Chronic myeloid leukemia as a stem cell-derived malignancy

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ABSTRACT

Chronic myeloid leukemia (CML) is a myeloproliferative disease of the hematopoietic stem cells, characterized by the presence of the Philadelphia (Ph) chromosome. Although imatinib inhibits the BCR-ABL kinase activity, clinical experiences confirm that imatinib may not target CML stem cells in vivo. The identification of signaling pathways responsible for the self-renewal properties of leukemic stem cells in CML will help in the discovery of novel therapeutic targets. Here we review signaling pathways including Wnt/β-catenin, Hedgehog, Alox5, and Foxo which play crucial roles in the maintenance of stem cell functions in CML. It is thought that inhibition of key genes that are part of self-renewal associated signaling pathways may provide an effective way to reduce aberrant stem cell renewal in CML.

Keywords: cancer stem cells; chronic myeloid leukemia stem cells; signaling pathway; microRNA

INTRODUCTION

Cancer stem cells are a small subpopulation of malignant cells identified in a variety of tumors (or leukemias) that are defined by their ability to undergo self-renewal, as well as multi-lineage differentiation, resulting in therapeutic resistance and cancer progression. Self-renewal is discriminated from other proliferating processes since at least one of progeny is identical to the primary stem cell. Dysregulation of stem cell self-renewal is a likely requisite for the initiation, progression, and therapeutic resistance of cancer [1-5]. The cancer stem cell hypothesis postulates that cancers are derived from a self-renewing cancer stem cell population that is also capable of initiating/maintaining cancer. Thus, according to the cancer stem cell hypothesis, these cells with the unique self-renewal ability are tumor-initiating cells that differentiate into non-self-renewing cells that comprise the bulk of the tumor [6-9]. Cancer stem cells were initially identified in leukemia [10,11] but has since expanded to solid tumors such as brain, breast, pancreas, colon, and head and neck cancer [3,12-22].

Chronic myeloid leukemia (CML) is a monoclonal myeloproliferative disorder of the hematopoietic stem cells, characterized by the presence of the Philadelphia (Ph) chromosome genetic abnormality which arises from the reciprocal translocation t(9; 22) (q34; q11). This translocation leads to the fusion of the Breakpoint Cluster Region (BCR) gene to the Abelson Tyrosine Kinase (ABL1) proto-oncogene and the formation of the BCR-ABL oncogene [23,24]. Depending on the precise translocation breakpoints and differential mRNA splicing, various molecular weight isoforms of BCR-ABL (P190, P210, and P230 isoforms) are generated. The BCR-ABL oncogenes produce a constitutively active non-receptor tyrosine kinase which deregulates several signal transduction pathways that ultimately lead to abnormal cell cycling, increased proliferation, and inhibition of apoptosis [24-26].

The natural course of the disease is usually characterized by three sequential stages (The chronic, accelerated, and blast-crisis). Initially, CML is a slowly progressive disease with symptoms that usually develop gradually [27]. As the disease progresses, the number of blasts in the bone marrow and peripheral blood is increased, and accelerated phase of the disease will evolve to an aggressive acute leukemia, referred to as a blast crisis [28], during which progressive resistance to therapy is acquired [29,30]. The transition from chronic to the accelerated and blast phases is presumed to occur due to secondary genetic changes [31].

CML STEM CELLS ARE RESISTANT TO BCR-ABL KINASE INHIBITOR IMATINIB

The development of the BCR-ABL kinase inhibitor imatinib was a breakthrough in the therapy of chronic-phase CML, establishing imatinib as the first-line therapy for newly diagnosed CML. A 5 year follow-up of patients receiving imatinib as initial therapy demonstrated complete hematological response in 98% of the treated patients, a complete cytogenetic response in 87%, and a complete molecular response in about 35%. The study showed a relapse rate of 17% during continuous treatment and 7% of patients progressed to the accelerated phase or blast crisis. However, 4% of patients discontinued therapy owing to an adverse event [32].

