Stem cell maintenance and disease progression in chronic myeloid leukemia

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Abstract Chronic myeloid leukemia (CML) is a cancer of blood cells driven by the BCR–ABL1 oncogenic protein tyrosine kinase, which is the product of a reciprocal chromosomal translocation known as the Philadelphia chromosome. Discovery of tyrosine kinase inhibitors targeting the BCR–ABL1 kinase revolutionized CML therapy, but these drugs are unable to eradicate the disease due to the presence of a drug-insensitive stem cell population that sustains continued growth of the malignant cells. Resistance to therapies also increases the risk of relapse and disease progression to a more advanced phase. This review discusses emerging issues in CML research, and describes recent progress in elucidating the mechanisms of CML stem cell maintenance and disease progression.

Keywords Chronic myeloid leukemia · Stem cell · Cancer progression · Blast crisis

Introduction

As we make progress toward effective therapies against cancer, several important issues remain, including therapy resistance, disease relapse following therapy cessation, and disease progression to advanced stages. Decades of studies on acute myeloid leukemia (AML) have provided sufficient evidence to conclude that leukemia is organized as a hierarchy of several distinct leukemic cell types with different degrees of self-renewal and differentiation potential [1]. There is a functionally, and often phenotypically, distinct population of tumor-initiating cells that exhibit the capacity to self-renew, the potential to generate all of the cell types present in the tumor, and the ability to proliferate actively and thereby drive sustained expansion of malignant cells [2, 3]. The tumor-initiating cells, or cancer stem cells, represent a therapy-resistant cell population and are primary drivers of cancer propagation and disease relapse after treatment and/or chemotherapy cessation [4, 5]. Recent studies have also supported the idea that several types of solid tumors, if not all, are a heterogeneous mixture of multiple cell types and are sustained by a subpopulation with higher tumorigenic potential [6, 7]. It is thus reasonable and appealing to speculate that targeting a population that can maintain cancer growth may provide clues to effective treatment strategies and, ultimately, a cure to many hematologic malignancies and cancers. Indeed, in the mouse model of *MOZ–TIF2*-induced AML, forced cell death of the leukemia stem cell population leads to eradication of the disease in vivo [8]. In this Perspective, I will discuss emerging issues and the roles of novel players in stem cell maintenance and disease progression in cancer with a specific focus on chronic myeloid leukemia.

Chronic myeloid leukemia

In the treatment of chronic myeloid leukemia (CML), chemotherapy resistance, disease relapse, and progression remain unsolved problems, and have been the focus of extensive research in recent years. CML is a clonal myeloproliferative disease characterized by the *BCR–ABL1*

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fusion oncogene, which is the product of a reciprocal chromosomal translocation between chromosomes 9 and 22, known as the Philadelphia chromosome [9, 10]. BCR–ABL1 functions as a constitutively active oncogenic tyrosine kinase, and drives increased production of mature granulocytes (Fig. 1). Development of the tyrosine kinase inhibitor (TKI) imatinib mesylate revolutionized CML therapy, and over 70 % of patients with chronic phase CML (CP-CML) attain complete cytogenetic response and improved survival through the use of TKI [11, 12]. However, despite its effectiveness in controlling CP-CML, TKI is unable to eradicate the disease at the CML stem cell level, leading to long-term dependence on TKI, and increased risk of drug resistance and relapse following drug discontinuation, even after molecular remission [13, 14].

Patients who show initial resistance to TKI and become resistant to primary TKI therapy are likely to relapse and progress to a more aggressive disease phase called blast crisis (Fig. 1) [15]. Compared with indolent CP-CML in which mature myeloid cells are produced, blast crisis CML (BC-CML) is characterized by the rapid proliferation of differentiation-arrested immature blast cells of either myeloid or lymphoid lineage [13, 14]. Interestingly, differentiation arrest and/or abnormal capacity of differentiation are shared features of hematologic malignancies and solid tumors with poor prognosis. Although the molecular mechanisms driving the transformation of CP to BC-CML are not fully understood, additional genetic mutations, defects in DNA damage repair and telomere maintenance

are implicated in this process [13–17]. Unfortunately, due to its resistance to current therapies, BC-CML remains a major challenge in the treatment of CML.

