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REVIEW

Molecular and cellular bases of chronic myeloid leukemia

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ABSTRACT

Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by the overproduction of granulocytes, which leads to high white blood cell counts and splenomegaly in patients. Based on clinical symptoms and laboratory findings, CML is classified into three clinical phases, often starting with a chronic phase, progressing to an accelerated phase and ultimately ending in a terminal phase called blast crisis. Blast crisis phase of CML is clinically similar to an acute leukemia; in particular, B-cell acute lymphoblastic leukemia (B-ALL) is a severe form of acute leukemia in blast crisis, and there is no effective therapy for it yet. CML is induced by the BCR-ABL oncogene, whose gene product is a BCR-ABL tyrosine kinase. Currently, inhibition of BCR-ABL kinase activity by its kinase inhibitor such as imatinib mesylate (Gleevec) is a major therapeutic strategy for CML. However, the inability of BCR-ABL kinase inhibitors to completely kill leukemia stem cells (LSCs) indicates that these kinase inhibitors are unlikely to cure CML. In addition, drug resistance due to the development of BCR-ABL mutations occurs before and during treatment of CML with kinase inhibitors. A critical issue to resolve this problem is to fully understand the biology of LSCs, and to identify key genes that play significant roles in survival and self-renewal of LSCs. In this review, we will focus on LSCs in CML by summarizing and discussing available experimental results, including the original studies from our own laboratory.

KEYWORDS BCR-ABL, leukemic stem cells, CML, therapeutic agents

PHILADELPHIA CHROMOSOME

In 1960, a major clue to the cause of chronic myeloid leukemia (CML) was provided by Nowell and Hungerford's landmark discovery of the Philadelphia (Ph) chromosome and its association with the development of CML (Nowell and Hungerford, 1962). In this study, they examined leukemia cells from patients with chronic phase CML and other leukemias. Surprisingly, the cells from patients with CML but not other leukemias showed a consistent "minute chromosome" abnormality, which they named the Philadelphia (Ph) chromosome (Nowell and Hungerford, 1962). This discovery was the first demonstration of a chromosomal rearrangement that is consistently linked to a specific cancer, and had sparked searches for associations of additional chromosomal aberrations with specific forms of cancer. Ph chromosome is now known to be present in over 90% of CML cases (Westbrook et al., 1992). In samples from patients with adult B-ALL, the Ph chromosome accounts for 10%–15% of such cases (Westbrook et al., 1992). Chronic phase CML has a consistent, relatively indolent presentation in patients, with an increase in immature and mature myeloid elements, and a retention of hematopoietic differentiation. This unique and consistent phenotype of chronic phase CML enables us today to easily and accurately identify CML patients.

The phenotypes of accelerated and blast crisis CML are much more diverse and aggressive than chronic phase CML, and these two phases are characterized by a severe reduction in cellular differentiation, with a displacement of mature cells by immature blasts. In blast crisis, more than 50% of patients enter a myeloid blast stage resembling acute myeloid leukemia (AML). A pre-B blast stage shown as B-cell acute lymphoblastic leukemia (B-ALL) accounts for 30% of CML patients, and erythroid blasts develop in 10% of patients

(Wong and Witte, 2004). The pleiotropic and aggressive phenotypes of blast crisis suggest that different oncogenic abnormalities could lead to the transition of chronic phase CML to blast phase CML. Numerous oncogenic events have been found to be associated with blast crisis; these include trisomy 8, i(17q) (Mitelman, 1993; Jennings and Mills, 1998), loss of p53 function (Feinstein et al., 1991; Prokocimer and Rotter, 1994), MYC amplification (Jennings and Mills, 1998), RB (RB1) deletion/rearrangement (Ahuja et al., 1991), and p16INK4A (CDKN2) rearrangement/deletion (Sill et al., 1995). Loss of p53 function has been exclusively linked with myeloid but not lymphoid blast crisis, which suggests that p53 signaling is critical for the regulation of normal myelopoiesis (Prokocimer and Rotter, 1994). Over 80% of blast phase CML cases have definable genetic aberrations in addition to the Ph chromosome (Mitelman, 1993). Recently, the development of B-ALL has been shown to be associated with mutations in Pax5 and EBF1 (Mullighan et al., 2007).

