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An overview of chronic myeloid leukemia and its animal models

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Chronic myeloid leukemia (CML) is a form of leukemia characterized by the presence of clonal bone marrow stem cells with the proliferation of mature granulocytes (neutrophils, eosinophils, and basophils) and their precursors. CML is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia (*Ph*) chromosome or t (9;22) translocation (*BCR-ABL*). CML is now usually treated with targeted drugs called tyrosine kinase inhibitors (TKIs). The mechanism and natural history of CML is still unclear. Here, we summarize the present CML animal disease models and compare them with each other. Meanwhile, we propose that it is a very wise choice to establish zebrafish (*Danio rerio*) CML model mimics clinical CML. This model could be used to learn more about the mechanism of CML, and to aid in the development of new drugs to treat CML.

chronic myeloid leukemia (CML), animal disease model, zebrafish

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1 Introduction—Chronic myeloid leukemia

Chronic myeloid leukemia (CML) is a cancer occurring in one to two of 100,000 adults, and comprises nearly 15% of the cases of adult leukemia [1]. CML is distinguished by clonal expansion of primitive pluripotent stem cells without the loss of their capability to differentiate into myeloid cells, and by a dramatic increase in proliferation of granulocytic cells that have the capacity to differentiate [2]. CML is a characteristic leukemia model which can be used to study the multistep processes of leukemogenesis and the essential nature of leukemia, as well as other cancers, in order to discover novel therapies.

During the past five decades, there has been significant progress in CML research. In 1960, an abnormal chromosome was found in nearly all human CML samples, which was designated as the Philadelphia chromosome (*Ph*) [3]. The Ph was recognized as deriving from a deleted chromosome 22 [4]. Shortly after this discovery, it was verified that the *Ph* positive chromosome resulted from a reciprocal translocation between chromosome 9 and 22 [5,6]. In 1984, the t (9;22) translocation was identified, where in an oncogene, *ABL* (Abelson murine leukemia), on chromosome 9 was fused with a formerly unknown gene, *BCR* (breakpoint cluster region), on chromosome 22 [7]. However, whether the acquisition of the characteristic translocation is secondary to, or is indispensable to the genetic lesion remains unknown.

There are three main *BCR-ABL* variants due to alternative splicing or translocation at different breakpoints between the *BCR* and *ABL* exons (Figure 1) [8]. In CML, the break in the *BCR* gene is in a region designated as the major breakpoint cluster region (*m-bcr*). While the break in the

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BCR gene in a distal region designated as the micro-bcr (*μ-bcr*). The μ-bcr is uncommon in CML, while chronic neutrophilic leukemia and/or thrombocytosis may be related to this breakpoint region [2]. However, the break in the *BCR* gene just distal to the first exon of *BCR*, in a region named for the minor breakpoint cluster region (*m-bcr*), is mainly associated with Ph-positive acute lymphoblastic leukemia (ALL). Consequently, BCR-ABL proteins are approximately 190, 210, and 230 kD, and the fusion protein in human CML is approximately 210 kD [2,9,10].

CML progression is generally triphasic, involving a chronic phase (CP), then progressing either to the accelerated phase (AP) and then to the blast phase (BP), or directly to the blast phase [9]. The detailed characteristics of these three phases are as follows (Table 1): The chronic phase; the white blood cell (WBC) count in patients progressively increases. In peripheral blood (PB), blasts account for less than 15% of the WBC count. Clinical symptoms like splenomegaly, weight loss, and B cell symptoms (fever, night sweats, and weight loss) may exist. The course from the chronic phase to the more aggressive phases without treatment is 3.5–5 years. The diagnosis is based on the aforementioned characteristics. The accelerated phase; patients have increasingly worse blood cell counts, which show blasts accounting for 15% to 30% in PB, and organomegaly. In addition, there may be a new chromosomal abnormality.

The blast phase; patients develop symptoms like acute leukemia, including bone pain and B cell symptoms. There are increasing numbers of blast cells, both in PB and bone marrow (BM). This stage is diagnosed by having more than 30% blasts.

