Blasts from the past: New lessons in stem cell biology from chronic myelogenous leukemia

Cancer can be viewed as a hierarchical system that is dependent on a small population of "cancer stem cells" with unlimited self-renewal potential for continued growth and propagation of tumors. The identity and nature of these cells remains enigmatic, but an improved understanding of their biology may allow for selective therapeutic targeting. A recent report by Jamieson et al. (2004) sheds new light on leukemia stem cells by identifying the cells with in vitro self-renewing properties in various phases of chronic myelogenous leukemia, and linking the self-renewal properties of this population to activation of β-catenin, a major effector of the canonical Wnt signaling pathway.

Unlimited self-renewal is an absolute prerequisite for any malignancy, and is the ultimate arbiter of the continuous growth and metastasis of tumors. It has been suggested that the self-renewal properties of a tumor are exclusively contained within a small population of "cancer stem cells" (Reya et al., 2001). Like normal adult somatic stem cells, these cells are purported to have capacity for self-renewal, and through a process of asymmetric cell division give rise to committed progenitors that comprise the bulk of the tumor, but lack the ability to self-renew. Populations of cancer stem cells, delineated by differential expression of surface markers or by functional properties, have recently been demonstrated in several cancers, including acute leukemias (Bonnet and Dick, 1997), breast cancer (Al-Hajj et al., 2003), and CNS tumors (Singh et al., 2003).

The cancer stem cell model may explain why many chemotherapeutic regimens result in dramatic reductions in tumor burden, but relapse of disease remains one of the most significant problems in cancer therapy. Current chemotherapeutics may target only the bulk, nonclonogenic tumor cells and spare the cancer stem cells, allowing for recrudescence of tumor following cessation of therapy. Thus, an improved understanding of cancer stem cells may allow for the development of more effective therapies that target this critical population.

What is the origin of the cancer stem cell? Some data suggests that they originate from normal adult somatic stem cells in which a transforming event has occurred. For example, human leukemia stem cells (LSC) are immunophenotypically similar to their tissue-specific counterpart, the pluripotent hematopoietic stem cell (HSC) in NOD-SCID mouse transplantation models (Bonnet and Dick, 1997). In addition, cancer-specific chromosomal abnormalities can be detected in stem cells, and tumor cells and stem cells express similar levels of telomerase. The inherent self-renewal properties of adult stem cells, accompanied by their longevity, may also predispose to acquisition of the minimum number of mutations required for malignant transformation. However, it is also possible that committed progenitor cells could be a source of cancer stem cells if the transforming event itself contributes properties of self-renewal.

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder that arises in the hematopoietic stem cell compartment and whose molecular signature is the *BCR-ABL1*

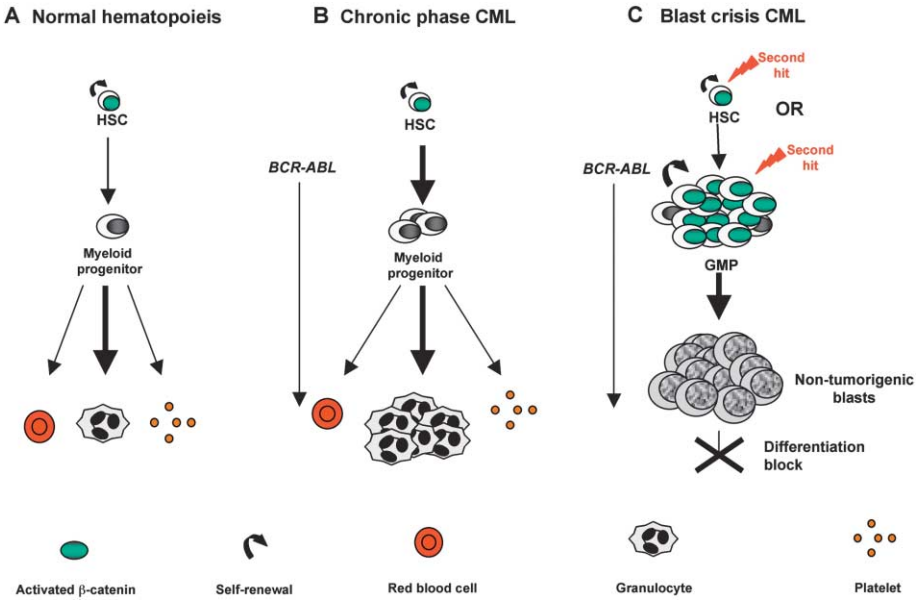

Figure 1. Comparison of stem cell ontogeny between normal hematopoiesis, chronic phase, and blast phase CML

A: In normal hematopoiesis, the hematopoietic stem cell (HSC) is the only cell which demonstrates activated β-catenin and self-renewal properties. In subsequent development, a combination of intrinsic and extrinsic signals ensures adequate production of mature effector cells.

B: In chronic phase CML, the leukemic stem cell (LSC) also resides within the HSC compartment. Similarly to its normal counterpart, it is the only cell to demonstrate activated β-catenin and self-renewal properties, a process for which BCR-ABL appears dispensable. However, activation of numerous signaling pathways by BCR-ABL in progenitor populations directs an expansion of these compartments and a subsequent expansion of terminally differentiated neutrophils in the peripheral blood.

