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Human Acute Myelogenous Leukemia Stem Cells Revisited - There's More Than Meets the Eye

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Abstract

In this issue of Cancer Cell, Goardon et al. revise earlier conclusions regarding acute myelogenous leukemia (AML) stem cells by demonstrating that in the majority of patients, they reside in two hierarchically related populations most similar to normal hematopoietic progenitors. These findings have implications for therapeutic targeting of these cells.

The cancer stem cell (CSC) model proposes that many human cancers are organized as cellular hierarchies initiated and maintained by self-renewing CSC. These CSC can be prospectively isolated based on their ability to serially transplant human cancer in immunodeficient mice. Ultimately, this model has numerous implications for both the cellular origin of cancer and cancer therapy.

CSCs were first enriched in human acute myelogenous leukemia (AML), an aggressive malignancy of immature hematopoietic cells in the bone marrow. Experiments by John Dick and colleagues originally demonstrated that the leukemia-initiating activity of primary human AML cells in immunodeficient mice was exclusively contained in the CD34+CD38- subpopulation of leukemia cells (Bonnet and Dick, 1997). As normal hematopoietic stem cells (HSC) share the CD34+CD38- immunophenotype, it was proposed that AML stem cells arise from HSC.

We had previously shown that HSC reside in the Lin-CD34+CD38-/loCD90+ fraction of normal bone marrow, and that CD34+CD38+ cells contained multiple progenitors. We tested the hypothesis that AML CSC were in the HSC compartment in patients with the t(8;21) AML1/ETO translocation, but found that while HSC contained the translocation and its transcripts, this fraction was non-leukemic; moreover, leukemia cells resided in an early CD34+CD38-CD90- progenitor, while leukemic blasts and CFU-L were CD34+CD38+ (Miyamoto et al., 2000). From these data and others investigating the myeloid blast crisis phase of chronic myelogenous leukemia (CML) (Jamieson et al., 2004), we proposed that leukemic progression occurred in self-renewing clones in the HSC compartment, while

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frank AML and myeloid blast crisis CML occurred in their clonal progeny at a non-HSC progenitor stage (Weissman, 2005).

Goardon et al. now revisit and revise these earlier conclusions through the investigation of a large number of primary human AML patient samples (Goardon et al., 2011). They found that the CD34+ cells in ~80% of these cases contained two predominant populations, one CD38-CD90-CD45RA+ and the other CD38+CD110+CD45RA+. Both of these corresponded to normal hematopoietic progenitor populations rather than HSC, with the normal CD38-CD90-CD45RA+ population of unknown function, and the CD38+CD110+CD45RA+ population representing granulocyte-monocyte progenitors (GMP). Significantly, the authors demonstrated that both of these populations from AML patients possess leukemia stem cell (LSC) activity based on their ability to serially transplant the disease in immunodeficient mice. These populations were hierarchically organized, whereby the CD38-CD45RA+ cells gave rise to the GMP-like cells, but not vice versa; moreover, the frequency of LSC was much higher in the CD38-CD45RA+ population compared to the GMP-like cells. Taken together, these results expand on the previous view of AML LSC and establish a hierarchy of populations with decreasing frequency of LSC.

The authors went further to investigate the normal counterparts of these LSC-containing populations. They demonstrate that the CD38-CD45RA+ and GMP-like leukemic populations were most like the corresponding normal progenitor populations rather than HSC based on microarray gene expression analysis. They then investigated the function of the previously uncharacterized CD38-CD45RA+ population and found that *in vitro*, it was able to produce granulocytes, monocytes/macrophages, and lymphocytes, but not erythroid cells or megakaryocytes. Based on these results, it was termed a lymphoid-primed multipotential progenitor (LMPP), the first description of such a population in human hematopoiesis. Unfortunately, as previously shown (Majeti et al., 2007), this immunophenotypic population failed to engraft in xenotransplantation assays, preventing definitive *in vivo* confirmation. In total, these data establish that most cases of CD34+ AML are primarily of hematopoietic progenitor origin, with at least two subpopulations containing LSC.