Many molecular signaling pathways essential to the pathogenesis of chronic leukemia have been found to be downstream of the BCR-ABL protein [8,10]. The BCR-ABL fusion protein can promote cell proliferation and increase anti-apoptosis, activating key signal molecules/pathways. Since 2001, tyrosine kinase inhibitors have been used as first line treatments for CML. However, leukemia will eventually recur unless CML patients receive lifelong tyrosine kinase inhibitor treatments, and at present, allogeneic stem cell transplant is another unique curative treatment [8,9].

2 Existing CML models

2.1 *BCR/ABL* transgenic mouse model

2.1.1 Conventional BCR/ABL transgenic mice

In conventional transgenic mice, BCR/ABL fusion proteins (including P190, P210, and P230) have been expressed in *BCR/ABL* transgenic mice under the control of a promoter/enhancer element. Different promoters, including

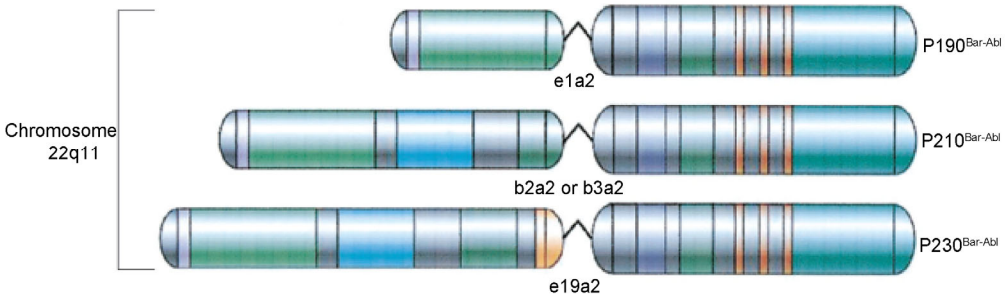

Figure 1 (color online) Schematic diagram of the various aberrant BCR-ABL counterparts [8]. Different junction breakpoints of BCR-ABL are shown. The left half of the oval indicates the N-terminus (amino terminus). Rectangles with different colors indicate different protein domains. The right half of the oval indicates the carboxy-terminal region. Thick lines represent the junction of the two genes. In CML, breaks in *m-bcr* join only the first exon of *BCR* to the entire *ABL* gene from exon 2 to the end of the gene (e1-a2 junction), breaks in *m-bcr* join all of *BCR* up to exons 13 or 14 (also known as exon b2 or b3 of *m-bcr* to *ABL* (again, the entire gene from exon 2 to the end) (b2-a2 or b3-a2 junction), and breaks in *μ-bcr* join all of *BCR* up to exon 19 to *ABL* (exons 2 to 11) (e19-a2 junction).

Table 1 The main characteristics of the three phases of CML[a)]

Phase of CML	Characteristics
Chronic phase (CP)	(i) The blasts account for less than 15% of the white WBC count in PB and BM. (ii) The diagnostic criteria do not reach the accelerated or blast phase.
Accelerated phase (AP)	(i) In peripheral blood, blasts account for from 15% to 30% of the WBC count, and/or progressive splenomegaly is observed. (ii) There may be a new chromosomal abnormality.
Blast phase (BP)	(i) There are increasing numbers of blasts both in PB and BM. This stage is diagnosed by more than 30% blasts. (ii) BM biopsy shows blasts together.

a) WBC: white blood cells; CML: chronic myeloid leukemia; PB: peripheral blood; BM: bone marrow

Eμ [11], *MPSV-LTR* (myeloproliferative sarcoma virus retroviral long terminal repeat) [11], *δMT-1* (metallothionein) [12–15], *tec* [16], *hMRP8* [17], and *Sca-1* [18], that drive *BCR/ABL* expression have been studied in mouse models. The models mentioned above demonstrate the expression of BCR/ABL fusion protein in mice. Nevertheless, there are several shortcomings of these models. First, a subset of mice only develops lymphoid leukemia rather than myeloid malignancies. Second, the disease progression of these models has a longer latency, which restricts the application of these models in CML treatment research. Third, the main problem of conventional transgenic mouse models is that the BCR/ABL fusion protein is produced in the transgenic mice throughout the lifetime, and this may account for embryo gene lethality. An approach to overcome this obstacle is the application of conditional promoters to prevent oncogene expression during embryogenesis and allow induction of gene expression under specified conditions after birth.