In the effort to identify the origin of the Ph chromosome, the advent of quinacrine fluorescence and Giemsa banding enabled Rowley to show that the Ph chromosome results from a reciprocal translocation between the long arms of chromosomes 9 and 22 t(9;22)-(q34;q11) (Rowley, 1973). During the next 9 years after the identification of the chromosome arms involved in the Ph chromosomal translocation, some key studies from several investigators including Heisterkamp, Groffen, Stephenson, and Canaani led to the definitive characterization of the Ph chromosome structure and its mRNA product. Chromosome mapping studies demonstrated that the human ABL gene mapped to chromosome 9 (Heisterkamp et al., 1982). This gene was shown to be present in the Ph chromosome in CML cells (Bartram et al., 1983; Groffen et al., 1983; Canaani et al., 1984; Stam et al., 1987). Breakpoints along the Ph chromosome were found to occur within the breakpoint cluster region (BCR) gene, suggesting that BCR is also be involved in the translocation that creates the Ph chromosome (Groffen et al., 1984). Hybridization of both BCR and ABL probes to the unique mRNA product of the Ph chromosome, demonstrating that Ph chromosome mRNA was composed of BCR-ABL (Shtivelman et al., 1985; Ben-Neriah et al., 1986; Stam et al., 1987). Later, through sequence analysis, the BCR-ABL transcript was shown to contain the first 13 to 14 BCR exons and exons1a or a2 through a11 of ABL (Melo, 1996). This BCR-ABL gene generated a large mRNA product that after splicing, encoded an 8.5-kb BCR-ABL chimeric transcript (Melo, 1996). These studies show that the molecular basis of the Ph chromosome is the BCR-ABL oncogene that encodes BCR-ABL protein.

BCR-ABL TYROSINE KINASE

c-ABL, a non-receptor tyrosine kinase, is expressed in most tissues. In a cell, it is distributed in both the nucleus and cytoplasm of cells, and shuttles between the two compartments. It transduces signals from cell-surface receptors for growth factors and adhesion receptors to regulate cytoskeleton structure (Woodring et al., 2003; Hernandez et al., 2004). Mice with a homozygous deletion of the Abl gene are variably affected, and the phenotypes include an increased incidence of perinatal mortality, lymphopenia and osteoporosis. ABL-null mice are also smaller, with abnormal head and eye development (Schwartzberg et al., 1991; Tybulewicz et al., 1991). BCR is also a signaling protein that contains multiple modular domains. BCR-deficient mice develop normally, and one obvious phenotype is that neutrophils produce excessive levels of oxygen metabolites following their activation (Voncken et al., 1995). The fusion of BCR to ABL during the translocation increases the tyrosine-kinase activity of ABL, and brings new regulatory domains/motifs to ABL, such as the growth factor receptor-binding protein 2 (GRB2) and SH2-binding sites (Li et al., 2001). Depending on the precise breakpoints in the translocation and RNA splicing, different forms of BCR–ABL protein with different molecular weights can be generated in patients, such as P190, P210, and P230 (Groffen et al., 1984; Fainstein et al., 1987; Saglio et al., 1990).