Recently, it has become clear that while differentiated CML cells are dependent on BCR–ABL1 activity for their survival and therefore remain sensitive to kinase inhibition, CML stem cells do not rely on its activity and persist despite the effective inhibition of the BCR–ABL1 by TKIs [18–20]. These studies clearly highlight the need for targeting the BCR–ABL1-independent, alternative mechanisms that protect and sustain CML stem cells to develop curative therapies for this hematologic malignancy.

Mouse models of CML and insights for cancer stem cell research

Development of animal models that recapitulate many features of human CML have been invaluable to our efforts to gain a better understanding of the pathology and biology of CML. To date, three mouse models are well established and widely used in the field (Fig. 2). The most commonly used model utilizes retroviral transduction coupled with bone marrow transplantation (Fig. 2a) [21, 22]. In this model, hematopoietic stem cell (HSC)-enriched bone marrow cells from healthy or 5-fluorouracil-treated mice are transduced retrovirally with the BCR–ABL1 oncogene and the BCR–ABL1-expressing cells are transplanted into lethally irradiated mice. The recipient mice develop
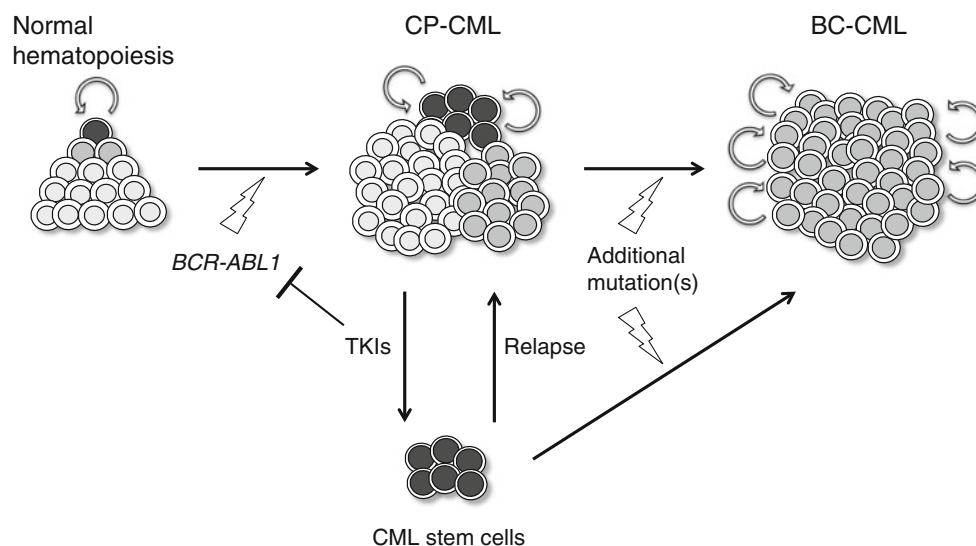

Fig. 1 Development and disease progression in chronic myeloid leukemia. Chronic myeloid leukemia (CML) is initiated by the BCR–ABL1 translocation in hematopoietic stem cells, which leads to myeloid cell expansion while allowing differentiation (chronic phase CML). Secondary chromosomal translocations such as NUP98–HOXA9 or AML1–EVI1, or mutations in p53 or INK4A trigger disease progression to a more advanced phase (blast crisis CML), with