2.1.2 Conditional BCR/ABL transgenic mice

The application of conditional transgenic systems has been taken into consideration to develop models of CML. Inhibition of the *BCR/ABL* transgene before birth prevents toxicity that may hinder transgene expression. Currently, the tetracycline (Tet)-inducible expression system is the most used of the inducible systems. There are primarily two kinds of tetracycline-inducible expression systems, the tetracycline-regulated transactivator (tTA) (Tet-off) system [19], and the reverse tetracycline-regulated transactivator (rtTA) (Tet-on) system [20]. However, all the conditional CML models utilize the tTA (Tet-off) system. Several transgenic mouse lines have been developed using the tTA gene under the control of the *MMTV-LTR* (mouse mammary tumor virus long terminal repeat) [21], the human *CD34* genomic locus [22,23], and the murine stem cell leukemia (*SCL*) gene 3′ enhancer [24]. The transgenic mice develop symptoms reminiscent of human B-cell ALL instead of CML, and a myeloproliferative disorder related to the megakaryocytic lineage, using *MMTV-LTR* and *CD34* promoters, respectively, after an induction of the *BCR/ABL* fusion gene expression, and then withdrawal of the tetracycline from the drinking water. In addition, after induction of *BCR/ABL* expression, all SCL tTA/BCR/ABL double transgenic mice developed a phenotype that had many common features with chronic phase CML [24]. Moreover, the phenotype could be induced again after thorough inhibition of *BCR/ABL* expression, indicating that the persistence of oncogene expression is not indispensable for the leukemic stem cell population that is maintained, even without the existence of *BCR/ABL*. Meanwhile, imatinib (a kind oftyrosine kinase inhibitor) failed to induce recovery in the mice.

2.1.3 Embryonic stem cell (ES cells)-based recombination introducing BCR/ABL ectopic expression

Using homologous recombination in ES cells, Castellanos et al. [25] constructed an in-frame fusion gene of *BCR/ABL* P190, mimicking the consequences of the human chromosomal translocation by fusion of *BCR/ABL* encoding sequences into the *BCR* endogenous gene. Of the total chimeric mice, 38 of 40 generated with the mutant ES cells developed B-cell ALL by 4 months after birth. The study demonstrated that the endogenous BCR product is not necessary for *BCR/ABL* oncogene activity in leukemogenesis.

Subsequently, differentiating cultures of ES cells were transfected with the CML-specific BCR-ABL oncoprotein to study leukemic transformation of ES cells derived from embryonic hematopoietic progenitors [26]. The transformed hematopoietic derivatives of ES cells demonstrated the relationship of *BCR/ABL* expression with interleukin-3 (IL-3) production that was reported in primitive hematopoietic progenitors from human CML patients. This study demonstrated the autocrine and paracrine effects of *BCR/ABL*-infected cells in mice.

2.1.4 Advantages and disadvantages of transgenic mouse models

Transgenic mouse models are time-consuming to develop because of founder selection, breeding, and genotyping processes. Compared to human disease, in which the Ph translocation exists in a single stem/progenitor cell type, the expression in mouse models is controlled by using specific promoter/enhancer constructs, and therefore the oncogene exists in all hematopoietic cells in the *BCR/ABL* transgenic mice. This may affect the pathophysiology of the leukemia or its response to drugs. However, highly reproducible expression among transgenic offspring, analysis of leukemic phenotypes under stationary conditions, and versatile mating with various transgenic mouse strains, especially gene knockout strains, makes them appropriate for CML modeling.