BCR-ABL DOMAIN FUNCTIONS AND CML MOUSE MODEL

The importance of some domains/motifs of BCR-ABL has been validated *in vivo* using a retroviral transduction/bone marrow transplantation (BMT) mouse model of CML (Fig. 1). This BMT-based CML mouse model is an excellent system for the *in vivo* structure-function analysis of BCR-ABL (Li et al., 1999), and various mutant forms of BCR-ABL have been expressed in mice using this model. Mice that express a form of BCR-ABL with a point mutation in the ATP-binding site of ABL, which inactivates its kinase activity, do not develop leukemia, even when the fusion protein is expressed in the long-term repopulating hematopoietic stem/progenitor cells, indicating that the ABL kinase activity is absolutely essential for BCR-ABL leukemogenesis *in vivo* (Zhang and Ren, 1998). This result is consistent with the finding that the kinase activity of ABL is required for BCR-ABL-mediated transformation in cultured cells (Ren, 2005). It also demonstrated that the ABL kinase is an excellent target for treating CML. In addition to the ABL kinase domain, there are other important domains/motifs in BCR-ABL, such as those that regulate the kinase activity of ABL or connect to other downstream signaling pathways (Fig. 2). The relative importance of various domains of BCR-ABL in neoplastic transformation has been examined *in vitro* and *in vivo*, including transformation of immortalized fibroblast cell lines, growth-factor-dependent hematopoietic cell lines and primary bone-marrow cells. The advantage of using cell lines is the relative ease of obtaining a large number of clonally derived cells for biochemical analysis, genetic

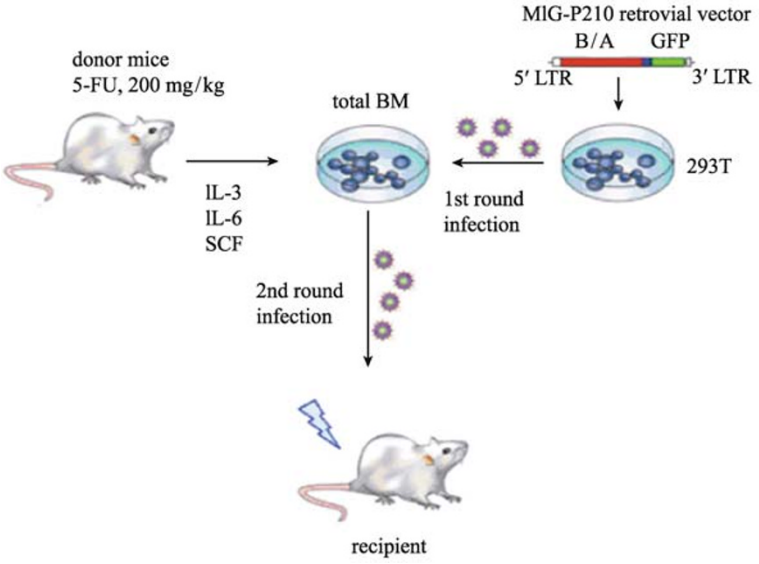

Figure 1. A retroviral transduction/transplantation model of BCR-ABL induced CML. Donor mice are pretreated with 5-FU, and bone marrow cells are stimulated with cytokines *in vitro*. After infected twice with MIG-BCR-ABL retrovirus, donor bone marrow cells are transplanted into lethally irradiated recipients for induction of CML.

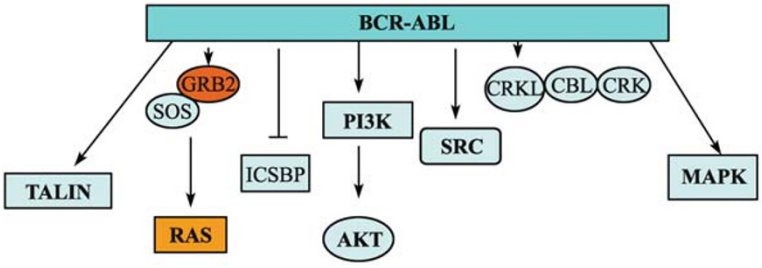

Figure 2. Signaling pathways activated or inhibited by BCR-ABL. GRB2: growth factor receptor-bound protein 2; PI3K: phosphatidylinositol 3-kinase; ICSBP: Interferon consensus sequence binding protein; CRKL: Crk-like protein; CRK: v-crk sarcoma virus CT10 oncogene homolog; CBL: Casitas B-lineage lymphoma.

