

# Acute Myeloid Leukemia Stem Cells

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**ABSTRACT:** A fundamental problem in cancer research is identification of the cells within a tumor that sustain the growth of the neoplastic clone. The concept that only a subpopulation of rare cancer stem cells (CSCs) is responsible for maintenance of the neoplasm emerged nearly 50 years ago; however, conclusive proof for the existence of a CSC was obtained only relatively recently. The evidence for the existence of CSCs was first derived from the study of human acute myeloid leukemia (AML), largely because of the availability of quantitative stem cell assays for the leukemic stem cell (LSC). These studies showed that only rare cells within the leukemic clone had the capacity to initiate AML growth after transplant into NOD/SCID mice, establishing the hierarchical organization of AML. Recent clonal-tracking studies showed that the LSC compartment is composed of different classes of LSCs, which can be distinguished on the basis of self-renewal potential. These findings have important implications for our understanding of the leukemogenic process as well as the design of more effective therapies to eliminate AML based on eradication of the LSCs. These studies are briefly reviewed here.

**KEYWORDS:** cancer stem cells; leukemic stem cells; hematopoietic stem cells

## INTRODUCTION

Our understanding of the leukemogenic disease process has, to a large extent, been formed from many decades of research on human subjects involving characterization of the cellular phenotype of acute leukemia and other aspects of the clinical picture. One of the major difficulties with this approach is the limited ability for experimental intervention in human subjects. Moreover, it is almost impossible to gain insight into the early events of the leukemogenic process before they become clinically apparent. Until the last decade, most experimental approaches have involved the study of naturally occurring animal (mostly murine) leukemia and experimentally induced disease after transgenic or gene knockout methods. However, while many aspects of these murine leukemias recapitulate the human disease, there can be significant differences with the human disease. Moreover, marked differences in genomic stability between humans and inbred mice strains suggest that the

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leukemogenic process might be subtly different. Ultimately, one would like to complement murine experiments with model systems that use human leukemia to ensure that they are relevant to the human situation and that therapies based on this knowledge will have a higher likelihood of efficacy in humans. The transplantation of normal and leukemic human cells into immune-deficient mice provides such a system.

## EXISTENCE OF DIFFERENT CLASSES OF HUMAN STEM CELLS

The hematopoietic hierarchy originates from hematopoietic stem cells (HSCs) that possess extensive proliferation and differentiation potential, which in turn produce clonogenic precursors that ultimately produce mature cells. Unique to the most primitive HSCs is the self-renewal potential, a property that enables life-long blood production. Progress to study human HSCs has lagged until our development of the NOD/SCID xenotransplant assay, a method that has revolutionized the study of both normal and leukemic human hematopoiesis (reviewed in Refs. 1 and 2). A detailed insight of the biology of human HSCs (SCID-repopulating cells, SRCs) is emerging in terms of frequency, cell surface phenotype, cytokine responsiveness, cell cycle kinetics, homing, and their relationship to colony-forming cells (CFCs) and long-term culture-initiating cells (LTC-ICs).<sup>1</sup> Clonal tracking and cell purification have revealed the existence of short- and long-term repopulating-capacity SRCs (ST-SRCs and LT-SRCs).<sup>3,4</sup> We and others have also demonstrated heterogeneous repopulation potential of different HSC subsets discriminated by Lin<sup>-</sup>CD34<sup>+</sup>CD38<sup>-</sup> (SRC), Lin<sup>-</sup>CD34<sup>+</sup>CD38<sup>low</sup> (ST-SRC), and Lin<sup>-</sup>CD34<sup>+</sup>CD38<sup>hi</sup> (no SRC, progenitors only).<sup>5-7</sup> These studies form the basis to purify and then identify the molecular factors regulating the developmental fate of each HSC class, and ultimately to determine how HSCs decide, on cell division, to generate daughter cells that self-renew (i.e., retain parental HSC properties) or that commit to differentiation pathways.

## LEUKEMIC STEM CELLS

Transplantation of human acute myelogenous leukemia (AML) and acute lymphocytic leukemia (ALL) into NOD/SCID mice generates a disease in the mice that recapitulates the human disease.<sup>1</sup> Morphologically, leukemia reflects abnormal development in one of the major blood lineages, but the blasts from different patients are heterogeneous with respect to the lineage antigens they express. Although our studies established leukemic stem cell (LSC) assays for both AML and ALL,<sup>8,9</sup> characterization of the LSCs is most advanced for AML, and there has been little characterization of ALL. The current reports on ALL-LSC are contradictory in that some studies suggest the ALL-LSC for pre-B cell lineage ALL is primitive and lacks expression of CD19,<sup>10</sup> while others suggest it is more committed and does express CD19.<sup>11</sup> Because of fundamental differences in developmental potential and because chromosomal rearrangements can mark the leukemic clone, it has been predicted that the ALL-LSC might be different from the AML-LSC.<sup>12</sup> For example, in contrast to the myeloid lineage, even mature lymphocytes possess self-renewal and express Bmi1.