Although continuous treatment of chronic-phase CML with imatinib was found to induce durable responses, several reports indicate that discontinuation of imatinib therapy even after achieving a molecular remission induces a relapse of the disease [33-37], and therefore, patients are forced to undergo lifelong therapy [33,38-40]. Further studies have been shown that BCR-ABL-positive leukemic stem cells are relatively resistant to therapies that target rapidly dividing cells, and thus contribute to avoid apoptosis, renew themselves, and survive long term [41-43]. This is supported because BCR-ABL-positive leukemic stem cells remain present in the patient's bone marrow even after long-term treatment with imatinib and can cause relapse of the disease [44,45]. These findings propose that inhibition of BCR-ABL tyrosine kinase activity alone is insufficient to eradicate leukemic stem cells and cannot cure CML. Therefore, development of efficient therapeutic strategies capable of eradicating CML stem cells would provide noticeably improved therapeutic benefits to patients suffering CML (Fig. 1) [46-50].

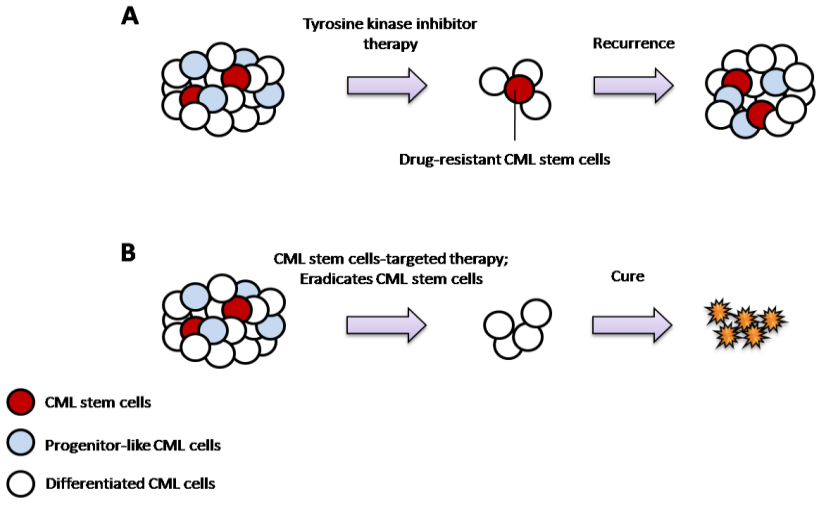

Figure 1. CML stem cells are resistant to BCR-ABL kinase inhibitor imatinib. A. Although tyrosine kinase inhibitor imatinib inhibits the BCR-ABL kinase activity and decreases the number of CML cells, it does not target CML stem cells. This leads to

relapse of the disease. B. By targeting key genes that are part of the self-renewal associated signaling pathways, it could be possible to reduce aberrant stem cell renewal in CML. Residual cells are not able to support cancer and undergo apoptosis or differentiation. This strategy may prevent drug resistance and disease recurrence associated with imatinib treatment of CML.

CRITICAL SELF-RENEWAL ASSOCIATED SIGNALING PATHWAYS IN CML STEM CELLS

Since both normal hematopoietic stem cells and CML stem cells are capable of self-renewing, it is not surprising that several signaling pathways which are involved in the regulation of normal stem cells may play significant roles in cancer stem cell biology. It has been shown that BCR-ABL promotes self-renewal of leukemic stem cells because all critical signaling pathways involved in the maintenance of survival of leukemic stem cells can be activated by BCR-ABL [51]. However, BCR-ABL oncogene cannot confer self-renewal capacity to committed progenitors to transform them and therefore rather utilizes and enhances the self-renewal properties inherent in existing self-renewing cells (i.e., hematopoietic stem cells) [52,53]. Here we review molecular pathways involved in the regulation of self-renewal properties in leukemic stem cells, and discuss their implication in regulating leukemic stem cell functions in CML. These self-renewal associated signaling pathways include Wnt/β-catenin, Hedgehog, Alox5, Foxo, and others.

WNT/β-CATENIN SIGNALING PATHWAY

The Wnt/β-catenin signaling pathway is involved in self-renewal of both normal hematopoietic stem cells and CML stem cells [54-57]. In blast crisis CML patients, β-catenin is activated in myeloid progenitors and the leukemic stem cells, which resemble granulocyte macrophage-progenitors (GMPs) have aberrant activation of β-catenin via canonical Wnt signaling pathway [58].