manipulations and biological examinations. However, cell lines are limited in that they are not likely to include all the physiologically relevant components of BCR-ABL-mediated leukemogenesis. The existence of unknown genetic abnormalities in established cell lines might also obscure the function of the gene studied. Inconsistent results have been obtained from cell line studies in assessing the role of certain functional domains or motifs, such as the GRB2 SH2-binding site at Y177 of BCR and of the SH2 domain of ABL, in transformation by BCR-ABL (Ghaffari et al., 1999). Deletion of the SH3 domain of ABL results in a mutant form of the protein with increased tyrosine kinase activity, and expression of this truncated protein can transform both fibroblast and haematopoietic cell lines *in vitro*. However, it only induces lymphoid leukemia with a greatly extended latency in mice (Gross et al., 1999). A mutant form of BCR-ABL with a deletion of the SH3 domain does, however, still effectively induce a fatal myeloproliferative disease (MPD) (Gross and Ren, 2000). These findings indicate that activation of ABL kinase alone (through the loss of SH3) is not sufficient to cause a CML-like MPD, and that other functional domains/motifs of BCR-ABL are also required for the induction of CML.

The amino-terminal coiled-coil (CC) oligomerization domain of BCR is an important activator of ABL kinase activity, and also promotes the association of BCR-ABL with ACTIN fibres (McWhirter et al., 1993). A mutant form of BCR-ABL that lacks the BCR-CC domain (ΔCC-BCR-ABL) failed to induce MPD in mice, but, rather, induced a T-cell leukemia/lymphoma only after a long latent period (Zhang et al., 2001; Smith et al., 2003). Reactivation of the kinase activity of ABL by mutating its SH3 domain (through deletion or a P1013L point mutation), rescued the ability of ΔCC-BCR-ABL to induce a CML-like MPD in mice (Smith et al., 2003). These results demonstrate that the BCR domain is essential for the induction of CML by BCR-ABL in mice, mainly owing to its ability to activate the kinase activity of ABL. Another important

motif in the BCR region of BCR-ABL is the GRB2-binding site. GRB2 binds SOS as well as the scaffolding adapter GRB2-associated binding protein 2 (GAB2). Formation of this complex depends on BCR phosphorylation at tyrosine 177 (Pendergast et al., 1993), leading to activation of downstream RAS and recruitment of protein tyrosine phosphatase (SHP2) and phosphatidylinositol 3-kinase (PI3K) (Pendergast et al., 1993; Sattler et al., 2002). Mutation of the tyrosine-177 residue of BCR-ABL to phenylalanine (Y177F) largely abolished its ability to bind GRB2 without affecting the kinase activity of ABL (Pendergast et al., 1993; Puil et al., 1994). In the BMT CML model, the Y177F mutant form of BCR-ABL has a greatly reduced ability to induce CML in mice, and these mice eventually developed T-ALL or abdominal T-cell lymphomas after a prolonged latent period (Million and Van Etten, 2000; Zhang et al., 2001). These results demonstrate that phosphorylation at Y177 is required for the induction of CML by BCR-ABL.

The SH2 domain of ABL is believed to activate RAS, at least partially, through binding to SHC, which, following tyrosine phosphorylation, can recruit GRB2 (Goga et al., 1995). Mutations in the SH2 domain of ABL reduced the ability of BCR-ABL to induce a CML-like MPD in mice (Zhang et al., 2001). The Y1294F point mutation in SH2 domain of BCR-ABL also attenuated leukemogenesis by BCR-ABL (Smith et al., 2003). The carboxy-terminal region of ABL is required for the proper function of normal ABL and for the lymphoid leukemogenicity of v-Abl (Prywes et al., 1983). However, deletion of actin-binding domain of ABL or the entire carboxy-terminal region downstream of the ABL kinase domain did not affect the ability of BCR-ABL to induce CML-like MPD in mice (Wertheim et al., 2003). Therefore, the functions of these domains are dispensable in BCR-ABL-mediated leukemogenesis. It is evident that certain domains/motifs of BCR-ABL bear overlapping functions. Deletions of both SH3 domain and carboxy-terminal proline-rich SH3-binding sites (ABL-PP) of ABL, but not point mutations of each domain, block the ability of BCR-ABL to stimulate spontaneous cell migration on fibronectin-coated surfaces, and greatly reduced BCR-ABL leukemogenicity in mice (Dai et al., 2001). Deletions of both SH3 and SH2 domains of ABL in BCR-ABL also showed more severe defects in CML induction than mutating either domain (Nieborowska-Skorska et al., 2002). Together these structure-function analyses of BCR-ABL have shown that the activation of the tyrosine-kinase activity of ABL is necessary but not sufficient to induce CML in mice.

