygous mice: severely anemic, dramatically reduced hemoglobin levels, abnormal red cell morphology, splenomegaly, markedly increased reticulocyte counts Homozygous mice: die in utero	Severe
βIVS-2-654 (murine knockout/ human knock-in)	$\Delta\beta^{maj}/\beta^{min}$/HuβIVS-2–654	Heterozygous mice: reduced mouse β globin chains and no human β globin Homozygous mice: do not survive postnatally	Moderate
Cooley's anemia (β thalassemia major) (murine knockout/human knock-in)	$\Delta\beta^{maj}/\beta^{min}$/Hu $\gamma\beta^{0}$ Δα1/α2/Hu Δα2/α1	Heterozygous $\gamma\beta^{0}$ knock-in mice: β thalassemia intermedia Homozygous mice: expire due to severe anemia upon completion of the HbF to adult switch after birth	Severe
Cooley's anemia (β thalassemia major) Preclinical model (murine knockout/human knock-in)	$\Delta\beta^{maj}/\beta^{min}$/Hu $\gamma^{HPFH}\delta\beta^{0}$ Δα1/α2/Hu Δα2/α1	Fully humanized mice survive postnatally by synthesizing predominantly HbF, some HbA2, completion of fetal to adult Hb switch after birth results in severe anemia marked by erythroid hyperplasia, ineffective erythropoiesis, hemolysis, and death	Severe
Berkeley α (murine knockout)	Δα1/α2	Homozygous mice: become hydropic, die late in gestation, model for human hydrops fetalis	Severe

α^{H}, γ^{H}, δ^{H}, human α-, γ- and δ-globin genes; β^{S}, human β-globin gene with HbS mutation; $\beta^{S\ Antilles}$, human β-globin gene with HbS Antilles mutation; β^{SAD}, human β-globin gene with HbS, HbS Antilles and HbD Punjab mutations; $\gamma^{Ha}/\gamma^{Hb}/\gamma^{Hc}$: human γ-globin genes expressing three different levels of HbF. See text for references

Table 2

Cre drivers

Gene promoter controlling expression of Cre-recombinase	Tissues/cell types/stage-specificity	Reference
Erythropoietin receptor (EpoR) promoter (ErGFPcre)	Erythroid progenitors/erythroid-specific lineage. EpoR expression initiates at the erythroid burst-forming unit stage (BFU-E), and reaches maximal expression at the erythroid colony-forming unit (CFU-E) and/or proerythroblast stage, and decreases thereafter.	[42]
Vav promoter (VavCre)	Hematopoietic tissues and vascular endothelium.	[43]
Mx1 promoter (Mx-Cre or Mx1-Cre)	This promoter is silent unless activated via the administration of interferon a, interferon p, or a synthetic poly I:C double-stranded RNA. Expression is not tissue-specific or developmental stage-specific, and expression can be activated at any point during development.	[44]
GATA-1 promoter (GATA1-Cre)	GATA1 is a transcription factor necessary for erythroid-specific gene expression and is essential for both primitive and definitive erythropoiesis. Low level expression is achieved in $CD34^{+}$ hematopoietic cells and high level expression is observed in eosinophils, mast cells, megakaryocytes, and erythroblasts.	[45]
Rosa26 promoter fused to the Cre ORF and the ligand- binding domain of the human estrogen receptor (Rosa 26CreER)	Rosa26 is highly and ubiquitously expressed in most cells and tissues of adult mice. CreER recombinase is inactive unless stimulated by the administration of the synthetic estrogen receptor ligand, 4-hydroxytamoxifen (OHT), allowing for temporal regulation of Cre activity.	[46]
Receptor tyrosine kinase Tek (Tie2) promoter/enhancer (Tie2Cre)	The Tie2 promoter/enhancer is expressed consistently in endothelial cells during embryogenesis and adulthood. The Tie2Cre system can delete *floxed* targets in vascular endothelial cells.	[47]
Mouse mammary tumor virus (MMTV) long terminal repeat (LTR) promoter (MMTV-LTR Cre)	MMTV-LTR promoter drives high levels of expression in virgin and lactating mammary gland, salivary gland, seminal vesicle, skin, erythrocytes, B cells, and T cells.	[48]
μ-LCR cassette-β-globin promoter-Cre construct (HBB-cre)	μ-LCR cassette-β-globin promoter stimulates high levels of Cre recombinase in a erythroid/megakaryocytic cell lineage-specific manner.	[49]
EllaCre	The adenovirus Ella promoter targets Cre recombinase expression to early mouse embryos. Cre expression is mosaic, and occurs in a wide variety of tissues including germ cells.	[50]
iCre	Mammalian codon-optimized Cre recombinase that improves Cre translation/function and reduces the high CpG content of the prokaryotic coding sequences, thus diminishing the chance of epigenetic silencing in mammalian systems. Included here for informational purposes as newer Cre drivers will utilize this variant with or without tamoxifen inducibility.	[51]