What accounts for the difference in LSC frequency and potential reported by Goardon et al. compared to the previous publications? One possibility is the use of different recipient mouse strains in the xenotransplantation assay. The earlier experiments identifying AML LSC exclusively in the CD34+CD38- population were conducted by xenotransplantation into NOD/SCID mice, whereas the current study used NOD/SCID/IL2Rγ-null mice. In addition to lacking mature B and T lymphocytes, the NOD/SCID/IL2Rγ-null mice also lack NK cells and other immune functions dependent on the IL2Rγ chain. As a result, these mice enable engraftment more robustly than the original NOD/SCID mice, which may result in detection of LSC activity in the CD34+CD38+ fraction.

In fact, the Bonnet lab has recently reported that CD34+CD38+ leukemia cells can exhibit AML engraftment potential in both NOD/SCID and NOD/SCID/IL2Rγ-null mice (Taussig et al., 2008). In the case of NOD/SCID mice, they showed that anti-CD38 antibodies inhibit engraftment of CD34+CD38+ leukemia cells through an Fc-dependent mechanism, and that when this mechanism was disrupted, these cells could then engraft. However, the situation becomes more complicated when considering another report from the same group investigating LSC identity in AML harboring nucleophosmin mutations where LSC activity was identified in the CD34- fraction in half the cases, and in both CD34- and CD34+ fractions in the others (Taussig et al., 2010). Ultimately, additional xenotransplantation experiments will be needed to investigate and clarify the identity of LSC in specific AML subtypes.

The new results reported by Goardon et al. implicate normal hematopoietic progenitors, LMPP and/or GMP, as the cell of origin for AML LSC in the majority of cases. A number of investigations of specific rare myeloid leukemia subtypes have hinted at a progenitor origin for LSC. For example, during myeloid blast crisis CML, GMP-like cells exhibit features of LSC (Jamieson et al., 2004). Additionally, a number of investigators have reported LSC activity in progenitor populations in mouse models of MLL-induced AML (Krivtsov et al., 2006).

One of the most important implications of a progenitor phenotype for AML LSC relates to the critical stem cell property of self-renewal. Since self-renewal is not a property of normal hematopoietic progenitors, being restricted to HSC, the acquisition of self-renewal ability in AML LSC is an aberrant event resulting from genetic and/or epigenetic changes. The identification of such events should yield novel candidate therapeutic targets, and analyses of the gene expression data reported by Goardon et al. would be a first step towards the identification of these LSC-specific genes and pathways.

The most significant implication of the leukemia stem cell model is that in order to eradicate the leukemia and cure the patient, all LSC must be eliminated. Development of novel methods to target LSC requires careful isolation and investigation of these cells. We recently demonstrated that both LSC and their progeny expressed CD47 and were eliminated with blocking anti-CD47 antibodies (Majeti et al., 2009). Moreover, we recently reported that high levels of an LSC gene expression signature were associated with worse clinical outcomes in several independent patient cohorts suggesting that the CSC model is clinically relevant for AML (Gentles et al., 2010).

The work reported by Goardon et al. is an essential step forward, but leaves several critical questions unanswered. First, in both the LMPP-like and GMP-like leukemic fractions, limiting dilution analysis indicates that LSC activity requires transplantation of many cells. Do LSC compose a very small fraction of these populations, and/or must subsets of these stem cells interact to support leukemia growth in vivo? Are there better surface markers aside from CD34 and CD38 that can be used to isolate these cells to much higher purity? Will surface markers alone be sufficient to isolate pure LSC? Second, the LSC hierarchy reported here does not apply to all AML cases. Can an LSC hierarchy be identified in the other cases? Will the identity of LSC vary amongst the known molecular and cytogenetic subtypes of AML? Finally, can methods be developed to track LSC during the course of therapy to provide guidance regarding therapy selection and/or intensification? Most significantly, will eradication of LSC ultimately prove to be curative?

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