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Lessons Learned from the Study of JunB: New Insights for Normal and Leukemia Stem Cell Biology

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JunB is important in maintaining normal hematopoietic stem cell functions, but the mechanisms underlying its activity are not well understood. In the current issue of *Cancer Cell*, a study by Santaguida et al. provides new insights into JunB's function and the genesis of myeloid disease.

Previous studies have indicated a role for JunB in the maintenance and preservation of normal hematopoietic stem cell (HSC) functions (Passegué et al., 2001, 2004). In this issue of *Cancer Cell*, Santaguida et al. (2009) now provide a detailed cellular and molecular analysis of *jun*B-deficient stem cells, which indicates that JunB functions to limit both cell-cycle entry and myeloid differentiation. Furthermore, in the course of their work, the investigators demonstrate some important limitations to the utility and interpretation of transplantation-based stem cell functional assays. Thus, their study provides not only new information about JunB but also some key considerations for analyses of HSCs.

Nearly 30 years ago, David Harrison first coined the term "competitive repopulation" to describe the assay that has since become the gold standard for functional analysis of HSCs (Harrison, 1980). Although variations on the method have emerged in recent years, the basic principle remains unchanged. By comparing the engraftment potential of an unknown source of HSCs to a known source of HSCs (in the same transplanted recipient animal), one can quantitatively estimate the frequency of stem cells in the unknown fraction. Experimentally, one transplants different ratios of HSCs from an unknown source and HSCs from a known source into appropriately conditioned recipient animals, typically by mixing different numbers of cells from an unknown source with a fixed number of cells from a known source (competitor). Using phenotypically distinct populations of known and unknown cells provides a simple means by which to determine the relative contribution of each stem cell source to repopulation of the hematopoietic system. A major advantage of this method is that it does not require purification of HSCs but rather can be performed using bulk populations, generally derived from bone marrow. Because only HSCs durably persist in vivo, while other more mature cell types turn over relatively quickly, by simply waiting an appropriate period of time, the constituent cells in a repopulated host are known to have derived from the stem cell source.

The competitive repopulation method has been used by countless investigators to assess the relative frequency and functional properties of HSCs and is arguably the most valuable tool researchers have for determining the in vivo potential of stem cell populations. Naturally, as molecular genetic methods have become more prevalent in recent years, virtually every mutation or genetic change relevant to HSCs has been investigated using this approach (Zon, 2008). However, as shown by Santaguida et al. in this issue, results from this assay can be more difficult to interpret than previously appreciated. In characterizing the properties of HSCs, these investigators initially observed reduced functional capacity of *jun*B-deficient cells in standard competitive repopulation assays. Such findings would generally be viewed as clear evidence that the activity of JunB is required for some basic stem cell function, such as self-renewal or homing. However, to their credit, the investigators delved much deeper into this problem and performed a detailed series of studies to better understand the nature of *jun*B deficiency. In so doing, they discovered that highly enriched populations of HSCs (defined using the so-called "SLAM" markers) (Kiel et al., 2005) are in fact entirely normal in *jun*B-deficient animals, but their presence is essentially masked by an overproduction of myeloid progenitors (Figure 1). Indeed, the relative abundance of progenitors, and even quiescent cell types within the primitive compartments, appeared to result in dilution of true long-term repopulating HSCs (LT-HSCs) when the overall marrow population was evaluated on a per cell basis. Collectively, the data indicate that loss of JunB does not impair the functional properties of LT-HSCs but rather skews early developmental programs such that their relative frequency is altered. In addition, these studies clearly demonstrate the importance of using highly enriched populations of LT-HSCs for competitive repopulation studies and suggest that it may be necessary to reevaluate findings from previous reports in which HSC functions were characterized using nonpurified cell populations (Zon, 2008).

Aside from developmental aberrancies in *jun*B-deficient HSCs, Santaguida et al. also found a striking increase in proliferation rates. In comparison to normal cells, almost double the proportion of *jun*B-deficient LT-HSCs underwent cell-cycle entry each day, suggesting that JunB functions to limit cell-cycle entry in primitive populations. Gene expression analyses revealed that *jun*B-deficient LT-HSCs had increased expression of cyclins and decreased expression of cyclin-dependent kinase inhibitors, suggesting that the absence of JunB induces otherwise quiescent cells to enter the cell cycle at a higher frequency. In many previous

