Chronic myeloid leukemia (CML) is typified by robust marrow and extramedullary myeloid cell production. In the absence of therapy or sometimes despite it, CML has a propensity to progress from a relatively well tolerated chronic phase to an almost uniformly fatal blast crisis phase. The discovery of the Philadelphia chromosome followed by identification of its BCR-ABL fusion gene product and the resultant constitutively active P210 BCR-ABL tyrosine kinase, prompted the unraveling of the molecular pathogenesis of CML. Ground-breaking research demonstrating that BCR-ABL was necessary and sufficient to initiate chronic phase CML provided the rationale for targeted therapy. However, regardless of greatly reduced mortality rates with BCR-ABL targeted therapy, most patients harbor quiescent CML stem cells that may be a reservoir for disease progression to blast crisis. While the hematopoietic stem cell (HSC) origin of CML was first suggested over 30 years ago, only recently have the HSC and progenitor cell–specific effects of the molecular mutations that drive CML been investigated. This has provided the impetus for investigating the genetic and epigenetic events governing HSC and progenitor cell resistance to therapy and their role in disease progression. Accumulating evidence suggests that the acquired BCR-ABL mutation initiates chronic phase CML and results in aberrant stem cell differentiation and survival. This eventually leads to the production of an expanded progenitor population that aberrantly acquires self-renewal capacity resulting in leukemia stem cell (LSC) generation and blast crisis transformation. Therapeutic recalcitrance of blast crisis CML provides the rationale for targeting the molecular pathways that drive aberrant progenitor differentiation, survival and self-renewal earlier in disease before LSC predominate.

Introduction

In 1960, Nowell and Hungerford described a small G group chromosome, the Philadelphia chromosome. The constitutively active tyrosine kinase product of the Philadelphia chromosome, P210 BCR-ABL, provided a pathogenetic explanation for the initiation of chronic phase chronic myelogenous leukemia (CML) as well as a critical molecular therapeutic target. These landmark discoveries together with the finding that chronic phase CML originates from a HSC fueled research into the genetic and epigenetic events driving aberrant differentiation, self-renewal and survival of stem and progenitor cells in CML. This review will examine the differences between normal and CML stem and progenitor cells as well as potential future clinical efforts to redirect CML stem and progenitor cells toward a normal developmental path.

Chronic Myeloid Leukemia Stem Cells

In CML and other malignancies, compelling research suggests that a population of cancer stem cells (CSC) is able to regenerate or self-renew resulting in therapeutic resistance and disease progression. CSC have been described in a number of cancers including acute myelogenous leukemia (AML), breast cancer, brain tumors, colon cancer, pancreatic cancer, as well as CML. A number of studies indicate that quiescent CSC, particularly in CML, are resistant to therapies that target rapidly dividing cells. Because CML has the propensity to evolve from a chronic phase to accelerated phase and blast crisis, one can pinpoint the phase and developmental stage at which mutations arise that promote blastic transformation. Research revealing the relative quiescence and resultant therapeutic resistance of primitive CML progenitor cells provided a framework for identifying the hematopoietic developmental stage of chronic phase initiating events, such as altered stem cell differentiation and survival, compared with molecular events that promote progression to blast crisis, such as aberrant acquisition of self-renewal capacity by committed progenitors. Hence, CML is an important paradigm for understanding both genetic and epigenetic events that drive aberrant stem and progenitor cell differentiation, self-renewal and survival during both early and advanced phases of disease.

Because CML was the first cancer to be associated with a pathognomonic chromosomal translocation called the Philadelphia chromosome that juxtaposes the c-abl gene on chromosome 9 and the bcr gene on chromosome 22 as t(9;22)(q34;q11), it has been the most extensively investigated cancer from a molecular standpoint. The fusion gene product of the Philadelphia chromosome, BCR-ABL, produces a constitutively active protein tyrosine kinase. The malignant transforming capacity of BCR-ABL was initially documented through its ability to support factor-independent growth of primary bone marrow cells in...
vitro. The first in vivo CML model involved transplantation of BCR-ABL–transduced mouse bone marrow cells into lethally irradiated syngeneic recipients.6,8,9 Retroviral overexpression of BCR-ABL was necessary and sufficient to induce a myeloproliferative disorder similar to chronic phase CML but rarely recapitulated all aspects of blast crisis phase, underscoring the complexity of malignant transformation.8,9 Nonetheless, these models provided an important step forward in developing targeted BCR-ABL inhibitor therapy that has induced hematologic and cytogenetic remissions in the majority of patients with chronic phase CML.11-20