Using a β-catenin-deficient mice model of CML, Zhao et al [59] demonstrated dependence of in vivo myeloid leukemia progression on β-catenin. They were able to show that loss of β-catenin impairs self-renewal of CML stem cells in vivo and subsequently reduces in vivo progression of CML, whereas it allows normal development of acute lymphoblastic leukemia (ALL). As CML is thought to be initiated in hematopoietic stem cells and ALL may be initiated in committed progenitors, these findings proposed that β-catenin is required for BCR-ABL-induced leukemias that originate in stem cells. Another study led by Hu et al [60] showed that β-catenin is essential for the maintenance of leukemic stem cells resistant to tyrosine kinase inhibition in mice model of CML. Delayed development of CML due to loss of β-catenin is attributed to a reduced ability of BCR-ABL to support long-term self-renewal property of leukemic stem cells. Overall, these findings suggest that Wnt signaling could be considered as a promising therapeutic target for curing CML.

HEDGEHOG SIGNALING PATHWAY

Hedgehog (Hh) signaling is a highly conserved developmental pathway which regulates self-renewal of normal hematopoietic stem cells and CML stem cells [61-64]. Hh signaling is triggered by the binding of the Hh protein ligands (Sonic hedgehog [Shh], Indian hedgehog [Ihh], and Desert Hh [Dhh]) to the 12 transmembrane receptor patched (PTCH), which is a negative regulator of the seven transmembrane receptor smoothened (Smo). Upon ligand binding, the inhibitory effect on Smo is relived. This signaling event leads to the induction of Gli transcription factors, which promotes transcription of Hh-responsive genes such as Gli1, Ptch1, cyclin D1, and Bcl-2 [65-69].

Hh signaling has been proven as a functional pathway for leukemic stem cells, and loss of this pathway impairs CML progression [38,70,71]. Dierks et al [38] indicated that Smo, which is specifically upregulated in BCR-ABL-positive cells, is essential for the expansion of the leukemic stem cell pool. While lack of Smo had no effect on long-term reconstitution of normal hematopoiesis, the absence of Smo expression

effectively reduced the development of BCR-ABL-positive leukemias in mice. Moreover, pharmacological inhibition of Hh signaling had no effect on regular hematopoiesis but reduced leukemic stem cells in vivo and enhanced time to relapse of the disease. Supporting the previous study, Zhao et al [71] have shown that the loss of Smo impaired hematopoietic stem cell renewal and decreased induction of CML by the BCR-ABL oncoprotein. Loss of Smo caused depletion of CML stem cells, whereas overexpression of Smo led to an increased percentage of CML stem cells and accelerates the progression of disease. As a possible mechanism, the inhibitory effects of the Smo deletion on leukemic stem cells might be attributed to the regulation of the cell fate determinant Numb, because the Smo deletion leads to an increase in the expression levels of Numb, which depletes CML stem cells. Taken together, these findings suggest that Hh inhibition might be a promising therapeutic strategy to reduce the leukemic stem cell pool in drug resistant CML.

ALOX5 SIGNALING PATHWAY

The arachidonate 5-lipoxygenase (5-LO) gene (Alox5) has been shown to be involved in several physiological and pathological process including oxidative stress response, inflammation, and cancer [72-74]. Alox5 function has been implicated in several critical signaling pathways such as p53 [72], NF-kB [75], and PI3k [75]. It has been also found that Alox5 is a critical regulator for leukemic stem cells and its function is essential for the induction of CML by BCR-ABL.

Transcriptional analysis-based studies have shown that Alox5 is differentially expressed in human CD34+ CML cells compared to their normal counterparts, proposing a possible role of Alox5 in CML stem cells [76]. A microarray analysis of gene expression in leukemic stem cells in mice model of CML indicated that the Alox5 gene was upregulated by BCR-ABL and this upregulation was not abolished by BCR-ABL kinase inhibitors [77]. This at least partially explains why imatinib had no effect on leukemic stem cells in mice model of CML [45]. It has been shown that the Alox5 deficiency impaired the function of leukemic stem cells through affecting differentiation, cell division, and survival of leukemic stem cells, consequently causing a gradual depletion of leukemic stem cells. This explains why BCR-ABL failed to induce CML in mice in the absence of Alox5 [72,74,77-79]. Importantly, the lack of Aox5 had no significant effect on the function of normal hematopoietic stem cells, suggesting a possible mechanism for how hematopoietic stem cells and leukemic stem cells distinctly self-renew and differentiate [77,80]. Altogether, these findings proposed that Alox5 could be a specific target gene in CML stem cells for developing curative therapies.