BCR-ABL KINASE INHIBITOR AND CML STEM CELLS

The BCR-ABL kinase inhibitor imatinib is a standard of care for Ph^+ leukemia, and has been shown to induce a complete hematologic and cytogenetic response in the majority of chronic phase CML patients (Druker et al., 2001). Although it is very effective in treating chronic phase CML patients, imatinib will unlikely provide a cure to CML patients for two obvious reasons. One reason is that BCR-ABL develops imatinib-resistant mutations in its kinase domains. Recently, other BCR-ABL kinase inhibitors have been developed to overcome imatinib resistance, including dasatinib and nilotinib. However, these drugs are still ineffective to BCR-ABL-T315I mutant that is present in about 15%–20% of imatinib-resistant CML patients (Bhatia et al., 2003). Another reason is that LSCs in CML are insensitive to imatinib treatment (Graham et al., 2002; Bhatia et al., 2003).

A stem cell has an ability to reproduce itself as well as to differentiate into mature functional cells. When applying the principles in the biology of stem cells to tumorigenesis, the cancer stem cell (CSC) population is believed to be a population of cells that are able to self-renew and differentiate to produce tumor in recipients. It was shown in the mid-1990s that a stem cell-like population isolated from human AML was capable of transferring human AML into an immunodeficient mouse host (Bonnet and Dick, 1997). A candidate CSC population should exhibit following properties: (1) the unique ability to engraft in recipients; (2) the ability to recapitulate the tumor of origin both morphologically and immunophenotypically in xenografts; (3) the ability to be serially transplanted (Park et al., 2009). LSCs in many types of hematologic malignancies are believed to be a cell population required for initiation and sustaining growth of the leukemia (Reya et al., 2001; Al-Hajj et al., 2003; Pardal et al., 2003; Singh et al., 2003; Wang and Dick, 2005; Jordan et al., 2006; Rossi et al., 2008). LSCs may arise from normal stem cells or normal progenitor populations (Park et al., 2009). In BCR-ABL induced CML, LSCs phenotypically appear to be similar to the normal hematopoietic stem cells (HSC). Fialkow and colleagues examined different cell types in chronic phase CML patients for the presence of the Ph chromosome (Fialkow et al., 1967, 1977). Surprisingly, both granulocytes and erythroid cells from chronic phase CML patients contained the Ph chromosome, even though only myeloid cells are expanded during chronic phase CML. The presence of the Ph chromosome in granulocyte and erythroid lineages suggests that the Ph chromosome is either generated in multiple cell types or originates in a HSC, from which it is passed down to other more differentiated cell lineages. Subsequent purification of HSCs and different lineage-restricted cells from CML patients by cell surface markers has confirmed the presence of the Ph chromosome in HSC (Takahashi et al., 1998). The discovery of a clonal HSC origin of CML suggests that elimination of Ph^+ HSCs and replacement of these cells with normal HSCs should be an effective therapeutic strategy.