Table 3

Cre-*loxP*-mediated conditional knock-outs or knock-ins affecting erythropoiesis

***Floxed* allele**	**Gene product description**	**Process under investigation**	**Phenotype/process affected**	**Reference**
Zbtb7a	Transcription factor: Zinc finger and BTB domain containing 7A	Hematopoietic Stem Cell (HSC) maintenance	Embryonic lethal, anemia	[52]
Myc	Transcription factor: Myc proto-oncogene protein	Primitive Hematopoiesis	Embryonic lethal, blocks HSC differentiation, absence of primitive erythrocytes	[53]
SCL/Tal-1	Transcription factor	Hematopoietic Stem Cells	Embryonic lethal, genesis of HSCs, differentiation into erythroid and megakaryocytic progenitors, anemia, thrombocytopenia	[54]
Asxl1 (also *Asxl1: nlacZ/nGFP* knock-in)	Transcription factor: Additional sex combs like transcriptional regulator 1	Erythropoiesis	Embryonic lethal, myelodysplastic syndrome	[55]
*Meis*1	Transcription factor: Homeobox protein Meis1	Erythropoiesis	Primitive hematopoietic repopulating cells and megakaryocytic/erythroid progenitor expansion, abnormal HSC function	[56]
Klf1	Transcription factor: Krüppel-like factor 1	Hemoglobin Switching	Embryonic lethal, β-like globin gene expression, anemia	[57]
Klf2	Transcription factor: Krüppel-like factor 2	Hemoglobin Switching	Embryonic lethal, β-like globin gene expression, anemia	[57]
Bcl1 1a	Transcription factor: C_2H_2 type zinc-finger	Hemoglobin Switching	γ-globin (HbF) repression	[58]
Mi2/β	Chromodomain helicase DNA binding protein 4	Hemoglobin Switching	γ-globin (HbF) repression	[59]
Atg7	Interacting protein: Ubiquitin - activating enzyme E1-like protein	Erythropoiesis	Mitochondrial autophagy, splenomegaly, death via severe anemia	[60]
Med1	Mediator complex subunit 1: required for SP1 activation as part of CRSP complex	Erythropoiesis	Block in erythroid development	[61]
Taf10	Interacting protein: TATA-box binding protein associated factor 10	Erythropoiesis	Embryonic lethal	[62]
Gfi 1b	Transcription factor: Growth factor independent 1 B transcription repressor	Erythropoiesis	Abnormal HSC function	[63]
Tsc 1/Rptor	Interacting protein: Tuberous sclerosis 1 interacting protein/regulatory associated protein of mTOR complex 1	Erythropoiesis	Role of mTORC1 in erythropoiesis, increased neonatal mortality, macrocytic/microcytic anemia	[64]
Ldb1	Interacting protein: LIM domain binding 1	Erythropoiesis	Embryonic lethal, abnormal erythroid gene expression, defective primitive erythropoiesis, continuously required for definitive erythropoiesis and megakaryopoiesis	[65]
Pit1	Interacting protein: Solute carrier family 20 (phosphate transporter) member 1	Erythropoiesis	Erythroid maturation, anemia, myelodysplastic syndromes	[66]
Sf3b1^{K700E} *knock-in*	Interacting protein: Splicing factor 3b subunit 1	Erythropoiesis	Impaired erythropoiesis and aberrant splicing, myelodysplastic syndromes	[67]