progressive loss of the capacity to differentiate and increased expansion of immature blast cells. While tyrosine kinase inhibitors (TKIs) targeting BCR–ABL1 kinase activity can kill the oncogene-expressing progenitor cells, they are unable to eliminate the CML stem cells, leading to an increased risk of disease relapse after discontinuation of TKI therapy
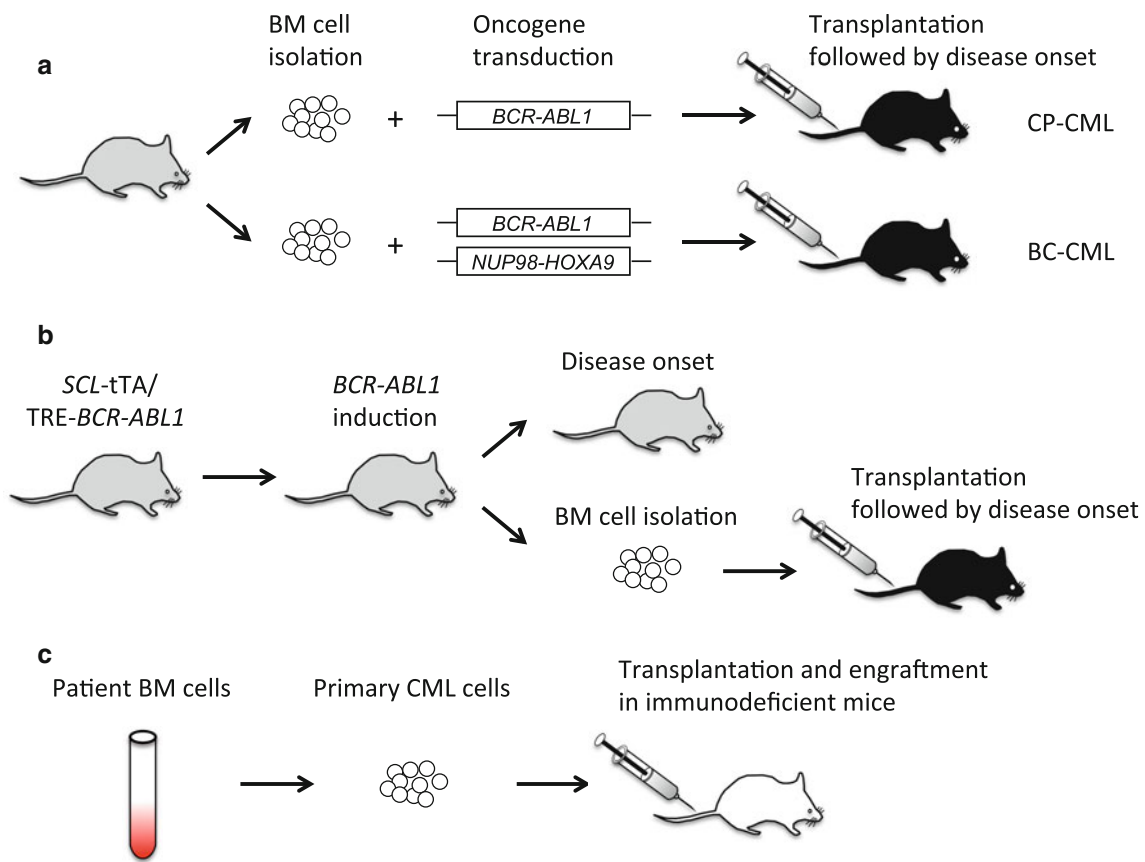
Fig. 2 Mouse models of CML. **a** Retroviral transduction-bone marrow (BM) transplantation model. To model CP-CML, hematopoietic stem cell (HSC)-enriched bone marrow cells from donor mice are transformed with *BCR–ABL1* retrovirus and transplanted into lethally irradiated syngeneic recipient mice. For BC-CML-like acute leukemia, bone marrow cells are transduced with *BCR–ABL1* and *NUP98–HOXA9* retroviruses, and doubly infected cells are transplanted in sub-lethally irradiated recipient mice. **b** Inducible *BCR–ABL1* transgenic mouse model. In a transgenic strain carrying the *BCR–ABL1* transgene under the control of a Tet-responsive element and a second transgene encoding a Tet-regulated transactivator protein (tTA) driven by the *SCL* gene enhancer, *BCR–ABL1* expression is induced by the removal of doxycycline, which leads to a CML-like disease in vivo. Alternatively, *BCR–ABL1*-expressing long-term HSC population is isolated by flow cytometry and transplanted into lethally irradiated recipients. **c** Human primary CML xenograft model. Primary CD34$^+$ CML cells are isolated from patient specimens and transplanted into sublethally irradiated immunodeficient NOD–SCID or NSG mice

