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Complexity of the Human Acute Myeloid Leukemia Stem Cell Compartment: Implications for Therapy

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INTRODUCTION

Emerging evidence suggests cancer stem cells (CSCs) sustain neoplasms; however, little is understood of the healthy cell initially targeted and the resultant CSCs [1-3]. The most advanced understanding comes from the hematologic malignancies because of the availability of quantitative functional assays for normal stem cells (hematopoietic stem cells; HSCs) and progenitor cells (colony-forming cells), as well as for leukemic stem cells (LSCs) and progenitor cells (acute myeloid leukemia [AML]-colony-forming cells). Advances in the ability to identify the biological properties of individual human HSCs and LSCs by using retroviral-mediated clonal tracking coupled with the nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mouse xenotransplantation assay have been critical to progress [4-6]. These studies demonstrated that LSCs are not functionally homogeneous but, like the normal HSC compartment, are composed of distinct hierarchically arranged LSC classes. Thus, the AML clone is organized as a hierarchy that originates from human SCID leukemia-initiating cells (SL-ICs), which produce AML colony-forming units (AML-CFUs) and leukemic blasts. Moreover, similarities between SL-ICs and normal HSCs support a hypothesis that the target cell of origin of AML LSCs often lies within the normal stem cell compartment, although, as noted below, under some circumstances LSCs could arise through the acquisition of additional mutations in downstream progenitors [1,3].

LSCs hold the key to understanding the origin and maintenance of AML and possess biological properties that are different from the bulk of the leukemic clones; this makes them difficult to eradicate. Thus, elucidation of these LSC-specific properties will aid in the development of more effective therapy that can be targeted to the most primitive LSCs.

NORMAL HSCs

The mammalian hematopoietic system is a hierarchy derived from stem cells that possess extensive self-renewal, proliferative, and differentiative capacity. HSCs maintain the hematopoietic system throughout life, and stem cell regulation is a critical element in the control of normal hematopoiesis. HSCs can be conclusively examined only by in vivo repopulation. We have used repopulation of immune-deficient mice to develop a quantitative assay for human stem cells that have been termed SCID-repopulating cells (SRCs). A detailed characterization of SRCs is emerging in terms of frequency, cell-surface phenotype, and cytokine responsiveness [3,7]. To understand the composition of the human HSC compartment, we tracked the in vivo fate of individual SRCs during repopulation of NOD/SCID mice by analysis of the unique clonal markers that were introduced with retroviral vectors [4,5]. The vector integration site provides a marker that is stably inherited by all progeny of an active stem cell. Analysis of serial bone marrow aspirations from NOD/SCID mice transplanted with transduced cord blood demonstrated that the repopulation was oligoclonal, with extensive variability in self-renewal capacity, as well as in the life span and proliferative capacity of individual SRC. Some clones contributed only for several weeks after the transplantation and disappeared, whereas others appeared later and persisted. Secondary repopulation experiments demonstrated that there was heterogeneity in the self-renewal capacity of the trans-

duced SRCs. These data point to the existence of different classes of human stem cells with short-term (ST) and long-term (LT) repopulating capacity (ST-SRCs and LT-SRCs).