To further identify CML stem cells in CML mouse model, we have tested whether BCR-ABL-expressing HSCs function as CML stem cells. C57BL/6 (B6) mouse bone marrow (BM)

cells transduced with BCR-ABL retrovirus were first sorted into two separate populations of Sca-1$^-$ and Sca-1$^+$ cells. These two populations of cells were then transferred, respectively, into B6 recipient mice. Only the mice receiving BCR-ABL-transduced Sca-1$^+$ cells developed and died of CML, diagnosed by detecting GFP$^+$ myeloid cells (Gr-1$^+$) in the peripheral blood of the mice, suggesting that early BM progenitors contain CML stem cells. To narrow down the specific cell lineages that function as CML stem cells, HSCs (Lin$^-$c-Kit$^+$Sca-1$^+$) were sorted out from BM cells transduced with BCR-ABL retrovirus, followed by transfer into recipient mice. The mice developed and died of CML. To confirm definitively that BCR-ABL-expressing HSCs contain CML stem cells, BM cells from primary CML mice were isolated and sorted for the BCR-ABL-expressing HSCs (GFP$^+$Lin$^-$c-Kit$^+$Sca-1$^+$) by FACS. The sorted cells were transferred into recipient mice, and the mice developed and died of CML, indicating that BCR-ABL expressing HSCs function as CML stem cells (Hu et al., 2006). These LSCs in CML mice were insensitive to imatinib and dasatinib (Hu et al., 2006). In CML patients, born marrow CD34$^+$Lin$^-$ cells, in which normal hematopoietic stem cells (HSCs) reside, are thought to contain CML stem cells and be responsible for disease initiation, progression and resistance to imatinib (Graham et al., 2002; Bhatia et al., 2003). Human CD34$^+$ CML stem cells were not effectively killed by imatinib treatment both *in vitro* (Graham et al., 2002) and *in vivo* (Bhatia et al., 2003). BCR-ABL transcripts could still be detectable in CD34$^+$ bone marrow cells from CML patients after a long-term treatment with imatinib (Bhatia et al., 2003), suggesting that these LSCs are eradicated through inhibiting BCR-ABL kinase activity. CD34$^+$ CML stem cells, especially the undivided CD34$^+$ cell population, are shown to be insensitive to the inhibition by imatinib in a cell culture assay (Graham et al., 2002). Similarly, LSCs could not be eradicated by imatinib in CML mice, as the total numbers and percentages of LSCs in bone marrow of imatinib treated mice continued to increase during the treatment, and the insensitivity of LSCs to imatinib is not associated with the development of imatinib-resistant mutations on BCR-ABL (Hu et al., 2009). Together, these results indicate that some unknown pathways contribute to the maintenance of survival and self-renewal of LSCs,

STRATEGIES FOR TARGETING CML STEM CELLS

Identification of novel genes that play critical role in regulating the function of LSCs will help to develop new therapeutic strategies through targeting LSCs. One strategy for inhibiting LSCs is to target essential genes for both normal HSCs and LSCs, which has been used often by researchers. Several genes including Hedgehog, Wnt/β-catenin, Bim-1, Mcl-1 were known to play important roles in the development of normal embryonic or hematopoietic stem cells, and were recently reported to regulate the function of CML stem cells (Taipale and Beachy, 2001; Lessard and Sauvageau, 2003; Reya et al., 2003; Zhao et al., 2007). Targeting of these essential genes could effectively inhibit the growth of LSCs (Zhao et al., 2007; Zhao et al., 2009). The cyclopamine, a Hedgehog inhibitor, reduces CML stem cell population and extend survive of CML mice (Zhao et al., 2009). Omacetaxine, a Mcl-1 inhibitor, is also shown to inhibit LSCs effectively (Chen et al., 2009a). Another strategy is to identify key functional pathways specific for LSCs but not normal HSCs, and target these pathways in treating CML without causing significant harm to normal HSCs (Fig. 3).