2.2 Murine bone marrow (BM) retroviral transduction and transplantation model of CML

2.2.1 Retroviral transduction and transplantation model

The initial murine BM retroviral transduction and transplantation model of CML was reported in 1990 [27]. There were three kinds of diseases including CML-like myeloproliferative syndrome, ALL, and a type of leukemia related to macrophages in the recipient mice at several months after transplantation. Soon afterwards, JW-RX [28], a retrovirus expressing *BCR/ABL*, was used to infect BM cells of donor mice that were pretreated by 5-FU (5-fluoro-2,4(1h, 3h) pyrimidinedione). The cells were applied to reconstitute mice after treatment with lethal irradiation. Approximately half of the mice developed a myeloproliferative syndrome

reminiscent of the CP of CML. Both of the studies suggested that *BCR/ABL* is the main cause of myeloproliferative syndrome in mice. Nevertheless, the recipient mice showed more than one kind of disease and most recipient mice developed CML with different disease latencies.

The development of P210-induced leukemia in primary, secondary, tertiary, and quaternary transplant recipients was recently explored [29]. The late-onset CML-like disease is a useful murine model reminiscent of human CML. Subsequent studies showed progress in the model system development, including improvement of the constructs, the transitory retroviral packaging system, and the modification of the virus infection conditions accounting for the increase in effective retroviral transduction and 100% efficiency of generating the CML-like disease [29–31].

An excellent model where all recipient mice developed CML with a shorter latency helped to evaluate drugs for CML therapy [32]. Either STI571 (a tyrosine kinase inhibitor) or placebo was given to mice reconstituted with P210 *BCR/ABL*-transduced BM cells. The retroviral model system provides a potent tool for exploring the disease mechanisms of CML, drug resistance, and the progression of CML.

2.2.2 Main applications of retroviral transduction and transplantation models

Retroviral transcription /transplantation model systems can be used to study the differences in activities of different functional domains to induce leukemia [33–35]. The models are useful to study the roles of different signal molecules/pathways in the pathogenesis of CML [36–38] or the roles of *BCR/ABL* in transformation [29,32], to detect the potential of acquisition of additional genetic or epigenetic aberrations causing blastic transformation in CML [39], and to test the effects of clinical drugs using BM retroviral transduction/transplantation murine CML models [40]. Moreover, the system also facilitates functional analysis of individual genes and signal pathways in CML. CML retroviral mouse models have been used for functional analyses of individual genes by transduction of transgenic cells for disruption of targeted genes such as signal transducer and activator of transcription 5 (*STAT5*) [36–38], *P53* [41], *Raf1* [42], and *Gads* (*Grb2*-related adaptor downstream of *Shc*) [43].

2.3 Model of CML primary BM cells transplanted into immunodeficient mice (xenograft models of CML)

2.3.1 Xenograft models of CML

Sawyers et al. [44] originally transplanted cells from patients with BP CML into SCID (severe combined immunodeficient) mice and observed reproducible growth of leukemic myoblasts in the hematopoietic tissues of immunodeficient mice [44]. Later, both normal and leukemic hematopoietic cells from patients with chronic phase CML proved to be capable of engraftment into the BM of sublethally irradiated SCID mice [45]. It is possible that the leukemic cells secreted cytokines supporting the engraftment of both leukemic and normal cells. Soon afterwards, Wang et al. [46] reported engraftment of chronic phase (CP) CML cells into nonobese diabetic (NOD)/SCID recipients with better results. The results suggested that the first step to identify the phenotype of CML stem cells was the ability of the leukemic cells to successfully reconstitute in NOD/SCID mice after implantation of concentrated populations of CD34+ cells from CP CML patients [46]. As might be expected, cells from CML patients in AP or BP engrafted faster than cells from patients in chronic phase [45,46]. Importantly, *in vitro* transfected *BCR/ABL* p210+BM cells (via coculture on stable retroviral producer cells), when injected intravenously into the tail vein of mice directly after retroviral infection, generated myeloproliferative CML-like disease [37]. In 2015, Schneckenleithner et al. [47] described the protocols in detail.

2.3.2 Application of xenograft models of CML

CML primary BM cells transplanted into immunodeficient mice can be used to study the transplantation of CML progenitor cells, the biological characteristics of CML, and can help to identify therapeutic treatments for CML.