However, BCR-ABL inhibition with currently available agents such as imatinib, dasatinib, nilotinib or SKI-606 rarely eliminates quiescent CML stem cells in chronic phase, a problem that is compounded by the inevitable progression of CML in patients who are diagnosed in advanced stages of disease.23-33 Notably, seminal studies demonstrated that a quiescent population of CML stem cells (CD34+CD38–CD45RA–CD71–HLA-DRlo) with BCR-ABL kinase domain mutations, detectable prior to initiation of imatinib therapy, gives rise to leukemic cells that persist because they are inherently resistant to imatinib.20,23-33 Moreover, the presence of BCR-ABL kinase domain mutations portended a greater risk of loss of complete cytogenetic remission (CCR).33 Other research revealed that quiescent CML stem cells have high BCR-ABL transcript levels.20,31 However, even 20-fold more potent BCR-ABL inhibition with nilotinib did not induce apoptosis of quiescent CD34+ CML cells nor did inhibition with a dual SRC-ABL kinase inhibitor, SKI-606 rarely eliminates quiescent CML stem cells in chronic phase.27,30,31 These findings suggest that BCR-ABL kinase mutations in primitive CML progenitors that are still detectable in patients in CCR on BCR-ABL inhibitor therapy contribute to CML persistence and may contribute to disease progression.27,33 Moreover, cumulative evidence indicates that aberrant differentiation, self-renewal program activation and enhanced survival triggered at an inappropriate developmental stage lead to accumulation of more mature and typically short-lived myeloid progenitors resulting in blast transformation.20,21

CML Stem and Progenitor Cell Differentiation

Although there is a relative dearth of gene expression profiling data comparing purified HSC (CD34+CD38–CD90+Lin–) and progenitor (CD34+CD38–Lin–) cells from different phases of CML, a number of studies have been performed on the CD34+ population that is composed primarily of progenitor cells and a less frequent HSC population.34-42 Genes that regulate cell-fate decisions in HSC and have been implicated in myeloid leukemogenesis, such as MEIS1 and HOXA9, were found to be overexpressed. Moreover, untreated chronic phase CML progenitors had upregulated expression of megakaryocyte-erythrocyte progenitor pool.20 In addition, quiescent chronic phase CD34+ progenitors increased expression of a number of chemokines associated with stem cell mobilization.38

In blast crisis CML progenitors, the characteristic block in myeloid differentiation appears to result, at least in part, from BCR-ABL and MAPK (ERK1/2)–induced hnRNP-E2 RNA-binding protein-mediated suppression of C/EBPα expression.36 Other research suggested that the imatinib and nilotinib resistance that derives, in part, from autocrine granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion in response to adaptive JAK2-STAT5 signaling in granulocyte-macrophage progenitors (GMPs), may be overcome with a JAK2 inhibitor.42 In addition to cell autonomous effects, results derived from a tetracycline-off mouse model revealed that an essential component of the stem cell niche, osteopontin, is upregulated in BCR-ABL–positive cells.43 Similarly, osteopontin levels are higher in the serum of patients with CML and may help to support the malignant clone at extramedullary sites.33,44

Progression of CML is associated with transcription factor–induced aberrant lineage priming of stem and progenitor cells. A notable insight into the role of transcription factor deregulation in CML stem cell differentiation was gained when a deficiency of a transcription factor, JunB, in a transgenic mouse model lead to the development of a CML-like disease with a propensity for myeloid differentiation in which serial transplantation potential existed only at the level of HSC.56,57 Moreover, GM-CSF–mediated survival and proliferation of the JunB-deficient GMP was associated with changes in anti-apoptotic proteins Bcl2 and Bclx, as well as cell cycle regulators p16ink4a and c-Jun.57 Emerging evidence suggests that a transcriptional repressor Bmi1 that is normally restricted to the stem-cell compartment is overexpressed in the aggressive forms of CML that progress to blast crisis within 3 years and during advanced phases of disease.36 In addition, GATA-2, a key myeloid transcription factor, is upregulated in CML CD34+ cells. Notably, stem cell fate determination by the large family of Hox genes is negatively regulated by Bmi1.58,59 Some Hox family members such as HoxA9 and HoxB4 as well as the Hox gene master regulator MLL are better known for transforming potential through enhanced HSC self-renewal and myelopoiesis skewing upon upregulation.59,60 Downregulation of Hox genes, such as HoxA5, impairs myelopoiesis, suggesting differentiation block at the level of hematopoietic stem cells. While, inactivation of HoxA4 and HoxA5 through hypermethylation has been detected in 34% of CD34+ cells from patients with chronic phase CML, it occurs in 90% of the samples from patients with CD34+ CML in blast crisis.60 Moreover, inactivation of HoxA4 and HoxA5 through hypermethylation is associated with a poor prognosis in other myeloid and lymphoid malignancies and may be a marker of more severe disease.60
A genetically defined mouse model of blast crisis CML demonstrates that coincident overexpression of genes that skew differentiation including BCR-ABL and NUP98/HOXA resulted in the production of an imatinib-resistant LSC population.37,41