AN ESSENTIAL ROLE OF THE Alox5 GENE IN LSCs

To identify critical specific pathways in BCR-ABL-expressing LSCs instead of normal HSCs, we performed a microarray study to compare gene expression profile between BCR-ABL expressing and non-BCR-ABL expressing Lin$^-$c-Kit$^+$Sca-1$^+$ cells. Among BCR-ABL regulated genes, one gene named Alox5 is noticeable. Alox5 is linked to many important pathways including P53 and PI3K, and is thought to play an important role in different diseases (Catalano et al., 2005; Wymann and Schneiter, 2008). We tested the function of Alox5 in BCR-ABL induced CML mice. Strikingly, recipients of BCR-ABL-transduced BM cells from Alox5$^{-/-}$ donor mice were resistant to the induction of CML by BCR-ABL, and the defective disease phenotype is correlative with gradually decreased leukemia cells in peripheral blood and bone marrow. In these mice, myeloid leukemia cells were found to grow initially, reached a peak after 2 weeks, then started to decline, and eventually disappeared after 7 weeks. However, normal myeloid cells in these CML mice were found to be at a low level initially, and started to grow as leukemia cells were disappearing. These results suggest that loss of Alox5 might lead to a defect in LSCs but not normal HSCs. Furthermore, we compared the functions of LSCs and normal HSCs. To do so, wild type (CD45.1$^+$) and Alox5$^{-/-}$ (CD45.2$^+$) LSCs sorted by FACS were mixed in a 1:1 ratio and transplanted into lethally irradiated recipient mice. More than 90% of myeloid leukemia cells in peripheral blood of the mice were wild type (CD45.1$^+$) leukemia cells, and all these mice developed CML and died. These indicate that Alox5 plays an essential role in regulating the function of LSCs but not normal HSCs (Chen et al., 2009b).

Alox5 AS A POTENTIAL TARGET FOR ERADICATING LSCs

Zileuton specifically inhibits the enzymatic activity of arachidonate 5-lipoxygenase (5-LO), the product of the Alox5 gene (Knapp, 1990). Therefore, we tested its therapeutic effect on CML in mice (Chen et al., 2009b). BCR-ABL transduced BM cells were transplanted into recipient mice to induce CML, and then the CML mice were treated with a placebo, Zileuton

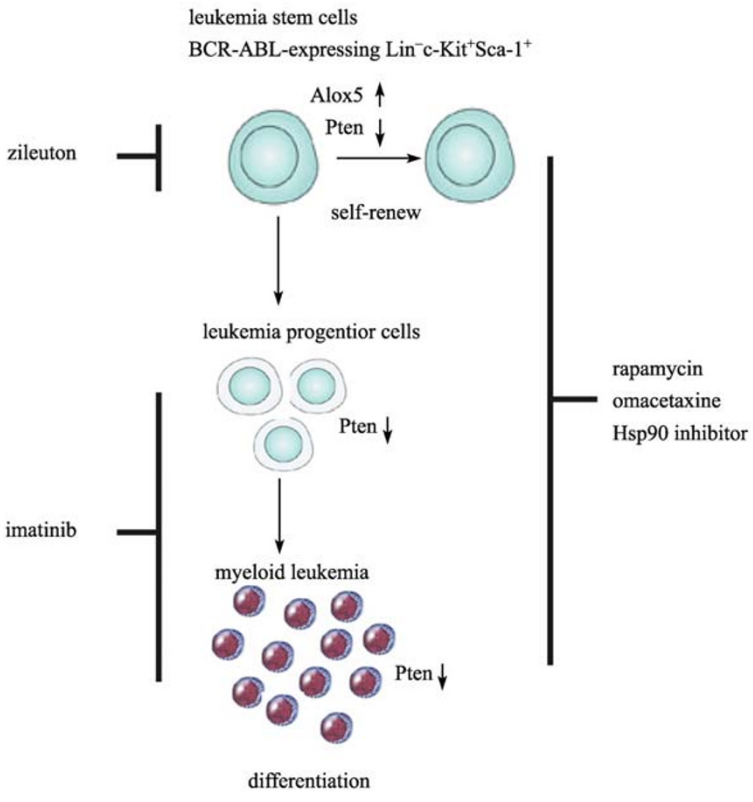

Figure 3. Targeting leukemia stem cells in CML. Described are some known drugs that inhibit leukemia stem cells, leukemia progenitor cells and more mature myeloid leukemia cells.