3 Advantages of the zebrafish (*Danio rerio*) model

3.1 Biological advantages of zebrafish

The mouse models mentioned above have limitations because studies have failed to establish an animal model that can fully recapitulate all the characteristics of CML. Compared to mice, zebrafish have the biological advantages of small size, productive fecundity, external fertilization, optical clarity of zebrafish embryos, easily identifiable phenotype, genes analogous with humans, and applicability to large-scale screening. Furthermore, the special characteristic of leukemia cells, using fluorescent proteins and *in vivo* direct visualization of leukemia processes in the zebrafish, can be ideally studied using this model for leukemia research. Compared with injections or feeding in mice, the zebrafish can absorb small molecular chemical compounds directly from the water, allowing high-throughput screening of drugs. The identification of small molecules through zebrafish screening procedures will aid in the development of novel drugs for the therapy of diseases, especially cancer.

The zebrafish therefore holds great promise for the study of hematopoiesis, hematopoietic system diseases, and leukemogenesis. Many malignant hematological diseases, which recapitulate crucial pathophysiological features of the homologous human disease, have been modeled in zebrafish.

3.2 Feasibility of using zebrafish as a CML model

In general, the development of zebrafish myeloid leukemia models emerged later than the lymphoid leukemia models, partially due to a lack of appropriate myeloid specific promoters. Not until 2006 was the *mpx* (myeloperoxidase) promoter applied for zebrafish transgenic line [48,49]. With the successful results of the mouse myeloid leukemia model, the significance of appropriate spatiotemporal expression of the oncogene was confirmed [50]. Although there are many acute myeloid leukemia (AML) zebrafish models [51,52], a CML model using zebrafish has not been reported.

The *TEL-JAK2* fusion construct generated from the zebrafish ortholog of the human *TEL* and *JAK2*, seen in a case of atypical CML [53] or derived from both T-cell ALL and atypical CML [54], was transiently expressed in developing zebrafish embryos under the control of either the white blood cell-specific *spi1* promoter (also known as the *pu.1* promoter) or the ubiquitously-expressed cytomegalovirus (CMV) promoter preferentially expressed in myeloid precursor cells. Although the study failed to develop a stable transgenic zebrafish line expressing the tel-jak2 fusion protein, mainly due to high lethality in the larval stage and later stages, the disruption of normal embryonic hematopoiesis showed that zebrafish were applicable for study in myeloid malignancy.

3.3 Strategies to establish a zebrafish CML model

3.3.1 Traditional approaches can be used in zebrafish models.

The zebrafish share significant homology with the *BCR* and *ABL* genes of humans. Therefore, it is possible to produce CML transgenic zebrafish lines expressing the P210 BCR-ABL fusion protein under control of a promoter/enhancer element. These promoters mainly include three types, the ubiquitous CMV promoter, the inducible heat shock protein (hsp) promoter, or the specific *coro1a* (coronin-1a) promoter, which is a specific promoter for myeloid cells. Besides the traditional transgenic approaches, the gene editing technique to cause chromosome translocation can also be used.

3.3.2 The gene editing technique can be used in zebrafish models.

Gene editing systems such as the Cre/loxP, zinc finger (ZFN), transcription activator-like effector nucleases (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system have been applied in chromosome translocation. In 2000, Buchholz et al. [55] reported the application of Cre recombinase to duplicate the t (8;21) translocation discovered in human AML in mice. They further demonstrated that the Cre/loxP system was a powerful tool to produce predesigned chromosomal translocations in mice, enabling recapitulation of human genetic cancer events. In 2013, the ZFN and TALEN were used to design chromosomal translocations [56]. The t(11;22)(q24;q12), found in Ewing sarcoma and t(2;5)(p23;q35) translocations, found in anaplastic large cell lymphoma(ALCL), were both induced with high efficiency, by using tailored nucleases ZFNs and TALENs designed to cut accurately at specific translocation breakpoints. The approach suggested that fusion gene expression could be inducted by the native promoter in cancer cells, allowing further study of the mechanisms and progression of cancer-relevant translocations in human cells. Recently, a novel type of site-specific nucleases, the CRISPR/Cas system, has been a revolutionary approach for genome engineering [57]. Using the ascendant RNA-guided CRISPR/Cas system, Torres et al. [58] generated human cell lines and primary cells bearing chromosomal translocations at high frequencies, which resembled those described in AML and Ewing's sarcoma. These gene editing techniques are completely applicable to zebrafish because of the advantages discussed above. Most notably, further development of the CRISPR/Cas system is promising for the creation of models to help identify the characteristic events that drive the myeloid leukemia. In the future, gene editing techniques to induce chromosomal translocations will also help determine the pathogenesis of CML.