CML Progenitor Self-renewal

Wnt/β-catenin pathway

Self-renewal is an essential stem cell property, but self-renewal pathway activation has also been increasingly recognized as a hallmark of cancer.20,21,45-57 Activation of the Wnt/β-catenin pathway, important in HSC self-renewal, was detected in samples from patients with CML in blast crisis by confocal fluorescence microscopic analysis.30 Additional studies revealed that inappropriate activation of the Wnt-signaling pathway in GMPs endowed these cells with self-renewal capacity, normally a property of HSC, as measured by generation of replatable myeloid colonies. Replacing efficiency was reduced with axin—a potent inhibitor of β-catenin.30 Self-renewal capacity is normally absent in GMP, suggesting that they have acquired a key property of a leukemia stem cell. Recently, xenogeneic transplantation of the blast crisis GMP population into highly immunocompromised mice demonstrated serial leukemia transplantation potential, suggesting that the GMP population is enriched for leukemia stem cells (Abrahamsson et al, submitted). In some cases β-catenin activation was associated with deregulation of GSK3β, an essential negative regulator of β-catenin, in both stem and progenitor cells, while other patients exhibited defective expression of another negative regulator of the pathway—axin 2 (Abrahamsson et al, submitted). Seminal work performed by Radich and colleagues comparing microarray data from CD34+ cells across the disease spectrum identified several critical differentially expressed genes that function in self-renewal, differentiation, survival, and DNA-damage response.61 The Wnt pathway, critical for HSC self-renewal and interaction with bone marrow niche, was found to be activated during progression to advanced phase. This study linked the abnormality in the free β-catenin pool to decreased JunB expression through the deregulation of MDFI (I-mfa—an inhibitor of myogenic basic helix-loop-helix transcription factors).61 The importance of β-catenin activation in myeloid blast crisis transformation was underscored by research demonstrating that progression to myeloid blast crisis was averted in a β-catenin knockout mouse model of CML.62 Notch-1 deregulation has been reported to induce acute leukemia in a P210 BCR-ABL mouse model.63 Finally, aberrant activation of another self-renewal program, sonic hedgehog, was recently shown to induce expansion of leukemia stem cells in a mouse model of CML, while another report suggested that there may be cross-talk between sonic hedgehog, Wnt and notch pathways in CML.64,65 Together these studies suggest that disruption of differentiation and enhancement of self-renewal in CML progenitor cells may be a critical component of disease progression.

CML stem cell survival

Deregulation of programmed cell death or apoptosis allows cancer stem cells to propagate even as they detach from the niche and accumulate genetic mutations. In CML, resistance to apoptosis begins with BCR-ABL, and notably, the final consequence of BCR-ABL inhibition with imatinib is induction of apoptosis.66 CML stem cell resistance to apoptosis involves the aberrant expression of the Bcl-2 family of apoptosis-regulatory proteins including anti-apoptotic members such as Bcl-2 and Mcl-1 and pro-apoptotic members such as Bad and Bim.57,82 The anti-apoptotic members inhibit mitochondrial release of cytochrome c and subsequent Apaf1 activation resulting in inhibition of caspase 9 activation. Initiation of CML by BCR-ABL leads to resistance to apoptosis.67-83 Moreover, BCR-ABL-induced PI3K activation of AKT results in downstream inhibition of Bad, a pro-apoptotic protein.63 In addition, BCR-ABL-induced STAT5 activation leads to increased Bal-xl expression, an anti-apoptotic protein.66-70 Finally, BCR-ABL expression increases with disease progression and enhances resistance to apoptosis by promoting increased bcl-2 expression.71,80 Notably, transgenic CML mouse model research showed that targeted BCR-ABL expression in myeloid progenitors led to a chronic MPD, while targeted overexpression of Bcl-2, but not Raps or My, promoted progression to blast crisis.81 In addition, activation of the Wnt/β-catenin pathway leads to increased c-myc expression and ultimately results in upregulation of Bcl-2 family proteins. Moreover, GSK3β, a critical negative regulator of the Wnt-signaling pathway, has been shown to regulate the activity of both pro-apoptotic and anti-apoptotic Bcl-2 family proteins. GSK3β can activate Bax, a membrane pore-forming Bcl-2 family member, by phosphorylation at serine 163 and can also regulate the stability of Mcl-1, one of the anti-apoptotic family members. Inhibition of GSK3β via the induction of glucose metabolism leads to stabilized Mcl-1 and resistance to apoptosis.82