For AML, the human AML-initiating cell—termed the SCID leukemia-initiating cell (SL-IC)—was identified and purified by transplantation into NOD/SCID mice.<sup>8</sup>

This represented the first direct identification of a stem cell from any cancer, providing direct evidence for the existence of cancer stem cells. The CD34<sup>+</sup>CD38<sup>-</sup> cell fraction that represents from 0.1 to 1% of the AML cell population contained all SL-ICs, whereas only clonogenic leukemia progenitors were found in other fractions. Upon transplantation of CD34<sup>+</sup>CD38<sup>-</sup> cells, the entire cellular diversity was recapitulated, conclusively establishing that leukemia is a hierarchy sustained by rare LSCs, closely resembling normal development. HSCs and SL-ICs are quiescent and share some similarities in cell surface phenotype,<sup>13</sup> although LSCs possess higher self-renewal.<sup>14</sup>

Although there are some differences in cell surface phenotype,<sup>13</sup> the similarity between normal and leukemic stem cells has been used as an argument for normal stem cells as being the “cells of origin.” However, comparisons based on cell surface phenotype are unreliable because the leukemogenic process may have altered the highly regulated expression of these markers. Thus it may not be possible to make accurate predictions of developmental stage by comparison with some normal stage of development. A functional comparison based on stem cell properties is more reliable. The recent data suggesting that the LSC pool, like the normal HSC compartment, is organized as a hierarchy of distinct stem cell classes with decreasing self-renewal capacity is the most compelling evidence to date of the stem cell origin of AML. Individual LSCs, detected by clonal marking, differ widely in self-renewal potential (creating LT- and ST-LSCs) demonstrating that the LSC pool is not homogeneous but still retains aspects of normal HSC hierarchical organization.<sup>15</sup> These data support a model in which the cellular targets for transformation are commonly HSCs, not progenitors or the bulk nonclonogenic blast population (detailed arguments reviewed in Refs. 1, 14, and 15). According to this model, heterogeneity of leukemic properties derive from variable differentiation of LSCs due to the direct influence of specific transformation or progression-related gene(s) and not on the degree of lineage commitment of targeted progenitors. This model predicts that LSCs and HSCs share many of the same properties that render them as stem cells, especially self-renewal potential. As noted below, the stem cell model of leukemogenesis was recently given a strong boost with the discovery that *Bmi1* is a key regulator of self-renewal in both LSCs and HSCs.<sup>16–18</sup>

These data point to self-renewal and the deregulation of self-renewal at the heart of the transformation process. Indeed, others had argued based on murine models that under some circumstances non-stem cells could be converted into LSCs if the correct self-renewal machinery were assembled in these cells through oncogenic processes.<sup>19,20</sup> It will be essential to establish whether this true for human leukemia using similar leukemogenesis assays and followed by *in vivo* leukemia read-outs in NOD/SCID mice.

## STEM CELL REGULATION

Little is known about the molecular program that defines human HSCs and controls their developmental program. When stem cells divide they have three choices: death, differentiation, or self-maintenance. Indeed, the latter property of self-renewal is the key feature that distinguishes HSCs from other hematopoietic cells. Genetic factors intrinsic to the HSCs randomly govern self-renewal and differentia-

tion decisions.<sup>21</sup> The identity of intrinsic factors and their upstream regulators is largely obscure, but evidence from other organisms and cell systems provides a starting point. Several molecules active during embryonic development, including wnt, bmp, shh, and notch family members, induce HSC self-renewal.<sup>22</sup> Several recent papers have demonstrated the important role that dysregulated wnt pathway signaling plays in leukemia and LSC biology.<sup>23–25</sup> HoxB4 was the first transcription factor that induced significant HSC expansion on overexpression in HSCs.<sup>26</sup> Recently, a polycomb group (Pc-G) family transcriptional repressor of homeo-box cluster genes, Bmi1, was found to play a key role in self-renewal.<sup>16,17</sup>

Bmi1<sup>-/-</sup> HSCs only transiently reconstitute primary recipients and possess no detectable self-renewal capacity in secondary transplants. Using an experimentally induced Hox/Meis leukemogenesis model, on a Bmi1<sup>-/-</sup> background the LSCs lost self-renewal capacity, indicating that Bmi1 is a key regulator of murine LSCs and HSCs self-renewal. Other studies have implicated the signal transducer and activator of transcription (STAT) pathway in self-renewal.<sup>27,28</sup> However, no studies have been done to examine the molecular regulators of human stem cells due to the absence of appropriate experimental systems. With the advent of reliable systems to overexpress genes as well as for gene silencing, it will be possible to directly test leukemogenic processes in primary human hematopoietic stem and progenitor cells.

## CANCER STEM CELLS IN SOLID TUMORS

Using LSC studies as a paradigm, recently cancer stem cells (CSCs) were identified in human breast cancer<sup>29</sup> and brain cancer.<sup>30</sup> In each case, cell sorting was done to isolate a minor fraction of the tumor that possessed all the CSC activity, while the remainder of the cells never showed tumor-initiating function, thus establishing that, like AML, solid tumor growth was driven from a minor fraction of the tumor tissue. Thus it may be that CSCs are at the apex of all neoplastic systems.

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