4 Application and future perspectives of the zebrafish CML model

The zebrafish CML model is useful for real-time observations of the development and transformation of leukemic cells, and for the analyses of the pathophysiology of signaling pathways that are affected downstream of the fusion gene. The model is also useful to more quickly and directly test the effects of drug treatments, by observing the abatement of the green leukemic cells marked by enhanced green fluorescent protein. The zebrafish CML model also provides a unique forward genetic screening for specific disease-related genes affecting the process of leukemia, such as genes related to tumorigenesis, cell specificity, disease progression speed, and metastasis processes. Moreover, the zebrafish CML model will also provide an ideal tool for chemical/drug library screening, to identify novel, targeted small chemical molecules with alternative, yet desired, *in vivo* pharmacological effects.

This review does not contain any studies with human participants or animals performed by any of the authors.

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1 Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2014 update on diagnosis, monitoring, and management. Am J Hematol, 2014, 89: 547–556
2 Pasternak G, Hochhaus A, Schultheis B, Hehlmann R. Chronic myelogenous leukemia: molecular and cellular aspects. J Cancer Res Clin Oncol, 1998, 124: 643–660
3 Hungerford DA, Nowell PC. A minute chromosome in human granulocytic leukemia. Science,1960, 132: 1497–1497
4 Caspersson T, Gahrton G, Lindsten J, Zech L. Identification of the Philadelphia chromosome as a number 22 by quinacrine mustard fluorescence analysis. Exp Cell Res, 1970, 63: 238–240
5 Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature, 1973, 243: 290–293
6 Rowley JD. Identificaton of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann Genet, 1973, 16: 109–112
7 Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell, 1984, 36: 93–99
8 Kurzrock R, Kantarjian HM, Druker BJ, Talpaz M. Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics. Ann Intern Med, 2003, 138: 819–830
9 Gore JM. Chronic myeloid leukemia and chronic lymphocytic leukemia. JAAPA, 2014, 27: 45–46
10 Inokuchi K. Chronic myelogenous leukemia: from molecular biology to clinical aspects and novel targeted therapies. J Nippon Med Sch, 2006, 73: 178–192
11 Hariharan IK, Harris AW, Crawford M, Abud H, Webb E, Cory S, Adams JM. A bcr-v-abl oncogene induces lymphomas in transgenic mice. Mol Cell Biol, 1989, 9: 2798–2805
12 Heisterkamp N, Jenster G, Ten HJ, Zovich D, Pattengale PK, Groffen J. Acute leukaemia in bcr/abl transgenic mice. Nature, 1990, 344: 251–253
13 Voncken JW, Griffiths S, Greaves MF, Pattengale PK, Heisterkamp N, Groffen J. Restricted oncogenicity of BCR/ABL p190 in transgenic mice. Cancer Res, 1992, 52: 4534–4539
14 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: 4603–4611
15 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: 2853–2861
16 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: 2067–2075
17 Jaiswal S, Traver D, Miyamoto T, Akashi K, Lagasse E, Weissman IL. Expression of BCR/ABL and BCL-2 in myeloid progenitors leads to myeloid leukemias. Proc Natl Acad Sci USA, 2003, 100: 10002–10007
18 Perez-Caro M, Cobaleda C, Gonzalez-Herrero I, Vicente-Duenas C, Bermejo-Rodriguez C, Sanchez-Beato M, Orfao A, Pintado B, Flores T, Sanchez-Martin M, Jimenez R, Piris MA, Sanchez-Garcia I. Cancer induction by restriction of oncogene expression to the stem cell compartment. Embo J, 2009, 28: 8–20