Key apoptosis proteins are also highly regulated by the PI3K/Akt pathway.82 In response to metabolic, survival, and growth signals, PI3K activates Akt, which then acts on a number of downstream targets. Akt directly inhibits Bad and Bax and modulates the activity of transcription factors such as those from the NFκB and FoxO families.83,84 NFκB protein activation leads to its nuclear localization, where it then activates the transcription of a number of pro-survival molecules including Bcl-2, Bcl-xl, and various caspase inhibitors.83 The importance of these transcription factors in CML has been highlighted by evidence that NFκB is
activated in transgenic models of CML and that BCR-ABL can activate NFκB.84-86 FoxO transcription factors, on the other hand, are targeted for proteolysis via Akt-mediated signaling.87 These factors normally induce the transcription of pro-death molecules including Bim and FasL. In CML, BCR-ABL causes constitutive repression of FoxO3a by continued activation of Akt leading to yet another mechanism of BCR-ABL-mediated apoptosis resistance.87,88 Finally, Akt inhibits GSK3β activity. Thus, the Akt pathway broadly modulates apoptosis and, via regulation of GSK3β, is perhaps intrinsically linked to the Wnt pathway in the deregulation of apoptosis in CML. Although many survival pathways are upregulated in CML, they are also active in normal stem cells. Thus, elucidation of the hematopoietic developmental stage of survival gene expression will be critical for devising therapies that effectively eradicate CML stem cells while sparing normal stem cells.

### Anti-leukemic stem cell therapy

Although allogeneic hematopoietic cell transplantation is the only known curative therapy for CML, transplant-related morbidity from graft-versus-host disease and mortality rates of 10% to 20% have greatly reduced its use since the advent of molecularly targeted therapy.89 The relative dearth of toxicity with BCR-ABL–targeted inhibitors, such as imatinib, and prolonged responses observed in chronic phase patients have significantly decreased morbidity and mortality rates, thereby revolutionizing therapy for CML.12-19 While a hematologic response is observed in over 95% of patients in chronic phase, the major molecular responses defined by a three-log decline of the BCR-ABL transcript, detected by reverse-transcriptase polymerase chain reaction (RT-PCR), have been sustained in less than 5% of patients. Patients in advanced phases of disease initially respond to single-agent tyrosine kinase inhibitor (TKI) but inevitably relapse with treatment-refractory disease because, in addition to BCR-ABL amplification or kinase domain mutations, they have acquired other mutations.12-19 This has provided the impetus for developing sensitive diagnostic assays for early detection of both genetic and epigenetic events that drive progression and may be amenable to targeted therapy.

The precise sequence of molecular events leading to recalcitrance to BCR-ABL inhibitor therapy as well as the cellular framework in which they occur has not been completely elucidated. Seminal studies have demonstrated persistence of quiescent CML stem cells that are impervious to BCR-ABL inhibitor therapy and contribute to disease progression.23-31 However, the diagnostic, prognostic and therapeutic relevance of the epigenetic and genetic mechanisms driving leukemic stem cell self-renewal, survival and aberrant differentiation in patients with advanced phase CML still needs to be investigated prospectively in the context of clinical trials.

Nonetheless, accumulating evidence regarding the cell type and context specific effects of mutations responsible for therapeutic resistance and disease progression suggests that combined therapy targeting the skewed proliferation, survival, differentiation and self-renewal of CML progenitors may benefit patients who do not sustain a molecular remission within a year of starting targeted BCR-ABL inhibitor therapy or who are diagnosed with advanced phase disease at presentation. Molecular mechanisms that promote CML progenitor survival such as bcl-2; self-renewal such as sonic hedgehog, bmi-1, notch and Wnt; and block differentiation such as JAK2/STAT5 activation and HOX gene hypermethylation may be targeted effectively using combinations of small molecule inhibitors and demethylating agents.90-101 Notably, a plethora of bcl-2 family member inhibitors are currently under development, JAK2/STAT5 inhibitors are currently being tested in clinical trials and self-renewal pathway inhibitors such as gamma-secretase inhibitors have been used clinically albeit with gastrointestinal toxicity. Although Wnt and sonic hedgehog inhibitors are still being evaluated in preclinical models, they hold great promise clinically for eradicating committed progenitors that have aberrantly gained self-renewal capacity as a result of oncogenic addiction to one of these pathways. With our increasing ability to enrich for leukemia stem cells combined with recent improvements in both transgenic mouse models and xenogeneic transplantation technology, it is currently possible to assess leukemia stem cell sensitivity to combinations of established and novel inhibitors in robust preclinical models. Moreover, implementation of strategies to augment both innate and adaptive immune responses against CML progenitors may accelerate disease eradication.102-103 Clinical trials involving highly active anti-leukemia stem cell therapy combining BCR-ABL inhibition and self-renewal pathway inhibitors with or without additional survival pathway antagonists may halt progression by eradicating the LSC population that represents a reservoir for relapse and progression to blast crisis.

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