

Human Leukemia Stem Cells

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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 following transgenic or gene knock-out 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 leukemogenic process might be subtly different. Ultimately, one would like to complement murine experiments with model systems that utilize 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.

Two fundamental problems in cancer research are identification of the normal cell within which cancer initiates and identification of the cell type capable of sustaining the growth of the neoplastic clone. There is overwhelming evidence that virtually all cancers are clonal and represent the progeny of a single cell. What is less clear for most

cancers is which cells within the tumor clone possess tumor initiating or “cancer stem cell” (CSC) properties and are capable of maintaining tumor growth. The concept that only a minor subpopulation of so-called cancer stem cells (CSC) is responsible for maintenance of the neoplasm emerged about 50 years ago¹ with the best evidence coming from the hematological malignancies. Key to these studies is the depth of understanding of normal hematopoietic development that has been gained in the past 4 decades. Functional *in vitro* and *in vivo* assays are available for all stem and progenitor cell types ranging from the primitive pluripotential stem cells to multipotential and unipotential progenitors. In addition, a rich collection of cell surface differentiation markers enable detailed characterization of normal hematopoietic development, as well as providing insight into how normal differentiation becomes disrupted in leukemia. It is clear that leukemic tissues, while abnormal, still retain remnants of normal differentiation and developmental programs.

With the advent of clonogenic assays for normal hematopoietic progenitors, it became possible to determine that the vast majority of acute myeloid leukemia (AML) blasts do not proliferate and only a minor proportion (~1%) of human leukemic cells are clonogenic progenitors (AML-colony-forming units-AML-CFU). However, it was not known if AML-CFU represented true leukemic stem cells (LSC). Conclusive evidence for the existence of LSC came from our identification of human SCID Leukemia-Initiating Cells (SL-IC) that were capable of propagating acute myeloid leukemia in a xenograft transplant system we developed for leukemic and normal stem cells². These studies provided functional proof that the AML clone in humans

is organized as a hierarchy that originates from SL-IC, which produce AML-CFU and leukemic blasts. SL-IC could be purified based on the CD34⁺CD38⁻ cell surface phenotype and transplanted into NOD/SCID mice where they “differentiate” albeit abnormally into AML-CFU and leukemic blasts characteristic of the donor indicating that the leukemic clone is not characterized by blocked differentiation. AML could be serially transplanted into secondary mice demonstrating that SL-IC possessed extensive self-renewal capacity, a key determinant of stem cell function. Interestingly the cell surface phenotype of SL-IC showed significant similarities to normal human hematopoietic stem cells (HSC), suggesting that there maybe a relationship between these two stem cell populations and that the cell of origin of AML derives from the pool of normal HSC^{3,4}.

Normal hematopoietic stem cells

The mammalian hematopoietic system is a hierarchy derived from stem cells that possess extensive self-renewal, proliferative, and differentiative capacity. Hematopoietic stem cells (HSC) maintain the hematopoietic system throughout life, and stem cell regulation is a critical element in the control of normal hematopoiesis. HSC can only be conclusively examined 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 (SRC). A detailed characterization of SRC is emerging in terms of frequency, cell surface phenotype, and cytokine responsiveness². To understand the composition of the human hematopoietic stem cell compartment, we tracked the *in vivo* fate of individual SRC during repopulation of NOD/SCID mice by analysis of the unique clonal markers that were introduced with retroviral vectors⁵. The vector integration site provides a marker that is stably inherited by all progeny of an active stem cell. Analysis of serial BM aspirations from NOD/SCID mice transplanted with transduced cord blood (CB) demonstrated that the repopulation was oligoclonal with extensive variability in self-renewal capacity as well as in the lifespan and proliferative capacity of individual SRC. Some clones only contributed

for several weeks after the transplant and disappeared, while others appeared later and persisted. Secondary repopulation experiments demonstrated that there was heterogeneity in the self-renewal capacity of the transduced SRC. These data point to the existence of different classes of human stem cells with short- and long-term-repopulating capacity (ST- and LT-SRC, respectively).

Leukemia stem cells

In order to determine whether SL-IC represent a homogenous population of LSC where each member possesses equivalent repopulating function or whether there is functional heterogeneity, we undertook an clonal tracking approach as outlined above⁹. We found that some clones contributed transiently while others were long term and stable indicating that SL-IC are in fact heterogeneous and the entire pool is comprised of different classes of short term (ST) and long-term (LT) SL-IC. We found that 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 carrying out serial transplant and assessment of whether the clone persisted or disappeared. Some SL-IC persisted in secondary and tertiary mice providing conclusive proof for the self-renewal of a CSC. In addition, we found that some LT-SL-IC generated a transient graft in secondary mice indicating that ST-SL-IC derive from LT-SL-IC. 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^{3,4,6}. Of course since leukemogenesis is a multistep process, the additional “hits” that are required could arise in these abnormal, but “pre-leukemic” stem cells or in more downstream progenitors to result in a fully transformed LSC. Our data shows 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 (i.e. regulation of self renewal) due to commitment processes,

of HSC targeted by the initial leukemogenic event(s) continue 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 ^{7,8}.

Conclusions

Leukemic stem cells hold the key to understanding the origin and maintenance of AML and possess biological properties that are different from the bulk of the leukemic clone making 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 LSC. The paradigm we have developed to examine the complexity of the LSC compartment should provide a roadmap to examine similar questions about the properties of CSC from other cancers.

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