19 Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc Natl Acad Sci USA, 1992, 89: 5547–5551
20 Gossen M, Freundlieb S, Bender G, Muller G, Hillen W, Bujard H. Transcriptional activation by tetracyclines in mammalian cells. Science, 1995, 268: 1766–1769
21 Huettner CS, Zhang P, Van Etten RA, Tenen DG. Reversibility of acute B-cell leukaemia induced by BCR-ABL1. Nat Genet, 2000, 24: 57–60
22 Radomska HS, Gonzalez DA, Okuno Y, Iwasaki H, Nagy A, Akashi K, Tenen DG, Huettner CS. Transgenic targeting with regulatory elements of the human CD34 gene. Blood, 2002, 100: 4410–4419
23 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: 3363–3370
24 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: 324–334
25 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: 2168–2174
26 Peters DG, Klucher KM, Perlingeiro RC, Dessain SK, Koh EY, Daley GQ. Autocrine and paracrine effects of an ES-cell derived, BCR/ABL-transformed hematopoietic cell line that induces leukemia in mice. Oncogene, 2001, 20: 2636–2646
27 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: 824–830
28 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: 6649–6653
29 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: 3780–3792
30 Li S, Ilaria R J, 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: 1399–1412
31 Zhang X, Ren R. 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, 1998, 92: 3829–3840
32 Wolff NC, Ilaria RJ. Establishment of a murine model for therapy-treated chronic myelogenous leukemia using the tyrosine kinase inhibitor STI571. Blood, 2001, 98: 2808–2816
33 He Y, Wertheim JA, Xu L, Miller JP, Karnell FG, Choi JK, Ren R, Pear WS. The coiled-coil domain and Tyr177 of bcr are required to induce a murine chronic myelogenous leukemia-like disease by bcr/abl. Blood, 2002, 99: 2957–2968
34 Million RP, Van Etten RA. The Grb2 binding site is required for the induction of chronic myeloid leukemia-like disease in mice by the Bcr/Abl tyrosine kinase. Blood, 2000, 96: 664–670
35 Zhang X, Subrahmanyam R, Wong R, Gross AW, Ren R. The NH_2-terminal coiled-coil domain and tyrosine 177 play important roles in induction of a myeloproliferative disease in mice by Bcr-Abl. Mol Cell Biol, 2001, 21: 840–853
36 Ye D, Wolff N, Li L, Zhang S, Ilaria RJ. STAT5 signaling is required for the efficient induction and maintenance of CML in mice. Blood, 2006, 107: 4917–4925
37 Hoelbl A, Schuster C, Kovacic B, Zhu B, Wickre M, Hoelzl MA, Fajmann S, Grebien F, Warsch W, Stengl G, Hennighausen L, Poli V, Beug H, Moriggl R, Sexl V. Stat5 is indispensable for the maintenance of bcr/abl-positive leukaemia. Embo Mol Med, 2010, 2: 98–110
38 Walz C, Ahmed W, Lazarides K, Betancur M, Patel N, Hennighausen L, Zaleskas VM, Van Etten RA. Essential role for Stat5a/b in myeloproliferative neoplasms induced by BCR-ABL1 and JAK2(V617F) in mice. Blood, 2012, 119: 3550–3560

39 Zhang SJ, Ma LY, Huang QH, Li G, Gu BW, Gao XD, Shi JY, Wang YY, Gao L, Cai X, Ren RB, Zhu J, Chen Z, Chen SJ. Gain-of-function mutation of GATA-2 in acute myeloid transformation of chronic myeloid leukemia. Proc Natl Acad Sci USA, 2008, 105: 2076–2081

40 El Eit RM, Iskandarani AN, Saliba JL, Jabbour MN, Mahfouz RA, Bitar NM, Ayoubi HR, Zaatari GS, Mahon FX, De The HB, Bazarbachi AA, Nasr RR. Effective targeting of chronic myeloid leukemia initiating activity with the combination of arsenic trioxide and interferon alpha. Int J Cancer, 2014, 134: 988–996