Floxed allele	Gene product description	Process under investigation	Phenotype/process affected	Reference
Miz-1	Transcription factor: Myc-interacting zinc finger protein 1	Erythropoiesis	Embryonic lethal, required for embryonic and stress-induced erythropoiesis, perturbed erythroid differentiation and development, severe anemia	[68]
Brg1	Transcription factor: Brahma-related gene 1	Erythropoiesis, Vascular Development	Embryonic lethal, altered globin expression, anemia, erythropoietic/vascular abnormalities	[69]
RhoA	Hub protein: Ras homolog family member A	Erythropoiesis	Embryonic lethal, cytokinesis in erythroblasts, failed definitive erythropoiesis, anemia	[70]
Spry1	Interacting protein: Sprouty RTK signaling antagonist 1	Erythropoiesis	Perturbed erythroid development, reticulocytosis, heightened splenic erythropoiesis, regulator of erythropoiesis during anemia, transducer of EPOR signals, and candidate suppressor of Jak2 activity	[71]
Vegf	Growth factor: Vascular endothelial growth factor	Erythropoiesis	Erythropoietic lineage development, increased Gata1 levels, increased erythroid differentiation	[72]
Stat5	Transcription factor: Signal transducer and activator of transcription 5A	Erythropoiesis	Reduced transferrin receptor gene expression, microcytic, hypochromic anemia	[73]
cdc42	Rho GTPase Cdc42: Cell division control protein 42 homolog	Erythropoiesis	Balance between myelopoiesis and erythropoiesis, altered gene expression, fatal myeloproliferative disorder, anemia, splenomegaly	[74]
Tfr2	Membrane protein: Transferrin receptor 2	Iron Metabolism	Liver iron overload, inadequate hepcidin levels, iron-deficient anemia	[75]
Mfrn1	Mitochondrial iron transporter: Mitoferrin-1	Iron Metabolism	Embryonic lethal, reduced erythroblast formation, severe anemia	[76]
Piga	Enzyme: Phosphatidylinositol glycan anchor biosynthesis class A	Erythropoiesis	Embryonic lethal, paroxysmal nocturnal hemoglobinuria (PNH)	[45]
Jak2V617F knock-in	Non-receptor tyrosine kinase: Janus kinase 2(JAK2)	Erythropoiesis	Polycythemia-like disease (all major features of human polycythemia vera)	[77]
Phd1, Phd2, *Phd3*	Enzyme: Prolyl hydroxylase domain protein 1, 2, or 3 (3 PHD isoforms)	Erythropoiesis	Erythrocytosis	[78]
Epo	Growth factor: Erythropoietin (EPO)	Erythropoiesis	Embryonic lethal, chronic, normocytic, normochromic anemia, pre-clinical model for anemia of Epo deficiency	[79]
pRb	Tumor suppressor protein: Retinoblastoma protein	Erythropoiesis	Defects in cell cycle regulation and mitochondrial function, anemia, myelodysplastic syndromes	[80]
Trim33	Transcriptional corepressor: Tripartite motif-containing 33, also known as	Erythropoiesis	Embryonic lethal	[81]