LEUKEMIA STEM CELLS

Although cancer is often regarded as overproliferation of neoplastic cells that do not differentiate properly, it has been known for more than a century that solid tumors and leukemias exhibit heterogeneity of cellular morphology. However, morphology and function are difficult to link, and consequently little is known of the cells that maintain the neoplasm. The concept that only a minor subpopulation of so-called CSCs is responsible for maintenance of the neoplasm emerged approximately 50 years ago. The best evidence comes from the discovery that the vast majority of AML blasts do not proliferate, and only a minor proportion (approximately 1%) of human leukemic cells are clonogenic progenitors (AML-CFU) [3]. However, parallel studies of normal clonogenic progenitors showed that most are not repopulating HSCs, thus calling into question whether AML-CFU is a true LSC. Conclusive evidence for the existence of LSCs came from our identification of a very rare population of human SL-ICs (1 per 10^6 leukemic blasts) that were capable of propagating AML in a xenograft transplant system we developed for LSCs and normal stem cells [8]. In this system, SL-ICs generated leukemic grafts that were highly representative of the original patient's disease: they had identical blast morphology and dissemination profiles. Cell purification, based on cell-surface markers that enrich for normal stem and progenitor cells, demonstrated that SL-ICs from a large number of AML samples were highly enriched in the $CD34^+CD38^-$ fraction and could not be found in any other fraction, including $CD34^+CD38^+$ cells [8-11]. Because AML-CFUs are contained in the $CD34^+CD38^+$ fraction, these studies provided functional proof that the AML clone is organized as a hierarchy that originates from SL-ICs, which produce AML-CFUs and leukemic blasts [8,9,12]. Normal HSCs, as measured by repopulation of NOD/SCID mice, are also highly enriched in the $Lin^-CD34^+CD38^-$ fraction. We hypothesized that the similar cell-surface phenotype between cell fractions highly enriched for normal HSCs and SL-ICs from AML samples, derived from a wide diversity of leukemic subtypes in terms of their differentiation properties, indicated that AML originated from the HSC pool as opposed to the committed progenitor pool. However, one limitation of this comparison is that the leukemogenic process disrupts cell differentiation, thus making a direct link based on cell-surface markers between LSCs and a representative stage of normal hematopoietic development tenuous. Indeed, some differences between normal HSC and LSC surface-marker expression have been identified [13-16]. Thus, the only comparison that is reliable must be based on functional stem cell properties.

The essential feature common to all stem cells is the capacity for self-renewal upon cell division, producing at least 1 daughter cell that possesses stem cell properties identical to those of the parent [17]. The progressive loss of self-renewal capacity is the likely mechanism underlying the existence of multipotential murine HSCs with ST and LT repopulation capacity. If LSCs and HSCs are related as we predict, the LSC pool should be similarly complex; they should possess self-renewal machinery and the ability to also regulate self-renewal to create a hierarchical structure.

To determine whether SL-ICs are a homogenous population of LSCs in which each member possesses an equivalent repopulating function or whether there is functional heterogeneity, we undertook a clonal tracking approach as outlined previously. We found that some clones contributed transiently, whereas others were LT and stable, thus indicating that SL-ICs are in fact heterogeneous and that the entire pool is composed of different classes of ST and LT SL-ICs [6]. The mechanism that underlies this heterogeneity is variation of the self-renewal capacity of each SL-IC type. The self-renewal capacity was determined by performing serial transplantations and assessing whether the clone persisted or disappeared. Some SL-ICs persisted in secondary and tertiary mice, and this provided conclusive proof for the self-renewal of a CSC. In addition, some LT SL-ICs generated a transient graft in secondary mice, thus indicating that ST SL-ICs derive from LT SL-ICs. The fact that both LSC and normal HSC compartments are structured as a hierarchy as a consequence of progressive loss in self-renewal capacity provides strong support for the hypothesis that in AML, the initial target cell for transformation lies within the HSC compartment [3]. Of course, because leukemogenesis is a multistep process, the additional "hits" that are required could arise in these abnormal, but "preleukemic" stem cells or in more downstream progenitors to result in a fully transformed LSC. These data show that the leukemogenic program does not abolish all the pathways that regulate normal hematopoiesis at the stem cell level. Thus, the intrinsic self-renewal capacity, as well as the decline in self-renewal capacity (ie, regulation of self-renewal) due to commitment processes, of HSCs targeted by the initial leukemogenic event(s) continues to function in the resultant LSC. Indeed, the recent finding that the stem cell–specific gene *Bmi-1* plays a key role in the self-renewal of both normal and leukemic murine stem cells supports this idea [18,19].

The discovery of functional complexity in the LSC compartment has critical implications for the

investigation of cancer-specific signaling pathways and the development of stem cell–targeted AML therapies. Because cancer pathways may function differently within each LSC subclass compared with the bulk leukemic blasts, differential responses to a given therapy may result. Effective therapy must target the highly self-renewing LT SL-ICs within a functionally heterogeneous SL-IC pool that is responsible for aggressively driving the growth and relapse of AML. Current AML therapies typically target proliferating cells; however, SL-ICs are quiescent, which makes them poorly responsive to such agents. Future therapies may be more successful if they target the altered self-renewal machinery of LSCs to more specifically eradicate the LSCs.

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