41 Wendel HG, de Stanchina E, Cepero E, Ray S, Emig M, Fridman JS, Veach DR, Bornmann WG, Clarkson B, McCombie WR, Kogan SC, Hochhaus A, Lowe SW. Loss of p53 impedes the antileukemic response to BCR-ABL inhibition. Proc Natl Acad Sci USA, 2006, 103: 7444–7449

42 Albers C, Illert AL, Miething C, Leischner H, Thiede M, Peschel C, Duyster J. An RNAi-based system for loss-of-function analysis identifies Raf1 as a crucial mediator of BCR-ABL-driven leukemogenesis. Blood, 2011, 118: 2200–2210

43 Gillis LC, Berry DM, Minden MD, McGlade CJ, Barber DL. Gads (Grb2-related adaptor downstream of Shc) is required for BCR-ABL-mediated lymphoid leukemia. Leukemia, 2013, 27: 1666–1676

44 Sawyers CL, Gishizky ML, Quan S, Golde DW, Witte ON. Propagation of human blastic myeloid leukemias in the SCID mouse. Blood, 1992, 79: 2089–2098

45 Sirard C, Lapidot T, Vormoor J, Cashman JD, Doedens M, Murdoch B, Jamal N, Messner H, Addey 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: 1539–1548

46 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: 2406–2414

47 Schneckenleithner C, Hoelbl-Kovacic A, Sexl V. Modeling BCR/ABL-driven malignancies in the mouse. Methods Mol Biol, 2015, 1267: 263–282

48 Renshaw SA, Loynes CA, Trushell DM, Elworthy S, Ingham PW, Whyte MK. A transgenic zebrafish model of neutrophilic inflammation. Blood, 2006, 108: 3976–3978

49 Mathias JR, Perrin BJ, Liu TX, Kanki J, Look AT, Huttenlocher A. Resolution of inflammation by retrograde chemotaxis of neutrophils in transgenic zebrafish. J Leukoc Biol, 2006, 80: 1281–1288

50 Rosenbauer F, Koschmieder S, Steidl U, Tenen DG. Effect of transcription-factor concentrations on leukemic stem cells. Blood, 2005, 106: 1519–1524

51 Zhuravleva J, Paggetti J, Martin L, Hammann A, Solary E, Bastie JN, Delva L. MOZ/TIF2-induced acute myeloid leukaemia in transgenic fish. Br J Haematol, 2008, 143: 378–382

52 Shen LJ, Chen FY, Zhang Y, Cao LF, Kuang Y, Zhong M, Wang T, Zhong H. MYCN transgenic zebrafish model with the characterization of acute myeloid leukemia and altered hematopoiesis. PLoS One, 2013, 8: e59070

53 Onnebo SM, Condron MM, McPhee DO, Lieschke GJ, Ward AC. Hematopoietic perturbation in zebrafish expressing a tel-jak2a fusion. Exp Hematol, 2005, 33: 182–188

54 Onnebo SM, Rasighaemi P, Kumar J, Liongue C, Ward AC. Alternative TEL-JAK2 fusions associated with T-cell acute lymphoblastic leukemia and atypical chronic myelogenous leukemia dissected in zebrafish. Haematologica, 2012, 97: 1895–1903

55 Buchholz F, Refaeli Y, Trumpp A, Bishop JM. Inducible chromosomal translocation of AML1 and ETO genes through Cre/loxP-mediated recombination in the mouse. Embo Rep, 2000, 1: 133–139

56 Piganeau M, Ghezraoui H, De Cian A, Guittat L, Tomishima M, Perrouault L, Rene O, Katibah GE, Zhang L, Holmes MC, Doyon Y, Concordet JP, Giovannangeli C, Jasin M, Brunet E. Cancer translocations in human cells induced by zinc finger and TALE nucleases. Genome Res, 2013, 23: 1182–1193

57 Zhang L, Zhou Q. CRISPR/Cas technology: a revolutionary approach for genome engineering. Sci China Life Sci, 2014, 57: 639–640

58 Torres R, Martin MC, Garcia A, Cigudosa JC, Ramirez JC, Rodriguez-Perales S. Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR-Cas9 system. Nat Commun, 2014, 5: 3964

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