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Common Signaling Networks Characterize Leukemia-Initiating Cells in Acute Myeloid Leukemia

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Identification and characterization of leukemia-initiating cells (LICs) is important to understand leukemogenesis and develop novel therapies for leukemia. In this issue of *Cell Stem Cell*, Gibbs et al. (2012) demonstrate that common active signaling pathways in LICs may be targeted to treat acute myeloid leukemia.

Previous studies have demonstrated that CD34+CD38− hematopoietic cells, but not the more mature CD34+CD38+ progenitors, could generate leukemia in immunocompromised NOD/SCID mice (Bonnet and Dick, 1997). This observation supported the notion that leukemia was initiated and maintained in vivo by a small fraction of leukemia stem cells, or leukemia-initiating cells (LICs), derived from hematopoietic stem cells (HSCs). In recent years, several laboratories have shown that even other less primitive fractions of hematopoietic cells, for example CD34+CD38+ cells, can generate leukemia in more immunocompromised NSG mice, supporting the notion that LICs can also arise from hematopoietic progenitors (Bonnet and Dick, 1997; Dick, 2009).

In the current issue of *Cell Stem Cell*, Gibbs et al. (2012) use a mouse model for acute myeloid leukemia (AML) engineered by overexpression of HoxA9 and Meis1 homeobox genes important for self-renewal (Kroon et al., 1998). To identify the LICs by immunophenotype, the authors purified hematopoietic stem/progenitor cells, which express c-kit (Lin−kit+), kit+ cells coexpressing lymphoid markers (Lym+kit+) or myeloid markers (Gr1+kit+), and mature myeloid cells (Gr1+kit^{lo}) from HoxA9-Meis1 (H9M) mice. These purified fractions were then transplanted into irradiated wild-type mice to determine their tumorigenicity. All transplanted cell fractions generated leukemia in the recipients except the Gr1+kit^{lo} cells. These findings show that the LICs represent malignant hematopoietic progenitors that are immunophenotypically heterogeneous. Furthermore, each immunophenotype of LIC from primary leukemias could recapitulate all the LIC immunophenotypes in secondary recipients. In rigorous experiments, the authors plated single Lym+kit+ and Gr1+kit+ leukemic cells from primary recipients into clonal liquid cultures and transplanted single colonies from these cultures into irradiated recipients. Interestingly, single colonies derived from different immunophenotypic LICs generated all three LIC immunophenotypes. Since the mature myeloid cells (Gr1+kit^{lo}) do not generate leukemia, LICs comprise a distinct lineage hierarchy. However, since the Lym+kit+ and Gr1+kit+ cells could generate Lin−kit+ LIC, the hierarchy is atypical (see Figure 1).

Because the three distinct LIC populations seemed to share developmental lineage potential, the authors hypothesized that they may possess common cell signaling and genetic properties. To characterize the genetic signatures of these three different immunophenotypic LICs, the authors analyzed and compared microarray data from each LIC population. The genetic signatures were found to be very similar between all three LICs. Furthermore, mass cytometry was used to analyze intracellular signaling in each LIC following cytokine stimulation, and the findings demonstrated that all three distinct LICs have largely conserved signaling networks. They exhibited activation of MAP kinase, DNA methyltransferase, receptor tyrosine kinases, and PI3K/Akt. Inhibitors against two of these signaling pathways were tested in vivo and shown to prolong survival. These findings demonstrate that immunophenotypic analysis to identify LICs may have to be complemented by molecular analysis to identify epigenetic regulators and signal transduction pathways that are altered and shared across LICs.

In human leukemias, it has also been difficult to find exact immunophenotypic markers that characterize all LICs. For example, two recent studies found that LICs represented hematopoietic progenitors with several different immunophenotypes (Goardon et al., 2011; Sarry et al., 2011). Goardon et al. (2011) examined a large group of primary AML patients and found two different progenitor-like LICs: one multipotent population and another representing granulocyte-macrophage progenitors (GMPs). Their relationship was hierarchical because the multipotent population could generate the GMP-like LICs, but not the other way around. These findings are analogous to those in the present paper, with the exception that no strict hierarchical relationship was found between the different LIC populations in murine AML. It is interesting that in humans, different AML subtypes harboring disparate genetic mutations are similarly defined by only two populations of LICs with different immunophenotypes. Another recent study also reported progenitor-like LICs in primary AML with different immunophenotypes, some without expression of lineage markers (Lin−CD38−) and others expressing lineage markers CD38 or CD45RA (Sarry et al., 2011). Interestingly, the different immunophenotypic LICs could generate each other in vivo, similar to murine LICs in the present paper by Gibbs et al. (2012).