Floxed allele	Gene product description	Process under investigation	Phenotype/process affected	Reference
	transcriptional intermediary factor 1 gamma (TIF1-γ)			
Bcl-x	Anti-apoptotic factor: Bcl-2-like protein 1	Erythropoiesis	Required for the survival of erythroid cells at the end of maturation, which includes enucleated reticulocytes in circulation, hemolytic anemia, hyperplasia of immature erythroid cells, splenomegaly	[82]
Nix	Pro-apoptotic factor: BH3-only-like protein	Erythropoiesis	Reticulocytosis, thrombocytosis, splenomegaly, splenic and bone marrow erythroblastosis, reduced apoptosis during erythrocyte maturation	[83]
Tr2/Tr4	Transcription factor: Testicular receptor 2 (TR2), Testicular receptor 4 (TR4)	Hemoglobin Switching	Embryonic lethal, γ-globin gene repression	[84]
Zbp-89 (Zfp148)	Transcription factor: Zinc finger protein 148	Hematopoiesis	Stress erythropoiesis, erythroid lineage development, anemia, thrombocytopenia, myeloid and B lymphoid lineage anomalies, altered HSC gene expression	[85]
Trim28	Transcriptional cofactor: Tripartite motif-containing protein 28, also known as transcriptional intermediary factor 1 beta (TIF1β)	Erythropoiesis	Cell- autonomous development of immature erythroblasts in bone marrow, essential for erythroblasts differentiation, anemia, increased apoptosis, reduced erythroid transcription factor expression, reduced heme biosynthesis	[86]
Ccbel	Lymphangiogenic factor: Collagen and calcium-binding epidermal growth factor domain-containing protein 1	Primitive Erythropoiesis	Secreted lymphangiogenic CCBE1 is essential for fetal, but not postnatal erythropoiesis, loss of CCBE1 signaling impairs erythroblastic island formation and function, not required in hematopoietic cells, severe anemia, increased apoptosis	[87]
Sur	Inhibitor of apoptosis: Survivin	Erythropoiesis	Essential for steady-state hematopoiesis and survival of adult, high level expression critical for proper erythroid differentiation, bone marrow depletion, pancytopenia, erythrocyte abnormalities, hypocellular spleen, hematopoietic cell death	[88]
Runx1	Transcription factor: Runt-related transcription factor 1	Hematopoiesis	Embryonic lethal, not required in adult hematopoietic compartment, abnormal HSCs, defective T- and B-cell maturation, inefficient platelet production, mild myeloproliferative phenotype	[89]
α4	Transmembrane receptor: α4 integrin subunit	Hematopoietic Stem Cells	Progenitor cell influx into the peripheral blood,	[90]

Floxed allele	Gene product description	Process under investigation	Phenotype/process affected	Reference
			delayed erythroid and myeloid regeneration, hematopoietic abnormalities	
Raf-1	Enzyme: RAF proto-oncogene serine/threonine-protein kinase	Erythropoiesis	Embryonic lethal, anemia, accelerated erythroid differentiation	[91]
Jak2	Non-receptor tyrosine kinase: Janus kinase 2	Hematopoiesis	Embryonic lethal, impaired hematopoiesis, splenic atrophy, severely diminished erythropoiesis and thrombopoiesis, modestly affected granulopoiesis and monocytopoiesis	[92]
Abcb7	Mitochondrial ATP-binding cassette sub-family B member 7	Hematopoiesis	X-linked sideroblastic anemia with ataxia (XLSA/A) due to inhibition of heme biosynthesis, damaged mitochondria, pancytopenia, hypocellular bone marrow	[93]
Ufbp1	Inhibitor of apoptosis in ER-stressed cells: Ufm1 binding protein 1	Hematopoiesis	Embryonic lethal, impaired hematopoiesis, defective erythroid development, pancytopenia, suppressed GATA-1 and KLF1 expression	[94]
Ezh2	Enzymatic component of Polycomb Repressive Complex 2: Enhancer of zeste homolog 2	Hematopoietic Stem Cells	Embryonic lethal, essential for fetal liver, but not bone marrow erythropoiesis, defective HSC expansion, compromised lymphopoiesis, reduced H3K27me3 levels suggesting epigenetic status of HSCs is developmentally regulated, anemia	[95]
Rcor1	Transcriptional corepressor: REST (repressor element-1 silencing transcription factor) corepressor 1	Hematopoietic Stem Cells	Embryonic lethal, promotes erythropoiesis by repressing HSC and/or progenitor genes, as well as genes and signaling pathways that lead to myeloid cell fate, block in fetal erythropoiesis at the proerythroblast stage, anemia	[96]
Setd8	Histone Methyltransferase: monomethylates histone H4 lysine 20 (H4K20mel)	Erythropoiesis	Embryonic lethal, defect in primitive erythropoiesis, abnormalities in cell cycle progression, regulates erythroid maturation, represses Gata2 expression, severe anemia	[97]
Smad4	Signal transduction: Mothers against decapentaplegic homolog 4	Erythropoiesis	Not required for adult erythropoiesis, severe anemia due solely to blood loss, iron deficiency	[98]
Ulk1	Enzyme: Serine/threonine-protein kinase	Erythropoiesis	Autophagy machinery that leads to the elimination of organelles in erythroid cells	[99]
Ck2β	Enzyme: serine-threonine kinase composed of two catalytic (α) and two regulatory (β) subunits	Hematopoiesis	Embryonic lethal, regulates definitive hematopoiesis of all hematopoietic cell lineages	[100]
Flvcr1a	Heme exporter membrane protein: Feline leukemia virus subgroup C receptor 1	Erythropoiesis	Embryonic lethal, heme export control, severe macrocytic anemia,	[101]