FOXO SIGNALING PATHWAY

Forkhead box class O (Foxo) transcription factors are involved in the maintenance of normal hematopoietic stem cells and CML stem cells. There are four members (Foxo1, Foxo3a, Foxo4, and Foxo6), all of which are functional downstream targets of phosphatidylinositol 3 kinase (PI3K)-AKT pathway [81-83]. In the absence of stimulation by growth factors or insulin, Foxos localize to the nucleus and activate their transcriptional targets regulating oxidative stress responses, cell cycle progression, and apoptosis [84-86]. Upon growth factor or insulin binds to the cell surface receptor, AKT is activated and directly phosphorylates Foxo members, leading to the exclusion of Foxo members from the nucleus and suppression of Foxo transcriptional activity. In the cytoplasm, Foxo members are degraded [87-89].

Tothova et al [82] demonstrated that Foxos are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress and that triple conditional deletion of Foxo1, Foxo3a, and Foxo4 in mice leads to a significant increase in the hematopoietic stem cell population. Several studies showed that Foxo3a alone is essential to maintenance the self-renewal capacity of normal hematopoietic stem cells and CML stem cells [83,90,91]. High levels of Akt phosphorylation and cytoplasmic localization of Foxo3a have been found in non-CML stem cells. In contrast, cells with low levels of Akt phosphorylation and

nuclear localization of Foxo3a were enriched in the CML stem cell population [90]. Foxo3a deficient mice showed a reduction of leukemic stem cells after serial transplantation in BCR-ABL driven CML mouse model. This was associated with decreased ability of leukemic stem cells to promote disease in vivo. These results suggest that Foxo3a is essential for long-term maintenance of leukemia-initiating potential in CML stem cells [90] and that targeting Foxo transcription factors might be a potential therapeutic approach to eradicate leukemic stem cells in CML.

THE NECESSITY OF TARGETING MOLECULAR PATHWAYS IN CML STEM CELLS

Since both hematopoietic stem cells and CML stem cells may share many survival signaling pathways, elucidation of the hematopoietic developmental stage of survival gene expression will be important for developing therapies which specifically target cancer stem cells while sparing normal stem cells. Moreover, by targeting key genes that are part of the self-renewal associated signaling pathways, it could be possible to reduce aberrant stem cell renewal in CML. This strategy may prevent drug resistance and disease recurrence associated with imatinib treatment of CML [45,92-96].

MICRORNA

MicroRNAs (miRNAs or miRs) are a novel class of small non-coding regulatory RNAs which control gene expression at the post-transcriptional levels. The official miRNA database miRBase lists that up to 30% of human genome is controlled by miRNAs. There is currently intensive research aimed at identifying all miRNAs, their target mRNAs and their biological functions [97-99]. MiRNAs have been shown to become involved in a variety of biological processes such as cellular proliferation, differentiation, apoptosis, and maintenance of stem cell potency [97,100-103]. Certain miRNAs have been shown to be aberrantly expressed human cancers and a large body of evidence points to their critical roles as oncogenes and tumor suppressors in the development of various human malignancies including leukemias [104-108]. Moreover, it seems that aberrant expression of certain miRNAs results in dysregulation of stem cell genes which, in turn, causes an increase in self-renewal potential of cancer stem cells [109,110]. Understanding the biological functions of miRNAs will require the identification of their multiple targets and the pathways that they control.

BIOGENESIS, PROCESSING AND FUNCTION OF MICRORNAS

MiRNAs are first transcribed by RNA polymerase II or III as primary miRNAs (pri-miRNAs), which are RNA hairpin structures up to several thousand nucleotides in length. In the nucleus, pri-miRNAs are processed by the "microprocessor complex" which consists of RNase III endonuclease Drosha bound to accessory protein DiGeorge syndrome critical region 8 (DGCR8). This converts pri-miRNAs to intermediate stem-loop structures approximately 70 nucleotides long called "precursor miRNAs" (pre-miRNAs) [111,112]. Following nuclear processing, pre-miRNAs are exported to the cytoplasm by exportin-5 in a GTP-dependent manner. The pre-miRNAs are subsequently processed by the cytoplasmic RNase III endonuclease Dicer, releasing double-stranded mature miRNAs. The strand which becomes the mature miRNA is the one whose 5' end is more unstable, thus more easily unwind by the helicase. Another strand is released and subsequently degraded in the cytoplasm. Mature miRNA is incorporated into the RNA-induced silencing complex (RISC), the effector of RNA interference (RNAi) pathway [99,100,113].