or imatinib alone, or two agents in combination. All placebo-treated mice developed and died of CML within 4 weeks after the induction of CML by BCR-ABL, and strikingly Zileuton alone was even more effective than imatinib in prolonging survival of CML mice. About 7 weeks after Zileuton treatment, $GFP^+Gr\text{-}1^+$ leukemia cells in peripheral blood of the mice gradually declined and dropped from over 50% to less than 2%, indicating that myeloid leukemia is eventually eliminated. Treatment of CML mice with both Zileuton and imatinib had a better therapeutic effect than with either Zileuton or Imatinib alone in prolonging survival of the mice. In these CMl mice, Long-term (LT)-LSCs were found to accumulate in BM of the treated mice; however, short-term (ST)-LSCs and MPP (multiple potent progenitor) cells were gradually depleted, suggesting that inhibition of 5-LO by Zileuton causes the blockade of differentiation of LT-LSCs. The inhibitory effect of Zileuton are consistent with the finding from above-described genetic studies using $Alox5^{-/-}$ mice, demonstrating that targeting of the Alox5 pathway is potentially curative for CML, and this idea needs to tested in human CML patients.

PTEN IS A TUMOR SUPPRESSOR IN LSCs

Another gene identified in our microarray study is Phosphatase and tensin homologue (PTEN). PTEN is often deleted or inactivated in many tumor types, including glioblastoma, endometrial carcinoma and lymphoid malignancies. PTEN is a phosphatase that dephosphorylates phosphatidylinositol (3,4,5)-trisphosphate (PIP3) (Maehama and Dixon, 1998; Stambolic et al., 1998). PIP3 is a direct product of PI3K activity, and plays a critical role in the regulation of cell survival and growth through activating the Ser/Thr protein kinase PDK1 and its downstream target Akt (Rameh and Cantley, 1999; Varnai et al., 2005). Loss of PTEN results in the development of AML and ALL in mice (Zhang et al., 2006). We found that PTEN is downregulated in LSCs and leukemia cells in CML mice (unpublished data), consistent with the down-regulation of PTEN in human leukemia cells (Bruns et al., 2009). In our study, overexpression of PTEN in CML mice prolonged survival of CML mice. Rapamycin, which inhibits the AKT downstream molecule mTOR, significantly decreased the number of LSCs *in vitro*. When BM cells from CML mice were isolated and cultured under the conditions that support survival and growth of LSCs (Chen et al., 2009b), we found that rapamycin inhibited LSCs effectively. We further tested the inhibitory role of PTEN in human K562 CML cells, and found that rapamycin significantly inhibited survival of the cells, and also significantly induced apoptosis of K562 cells. Our finding that PTEN is a critical tumor suppressor in CML leukemia stem cells and

inhibition of PI3K-AKT-mTOR pathway by rapamycine provides a promising strategy in CML therapy (Chen et al., 2009b).

FUTURE DIRECTIONS

We believe that LSCs serve as key target cells for curing CML, although it is difficult to identify effective and specific target genes in LSCs. Further comparative analysis of gene profiling between normal HSCs and LSCs by DNA microarray will help to identify candidate genes, as exemplified by our identification of the role of Alox5 in regulating the function of LSCs (Chen et al., 2009b). Several important questions remain to be answered: (1) How does BCR-ABL activate these genes in LSCs? (2) How these genes function to regulate LSCs? (3) What are related signaling pathways involved in regulating the function of LSCs? We believe that our strategy for identifying and testing LSC-specific genes will help to develop a curative therapy of CML.

ABBREVIATIONS

AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia; BCR, breakpoint cluster region; BM, bone marrow; CBL, Casitas B-lineage lymphoma; CC, coiled-coil; CML, chronic myeloid leukemia; CRK, v-crk sarcoma virus CT10 oncogene homolog; CRKL, Crk-like protein; CSC, cancer stem cell; GAB2, GRB2-associated binding protein 2; GRB2, growth factor receptor-binding protein 2; HSC, hematopoietic stem cells; ICSBP, Interferon consensus sequence binding protein; LSC, leukemia stem cells; MPD, myeloproliferative disease; MPP, multiple potent progenitor; Ph, Philadelphia; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, Phosphatase and tensin homologue

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