Floxed allele	Gene product description	Process under investigation	Phenotype/process affected	Reference
			splenomegaly, iron accumulation in duodenum, liver and spleen, reduced BFU-E and CFU-E, defective erythroid differentiation	
Rhau	RNA helicase: RNA helicase associated with AU-rich element	Hematopoiesis	Embryonic lethal, hemolytic anemia, differentiation defect at the proerythroblast stage	[102]
Add3	Adducins: Skeletal protein involved in the assembly of spectrin-actin network	RBC membrane skeleton structure	Animals were viable and presented no obvious erythroid cell defects	[103]
cyclin E$^{T74A\ T393A}$ knock-in	Cell cycle regulator: Complexes with the CDK2 cyclin-dependent kinase subunit	Erythropoiesis	Links ubiquitin-proteasome pathway control of G1-to-S-phase progression to regulation of metabolism and gene expression in terminally differentiating bone marrow erythroid cells, abnormal erythropoiesis characterized by a large expansion of abnormally proliferating progenitors, impaired differentiation, dysplasia, and anemia, models early stage human refractory anemia/ myelodysplastic syndrome	[104]
Rps14	40S ribosomal subunit protein S14	Erythropoiesis	Erythroid differentiation defect dependent on p53 characterized by apoptosis during transition from polychromatic to orthochromatic erythroblasts, age-dependent progressive anemia, megakaryocyte dysplasia, block in erythroid differentiation, loss of HSC quiescence	[105]
Rps6	40s Ribosomal subunit protein S6	Erythropoiesis	Hypoproliferative macrocytic anemia, granulocytopenia, thrombocytosis, lymphopenia (model for Diamond-Blackfan Anemia and myelodysplastic syndrome)	[106]
Ppp2ca	Enzyme: Serine/threonine phosphatase, protein phosphatase 2A	Erythropoiesis	Perturbed definitive erythropoiesis characterized by fetal liver atrophy, reduced Terll9^{+} cell number, atypical expression patterns of molecular markers, reduced colony formation, reduction in definitive globin gene expression	[107]
Dicer	Enzyme: Endoribonuclease	Hematopoietic Stem Cells	Down-regulation of erythroid genes, defective erythroid linage differentiation	[108]
*Mll*1	Enzyme: Mixed-lineage leukemia	Hematopoietic Stem Cells	Embryonic lethal, reduced numbers of HSCs, abnormal HSC function	[109]
Ufm1	Ubiquitin-like modification system: Ubiquitin-fold modifier 1	Hematopoietic Stem Cells	Embryonic lethal, diminished hematopoietic	[110]

***Floxed* allele**	**Gene product description**	**Process under investigation**	**Phenotype/process affected**	**Reference**
			development, increased HSC death, severe anemia, cytopenia, elevated endoplasmic reticulum stress, blocked autophagic degradation, increased ROS	
Rac1, Rac2	Rac GTPases: Racl and Rac2 regulates many aspects of intracellular actin dynamics	Erythropoiesis	Abnormal BFU-E and CFU-E morphology, decreased megakaryocyte-erythrocyte progenitors in bone marrow, increased splenic erythropoiesis	[111]
Adar1	RNA-editing enzyme: Adenosine deaminase acting on RNA 1	Hematopoietic Stem Cells	Embryonic lethal, abnormal HSC function, increased HSC apoptosis	[112]
Sod2	Enzyme: Superoxide dismutase 2	Hematopoietic Stem Cells	Increased ROS in erythroid progeny, diminished erythrocytes, decreased ferrochelatase activity, extramedullary hematopoiesis, systemic iron redistribution, abnormal gene expression (hematopoietic transcription factors, globins, and iron-response genes), changes in histone posttranslational modification	[113]