The RISC-miRNA complex typically recognizes and targets the 3'-untranslated region (3'-UTR) of specific mRNAs bearing a perfectly complementary target site for degradation or can repress the translation of an mRNA that contains several partly mismatched target sites [114]. Recently, however, evidence indicates that miRNAs can also regulate gene expression through binding "seedless" 3'-UTR miRNA recognition elements [115] or by binding to sites

located within the coding regions of transcript [116].

MICRORNA INVOLVEMENT IN CML

MicroRNA involvement in CML presents an additional layer of complexity to understanding the development and progression of the disease. The involvement of miRNAs in the regulation of several cellular processes altered in CML, such as cell cycle, apoptosis, and adhesion [107], establishes these small RNA molecules as potential players in pathogenesis of CML. Functional analysis of individual miRNA is necessary to understand altered cellular process. The first evidence for the involvement of miRNAs in hematologic malignancies was described in chronic lymphocytic leukemia (CLL) [117]. Subsequent miRNA expression profiling studies based on sequencing [118,119], microarray [117,120], and quantitative real-time PCR [120-122] revealed miRNA signatures characterizing ALL, acute promyelocytic leukemia (APL), and acute myeloid leukemia (AML) associated with various cytogenetic abnormalities [123-125]. Information about the involvement of miRNAs in CML pathogenesis is restricted to the description of downregulation of certain miRNAs (miR-15a, miR-16-1, miR-155, miR-181, miR-221, and let-7a) in the CML cell line k562 [104,126,127] and upregulation of miR-17-92 in CML patients [128].

Venturini et al [128] were able to show that expression of the polycistronic miR-17-92 cluster which is transcriptionally regulated by c-MYC, are increased in CML CD34+ cells from patients in chronic phase but not in blast crisis compared with normal CD34+ cells, suggesting a BCR-ABL-c-MYC-miR-17-92 pathway in CML cells. Agirre et al [126] identified an abnormal miRNA expression profile in mononuclear and CD34+ cells from patients with CML compared with healthy controls. Expression analysis of 157 miRNAs in patients with newly diagnosed CML revealed that miR-10a, miR-150, and miR-151 were downregulated, whereas miR-96 was upregulated in CML cells. Interestingly, this study showed that downregulation of miR-10a was not dependent on BCR-ABL activity and resulted in the increased upstream stimulator factor 2 transcription factor (USF2)-mediated cell growth of CML cells, supporting the potential role of a miRNA in the abnormal behavior of CML.

MiR-15a and miR-16-1, two p53-induced miRNAs with tumor suppressive activity, negatively regulate expression of the anti-apoptotic proto-oncogene Bcl-2 at the posttranscriptional level, inducing apoptosis in leukemic cells [129]. It has been shown that Bcl-2 overexpression results in the prevention of apoptosis which, in turn, leads to an increase in number and repopulation potential of stem cells in vivo [130,131]. Therefore, it seems that apoptosis has a possible role in regulating the microenvironments of stem cells. This highlights the importance of the Bcl-2 signaling pathway for the survival of stem cells and suggests a possible role for miR-15a/16-1 in regulating apoptosis in stem cells.

It seems that miRNA antagonists, referred as "antagomiRs", can block the oncogenic properties of the miRNAs and miRNA mimics can restore the expression levels of miRNAs with tumor suppressive activity [132-138]. Therefore, miRNAs could be considered as promising therapeutic targets in addressing cancer stem cell dysregulation.

CONCLUSION AND FUTURE PERSPECTIVES

The identification of key genes that are part of self-renewal signaling pathways in CML stem cells provides new opportunities in the future of leukemia therapy. As aberrant expressions of miRNAs are associated with cancer stem cell dysregulation, it seems that miRNA-based molecular leukemia therapy eliminates the self-renewal capabilities of CML stem cells. Obviously, further investigations are needed to gain more insights into the therapeutic potential of miRNAs against cancer progression, resistance, and relapse.

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