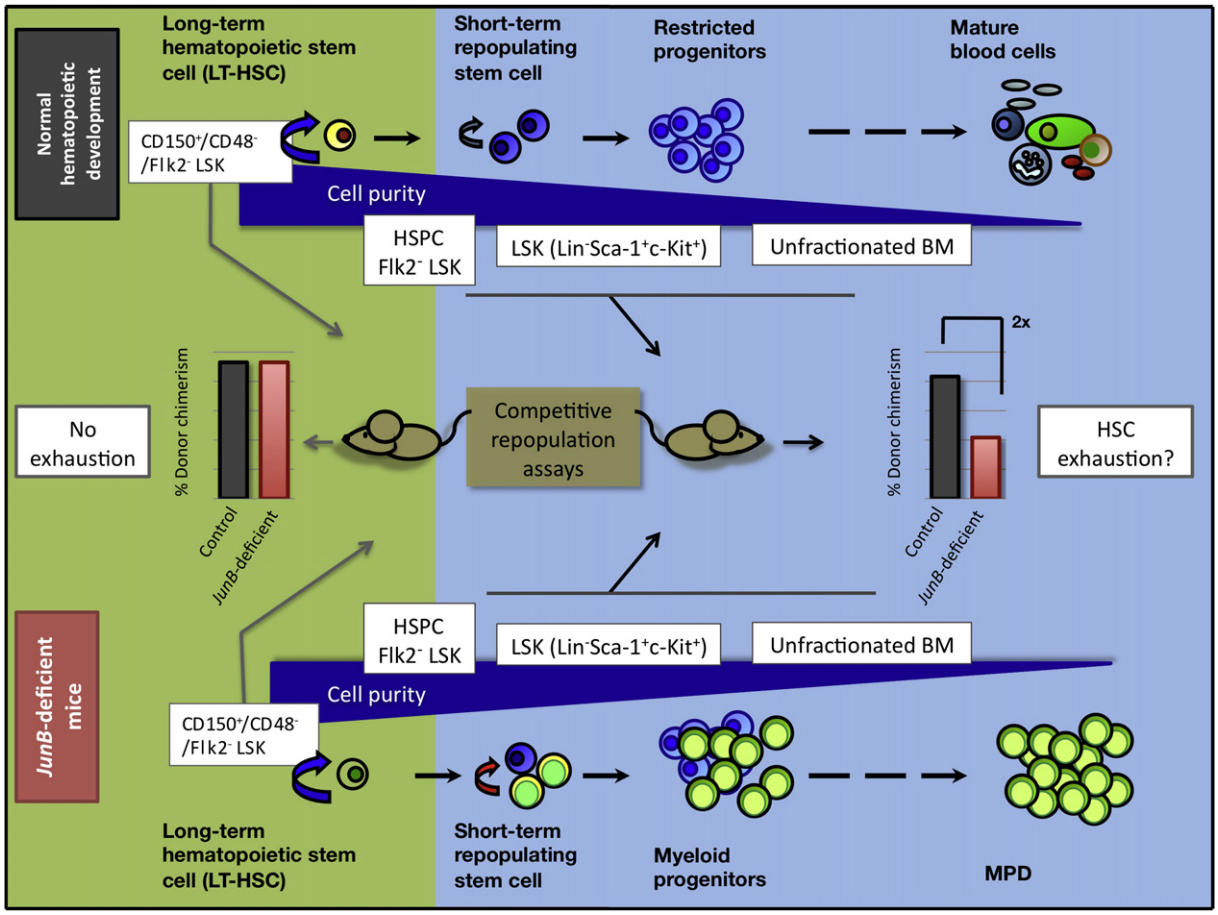

Figure 1. Competitive Repopulation Assays and Hematopoietic Stem Cell Frequency

Studies in normal hematopoietic cells (top) are typically performed with either unfractionated bone marrow (BM) or enriched stem cell populations (blue panel at right). In perturbed genetic systems, such as *jun*B-deficient mice (bottom), higher proliferation and/or skewed differentiation cause an increased pool of myeloid progenitors. The accumulation of such cells can lead to erroneous conclusions when nonpurified populations are used for the assay (blue panel at right). Using the same assays with highly purified LT-HSC populations (green panel at left), Santaguida et al. (2009) revealed that there are no functional differences in the LT-HSCs of *jun*B-deficient mice in comparison to wild-type controls. HSPC, hematopoietic stem and progenitor cell; MPD, myeloproliferative disease.

studies, it has been reported that mutations causing increased cycle rate typically result in so-called stem cell "exhaustion" (Miller and Van Zant, 2006). However, as shown by Santaguida et al., *jun*B-deficient LT-HSCs have regenerative potential similar to control stem cells, with the only notable difference being higher levels of myeloid reconstitution. This surprising finding contradicts the long-standing notion that cycle activation/entry of HSCs is associated with loss of their functional potential (Quesenberry et al., 2007).

Given the observations noted above, the challenge for these investigators was to provide a mechanism explaining how JunB could simultaneously orchestrate the increases in cell-cycle rate and myelopoiesis while maintaining stem cell functions. To this end, their molecular studies led to the identification of two important JunB-mediated signaling pathways that appear to regulate homeostasis of the hematopoietic system. They found that Notch and TGF-β pathways inhibit the differentiation of LT-HSCs to myeloid progenitors by acting through a common target, Hes1, the expression of which is dependent on JunB. Therefore, *jun*B-deficient cells cannot respond to Notch and TGF-β signals that limit cycle rate and myeloid differentiation. These findings provide important insight into possible early stages of the leukemogenic process. Indeed, it is attractive to speculate that the first steps of leukemic transformation involve very primitive cells undergoing mutations that cause loss of control over growth and differentiation. The resulting increase in cycle rate and myelopoiesis would then lead over time to myeloproliferative disease. Presumably, further genetic or epigenetic alterations in the aberrantly accumulating clones could then give rise to a fully malignant leukemic stem cell that would be sufficient to produce and maintain acute leukemia.

Taken together, the studies by Santaguida et al. have provided a valuable lesson on the interpretation of classical stem cell biology assays in genetically modified systems, described new insights into the role of JunB in HSC biology, and provided exciting clues as to the early pathogenesis of leukemia stem cells.

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