myeloproliferative disease within a few weeks that resembles many aspects of human CP-CML, including increased numbers of mature granulocytes in bone marrow and blood, splenomegaly, and anemia. In addition to CP-CML, human myeloid BC-CML can also be modeled in mice by combined retroviral expression of *BCR–ABL1* and a second oncogene, such as *NUP98–HOXA9* or *AML1–EVI1* [23–26]. *NUP98–HOXA9* is a product of the t(7;11) chromosomal translocation identified in humans, and is associated with blast crisis as well as de novo AML [26–28]. Recipient mice transplanted with cells expressing *BCR–ABL1* plus *NUP98–HOXA9* develop acute myeloproliferative disease that closely mimics human BC-CML. In contrast to the disease induced by the *BCR–ABL1* oncogene alone, cells harboring the two oncogenic hits show immature morphology and immunophenotype and are highly tumorigenic upon serial transplantations [26].

Another model of CML takes advantage of genetically engineered mice expressing the *BCR–ABL1* transgene. Several distinct transgenic strains are available [29–32], and among those, a tetracycline(Tet)-regulated binary transgenic model is the most promising (Fig. 2b). This model carries the *BCR–ABL1* transgene under the control of a Tet-responsive element as well as a second transgene encoding a Tet-regulated transactivator protein (tTA) driven by the *SCL* gene enhancer. The combination of these two transgenes results in Tet-regulated *BCR–ABL1* expression in HSCs. *BCR–ABL1* gene expression can be induced by the removal of doxycycline, a tetracycline analog, leading to a CML-like disease characterized by neutrophilia and splenomegaly. Using the retroviral/transplantation model, CML stem cells have been identified in the population with a Lin$^-$ Sca-1$^+$ c-Kit$^+$ (LSK) phenotype [33]. In a recent study using the binary *BCR–ABL1*-

inducible model, Passegue and colleagues found that CML stem cells share the same cell surface phenotype as long-term HSCs (LSK Flk2$^-$ CD48$^-$ CD150$^+$) [34]. Although the transplantation of *BCR–ABL1*-expressing cells with a short-term HSC phenotype (LSK Flk2$^-$ CD48$^-$ CD150$^-$) can cause transient myeloid hyperplasia, these cells are unable to induce CML-like disease in vivo. In contrast, as few as 50 *BCR–ABL1*$^+$ long-term HSCs lead to robust and long-lasting engraftment, and most of the engrafted mice eventually develop CML with an average latency of 4 months, demonstrating that CML stem cells reside exclusively in the long-term HSC compartment.

The most physiologically relevant mouse model currently available is a xenograft model of human primary CML cells (Fig. 2c). Flow-sorted primary CD34$^+$ leukemia cells from patient bone marrow specimens are transplanted into immune-compromised recipient mice, such as NOD–SCID mice [35, 36]. Although the model remains technically challenging due to low efficiencies of long-term engraftment and/or biologic variations observed in primary leukemia specimens, development of the novel immuno-deficient mouse strain NSG (NOD–SCID IL-2Rγ-chain-deficient) has generated an improved model with robust engraftment, providing a better way to analyze human CML stem cells in vivo and test the effectiveness of novel therapies against human CML [37].

Emerging players mediating stem cell maintenance and disease progression in CML

In the past decade, multiple signaling pathways have been identified as critical regulators of CML pathogenesis by the use of the aforementioned mouse models of CML. These include classic developmental pathways such as Wnt/beta-catenin and Hedgehog [38–40], as well as the multifunctional signaling scaffold protein β-arrestin [41], the pro-myelocytic leukemia protein (PML), transcriptional regulators of FoxO and HIF1α [42–44] and the arachidonate 5-lipoxygenase Alox5 [45] (well reviewed in [46–48]). Discussed below are the emerging players that sustain stem cells and drive cancer progression in CML.