C: In blast crisis (BC) CML, a second mutation results in self-renewal properties within the GMP compartment, presumably dependent upon β-catenin activation. This second mutation may occur within the HSC compartment, but as has been demonstrated prospectively in mouse models, may also occur within the more committed GMP progenitor population. In contrast to chronic phase CML, *BCR-ABL* appears to contribute to the self-renewal properties of this compartment, and in cooperation with activated β-catenin and possibly other mutations that block differentiation and lead to the development of an acute leukemia.

gene rearrangement. BCR-ABL1 is a constitutively activated protein tyrosine kinase that transforms hematopoietic cells through the activation of multiple signaling pathways (Deininger et al., 2000). The stem cell nature of CML is confirmed by demonstration of the BCR-ABL rearrangement in multiple mature hematopoietic lineages, and by the potential to generate acute leukemias of various lineages upon blast transformation (Deininger et al., 2000). CML is characterized by step-wise progression, driven by the accumulation of additional mutations (Deininger et al., 2000). In the initial chronic phase (CP), the myeloid compartment is expanded, but cells retain their capacity to differentiate and function normally, and drug treatment is usually effective. Progression to blast crisis (BC) is characterized by a loss of differentiation capacity and refractoriness to therapy.

The development of imatinib mesylate (Gleevec) as a selective small molecule inhibitor of the BCR-ABL1 kinase represents a dramatic advance in treatment of CML, and has been a harbinger for effective therapy of solid tumors using kinase inhibitors. Imatinib therapy of CML results in hematological and cytogenetic responses in CP and some BC patients. However, these successes have been tempered by the development of resistance due to ABL kinase mutations, the transient response to imatinib in most BC patients, continued detection of the BCR-ABL transcript at the molecular level in most patients treated with imatinib, and the finding of quiescent LSC in CML that are resistant to imatinib (Melo et al., 2003), indicating that additional therapies will be necessary to cure CML.

Jamieson and colleagues have shed new light on leukemia stem cells (LSC) by studying patients with CML (Jamieson et al., 2004). They provide evidence for a dynamic LSC compartment during disease progression of CML, demonstrate that self-renewal programs can apparently be reactivated in committed hematopoietic progenitors, and annotate a role for interaction between BCR-ABL1 and the WNT signaling pathway in sustaining self-renewal. In this elegant study, distinct populations of hematopoietic progenitors were prospectively isolated by multiparameter flow-sorting (Manz et al., 2002) from patients with differing phases of CML. Surprisingly, the size of the HSC compartment was the same in all phases of CML, whereas there was progressive expansion of the myeloid progenitor compartment with disease progression. In BC patients, there was an increase in the size of the granulocyte-macrophage compartment (GMP), and subsequent analysis demonstrated that these GMPs had properties of self-renewal in vitro, as assessed in serial replating assays. Although additional work is needed to characterize the stem cell properties of this population, these data indicate that the GMP population, normally lacking any capacity for self-renewal, may acquire certain properties of leukemic stem cells with disease progression (Figure 1).

Based on data from this same group showing that WNT signaling is important for normal HSC self-renewal (Reya et al., 2003), the role of β-catenin, the major effector of this pathway, in the self-renewal of these populations was examined. Nuclear colocalization of β-catenin detected by immunofluorescence and an in vivo lentiviral LEF/TCF reporter assay were used as surrogates for WNT pathway activation. In CP CML, β-catenin activity was restricted to the HSC fraction, but in patients with BC, β-catenin activation was detected in the GMP population. A direct association between β-catenin activation and self-renewal was supported by the ability of lentiviral transfer of β-catenin to increase the in vitro replating ability of normal GMP, and by inhibition of serial replating of BC GMP by transfer of the β-catenin inhibitor Axin. In addition, in CP CML, BCR-ABL transcripts were more numerous in stem cells than progenitors, but this pattern was reversed with progression to BC. Taken together with the demonstration of a decrease in β-catenin activation in the GMP of BC patients treated with imatinib, these data suggest a link between BCR-ABL kinase activity and self-renewal in BC CML.

These findings have interesting implications for the origin and organization of the LSC compartment. First, they suggest that the LSC compartment either changes, or operates on two tiers with progression of CML (Figure 1). Second, they demonstrate that oncogenic mutations in humans may confer properties of self-renewal to cells that lack these properties. Similar findings have been reported by the Weissman/Cleary groups in murine models of leukemia induced by the MLL-ENL fusion (Cozzio et al., 2003), and we have made similar observations with the MOZ-TIF2 fusion oncogene (unpublished observations). However, a recent report suggests that there may be added complexity in the interpretation of these findings. Lentiviral marking of human AML cells, followed by serial transplantation into NOD-SCID mice, identified LSCs with differing capacity for long term self-renewal (Hope et al., 2004). These data are consistent with maintenance of a normal HSC hierarchy in the LSC compartment, as suggested by the authors. However the work of Jamieson et al. raises the possibility that ongoing acquisition of second mutations that confer properties of self-renewal to leukemic progenitors could also contribute to such a hierarchy.

There are several important avenues for further investigation, including rigorous characterization of the stem cell properties of CML GMPs and determining how broadly applicable these findings are among all CML patients and in other malignancies. These data provide further impetus to dissect the WNT signaling pathway in hematopoietic and in solid tumors, and raise the possibility of therapeutic targeting of the WNT pathway in cancer. The observation that progenitors may acquire an ability to self-renew is fascinating, and provides support in humans for previous observations in murine models of disease suggesting that certain leukemia oncogenes can reprogram cells for self renewal potential. These in turn may be useful tools for understanding the transcriptional programs that control self-renewal. In particular, the data suggesting an interaction between BCR-ABL1 and WNT signaling to confer properties of self-renewal in GMP should be pursued. The capacity of an "old" disease such as CML to educate us about diverse topics in cancer biology, including the role of chromosomal translocations in malignancy, molecularly targeted therapy of kinase-driven cancers, and now leukemic stem cell ontogeny, continues to amaze.

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