From the above findings, it is clear that identifying LICs based on one defined immunophenotype can be a challenge. First, there are many different leukemias with different histological subtypes caused by different genetic mutations. The AML studied by Gibbs et al. is more homogenous than the human leukemias
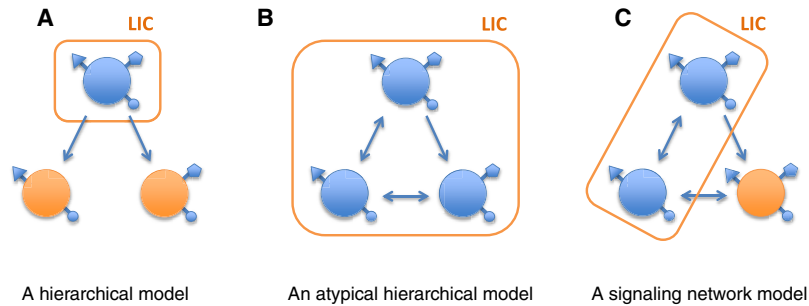
Figure 1. Different Models for Leukemia-Initiating Cells
(A) A classical hierarchical model: Leukemia-initiating ability is restricted to a small fraction of hematopoietic-stem-cell-like cells, defined by a particular immunophenotype, that generate progeny cells that lack tumor-initiating ability. (B) An atypical hierarchical model: Various types of hematopoietic-stem-cell-like or progenitor-like cells comprise leukemia-initiating cells (LICs), and these cells can independently generate other LICs with distinct immunophenotypes and therefore exhibit an atypical hierarchy. Hence it is not possible to identify LICs with a single immunophenotype (as shown in Gibbs et al., 2012). (C) A signaling network model: LICs with different immunophenotypes exhibit activation of common signaling pathways (as shown in Gibbs et al., 2012). Therefore, characterization of intrinsic signaling networks as well as immunophenotypic analysis will be needed for more effective identification of LICs. Please note that the models in (B) and (C) are not mutually exclusive. Cell bodies in blue are LICs, while the orange cells do not have leukemia-initiating activity.

because the initial mutation, overexpression of HoxA9-Meis1, is the same in all animals. Despite this, the authors find LICs with three different immunophenotypes. However, it is also possible that the authors did not find an immunophenotype that may be common to all LICs. The present findings and previously published papers report clearly that LICs are not restricted to HSCs, but can also be progenitor-like, provided that the LIC progenitors can undergo effective self-renewal. Expression profile analysis of LICs demonstrates that they have active self-renewal gene signatures, an important definition of LICs. The findings in Gibbs et al. (2012) present a novel and more functional definition of LICs, where common genetic and signal transduction pathways may characterize LICs better than the cell surface characteristics or

immunophenotype. While cell surface characteristics may not define LICs, recent studies have identified several cell surface markers that predict the ability of LICs to grow and metastasize. For example, antibodies against CD44 expressed on LICs could markedly deplete human LIC engrafted in NOD/SCID mice. Similarly, in HOXA10-Meis1 induced murine leukemia, prevention of secondary leukemias was achieved upon treatment of the recipients with antibodies against CD44 (Jin et al., 2006; Quéré et al., 2011). In the latter study, the expression of the CD44 gene was unaltered. However, increased protein levels of CD44 were discovered by screening the LICs by proteomics, indicating that expression profile analysis may not always be sufficient to find key molecules that are dysregulated in LICs (Quéré et al.,

2011). Therefore, in developing better therapies, it will be essential to determine the immunophenotypes that enable LICs to sustain growth of leukemic cells. However, as the findings of Gibbs et al. (2012) emphasize, in addition to immunophenotypes, LICs also have to be better characterized with regard to their functional properties. What cell surface molecules, signal transduction pathways, and epigenetic regulators allow these cells to self-renew and metastasize throughout the body, and how can we effectively target or block these pathways? The paper by Gibbs et al. (2012) has uncovered the first steps needed to answer these questions.

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