Oncogenic RNA binding proteins

RNA binding proteins (RBPs) are key components in cellular RNA metabolism. RBPs regulate maturation, alternative splicing, nucleocytoplasmic transport, translation and stability of mRNAs and non-coding RNAs, and therefore altered RBP activity and expression can have profound effects on cellular events. Indeed, CML progression from chronic to blast crisis phase has been shown to accompany dysregulated expression of distinct RBPs,

including heterogeneous nuclear ribonucleoproteins (hnRNPs), Lin28, and Musashi (Msi) (Fig. 3).

Pioneering work on hnRNP E2 clearly showed that its gene expression is highly upregulated in human BC-CML compared with CP-CML, and that the hnRNP E2 protein binds to *C/EBPα* mRNA to repress its translation [49]. The transcription factor C/EBPα is essential for granulocyte differentiation, and functional inactivation of C/EBPα results in a block to myeloid differentiation. Thus, suppression of *C/EBPα* mRNA translation by upregulated hnRNP E2 protein is one of the mechanisms of differentiation arrest leading to the expansion of immature myeloid cells in BC-CML. A recent study has shown that *miR-328*, one of the microRNAs (miRNAs) downregulated during CML progression, competes with *C/EBPα* mRNA for binding to hnRNP E2 [50]. Forced expression of miR-328 increases C/EBPα protein levels in BC-CML cells and functionally impairs the clonogenic potential of human BC-CML cells, indicating that *miR-328* functions as a decoy RNA to modulate hnRNP E2 activity.

RBPs also mediate regulation of miRNA biogenesis. The RNA binding protein Lin28 binds to *let-7* miRNA precursors and inhibits their processing and maturation. While the *let-7* family of miRNAs is abundant in differentiated cells, its expression is repressed in undifferentiated pluripotent ES cells and human cancers [51]. Consistent
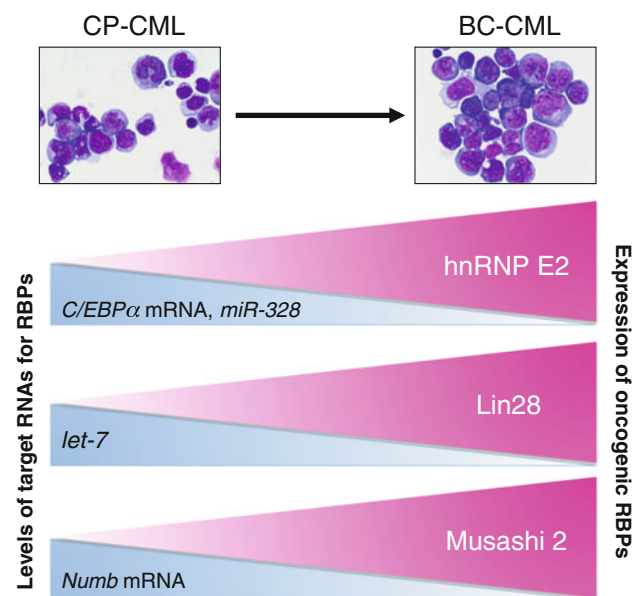

Fig. 3 Dysregulation of RNA-binding proteins during CML progression from chronic to blast crisis phase. Expressions of RNA-binding proteins (RBPs) become elevated in BC-CML with concomitant decrease in the levels of their target RNAs. Inactivation of these RNA-binding proteins, or forced expression of the target RNA species can functionally rescue the arrested differentiation in BC-CML, suggesting that targeting these pathways may provide novel therapeutic strategies to block and reverse CML progression

with its role in *let-7* miRNA maturation, *Lin28* is overexpressed in a broad range of human cancers [52]. *Lin28* expression is more frequent in BC-CML specimens than in CP-CML, and the inhibition of *Lin28* expression by RNA interference in K562 cells restores the levels of mature *let-7* miRNA and impairs the proliferative and clonogenic capacities with concomitant induction of cellular differentiation, suggesting that targeting the *Lin28-let-7* axis might delay or block the blast crisis transformation.

The Musashi gene (Msi) encodes an RBP originally identified as a developmental regulator of neural cell fate in the fruit fly. Later studies showed that there are two paralogous *Msi* genes in mammals (*Msi1* and *Msi2*), and that the *Msi1* gene expression marks tissue stem cells in brain, eye, and intestine [53]. Msi1 binds to and inhibits the translation of the mRNA for *Numb*, which specifies a differentiated cell fate in neural progenitors and HSCs [54, 55]. The functional importance of *Msi1* in neural stem cells, however, remains elusive. *Msi1* inactivation alone results in barely appreciable defects during embryonic neural development, which may be due to functional redundancies among the two paralogs [56]. The second gene, *Msi2*, is predominantly expressed in the most immature HSC compartment within the hematopoietic organ, and during CML disease progression, *Msi2* expression levels are significantly elevated in the blast crisis phase [57]. Importantly, NUP98–HOXA9 can trigger *Msi2* expression, which in turn represses Numb expression. Genetic loss of *Msi2* results in increased Numb levels and, as a consequence, functionally impairs the development and propagation of BC-CML in vivo. Interestingly, *Msi2* is not only highly upregulated during human CML progression, but is also a marker for poorer prognosis in human BC-CML and de novo AMLs [57, 58]. Collectively, these studies define the essential role of the RNA binding factor Msi2 as a molecular driver of cancer progression by regulating the balance between stem cell self-renewal and differentiation.

SIRT1 histone deacetylase

Histone deacetylases (HDACs) are among the promising candidates for targeted therapies in CML [36]. A recent study identified a non-canonical class III HDAC protein, SIRT1 as an exciting new target for eradicating CML stem cells [37]. SIRT1, the founding member of the Sirtuin family of proteins, is a mammalian homolog of the yeast protein Silent Information Regulator 2 (Sir2), and exerts a powerful influence on a variety of cellular processes, including DNA repair, cell cycle, metabolism and aging in diverse organisms [59]. Unlike canonical HDACs, SIRT1 requires the coenzyme nicotinamide adenine dinucleotide to deacetylate its targets, and it is not inhibited by pan-

HDAC inhibitors [60]. In the CD34$^+$ population from human CP- and BC-CML cells, *SIRT1* is expressed at higher levels than in the CD34$^+$ population in normal bone marrow [37]. *SIRT1* downregulation by RNA interference or pharmacological inhibition of its activity by tenovin-6, a selective small molecule inhibitor of SIRT1, induces cell death and impairs the proliferation of the quiescent, TKI-insensitive CML stem cell population in culture. SIRT1 inhibition ex vivo reduces the growth of CML cells and compromises the long-term engraftment of human CD34$^+$ CML cells upon transplantation into immunodeficient NSG mice. Spurred by the successful inhibition of CML stem cell growth in vitro, Li et al. tested whether tenovin-6 could impair the CML development in vivo by using the Tet-regulated *BCR–ABL1* transgenic model. Although imatinib treatment alone reduced tumor burden, it failed to impact the CML stem cells. In contrast, combined treatment of imatinib and tenovin-6 effectively inhibited the maintenance of the *BCR–ABL1*$^+$ long-term HSC population and significantly prolonged overall survival after discontinuation of the drug treatments. These results suggest that targeting SIRT1 could provide a novel therapeutic approach for the eradication of the CML stem cells.

Concluding remarks

Recent basic and translational research has contributed to our understanding of the biology of CML stem cells and has uncovered several potential candidates for targeted therapies in the myeloid leukemia. An accumulating body of evidence supports the idea that the properties of CML stem cells are shared by a wide array of cancer stem cells in other hematologic malignancies and solid tumors. Thus, the knowledge gained thus far from studies of CML is likely to have broader implications and extend to other cancers.

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