Searching for leukemia stem cells—Not yet the end of the road?

Defining the characteristics of leukemia stem cells is critical in order to better understand both the genesis of leukemic disease and strategies by which such cells may be eradicated. In this issue of *Cancer Cell*, Somervaille and Cleary describe studies in which the properties of malignant stem cells are elucidated in a mouse model of leukemia induced by expression of the MLL-AF9 translocation. Biological features of leukemia stem cells in this system challenge previous thinking in several ways and suggest an unexpected degree of heterogeneity among stem cells in various forms of leukemia.

In 1994, a landmark study by Lapidot et al. first described the immunophenotype and isolation of stem cells from primary human acute myeloid leukemia (AML) specimens (Lapidot et al., 1994). From that study and several others over the next 6–7 years, a seemingly clear picture emerged—AML stem cells (AML-SCs) could be identified by a specific set of immunophenotypic markers. While many of these markers were shared with normal hematopoietic stem cells (HSCs), some differed as well. Nonetheless, a profile emerged that was apparently shared among the majority of AML-SCs and seemed to indicate that such cells are closely analogous to HSCs. Indeed, it was easy to imagine that oncogenic subversion of a normal stem cell would be the most logical manner in which an leukemia stem cell might arise.

Following the initial human studies, a more recent series of reports has exploited the power of mouse molecular genetics to model the stem cell biology of myeloid leukemia. Most of these studies have employed retroviral vectors to introduce specific oncogenic mutations into primitive hematopoietic cells. Experiments of this nature have shown that primary AML can be readily generated in the mouse, with pathological features that closely mimic human disease. As anticipated from the earlier human studies, expression of appropriate oncogenes in mouse HSCs leads to the formation of de novo AML (Huntly and Gilliland, 2005). Intriguingly, two recent reports indicate that expression of some oncogenes in more differentiated cell types is also sufficient to induce AML (Cozzio et al., 2003; Huntly et al., 2004). Specifically, those studies showed that expression of the MLL-ENL or MOZ-TIF2 translocations in either common myeloid progenitors (CMPs) or granulocyte-macrophage progenitors (GMPs) was able to cause AML, albeit at a lower efficiency than HSCs. Thus, the precedent has been formally established that the originating normal cell for AML in the mouse need not necessarily be a HSC.

In the current issue of *Cancer Cell*, a study by Somervaille and Cleary (2006) now takes the important next step of defining the immunophenotype and properties of leukemia stem cells in a mouse model of AML. Using retroviral transduction of normal hematopoietic cells with the MLL-AF9 translocation, the authors observe the expected development of AML and perform experiments to identify the subset of cells that can be functionally defined as AML-SCs. As is done for analyses of normal stem cells, establishing such functionality is accomplished by transplanting a small number of putative stem cells into secondary recipient animals and asking for regeneration of the overall (hierarchically organized) population. Surprisingly, their findings indicate that most AML-SCs are not found among the more phenotypically primitive cell types, but rather express both the Mac1 and Gr1 antigens, markers typically found on more mature myeloid cells. Interestingly, the majority of AML-SCs in this system also express the stem cell antigen c-kit, thereby indicating at least some primitive features. A second surprising observation is the relatively high frequency of AML-SCs in this model, estimated at approximately 25% of the overall leukemic population, much higher than the frequency of leukemia-initiating cells in other systems, which ranges from approximately 1.0% to approximately 0.0001%. The abundance of AML-SCs was established by first plating leukemia cells in semisolid medium and then isolating and transplanting individual colonies arising from the in vitro cultures. These experiments showed that 25%–30% of leukemia cells were able to generate colonies in vitro and that all of those colonies were able to induce AML upon transplantation into recipient mice. Thus, the overall frequency of AML-SCs appeared to correlate directly with the frequency of in vitro colony-forming cells. Notably, when conventional limiting-dilution studies were performed by directly transplanting graded doses of leukemia cells into secondary recipients, the frequency of AML-SCs was much lower (1 in 121). The authors conclude that the relative homing efficiency of AML-SCs in their model is low and suggest that this may be a feature of other tumor stem cells.

Another very recent report has also employed retroviral gene transfer of the MLL-AF9 as a means to characterize leukemia stem cells (Krivtsov et al., 2006). Krivtsov et al. performed studies in which highly purified populations of normal GMPs were transduced to express MLL-AF9. Transplantation of such cells into recipient mice yielded AML in which a phenotypically defined leukemic GMP population showed the functional characteristics of AML-SCs.

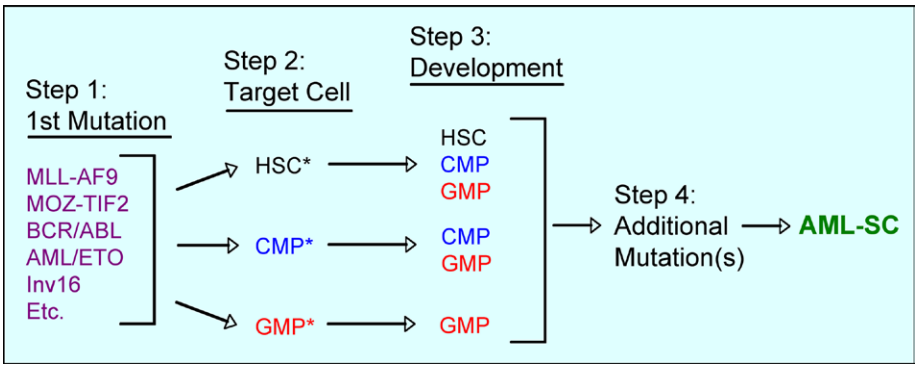

Figure 1. Hypothetical parameters affecting the genesis of AML stem cells

Initial mutations occur at the level of normal stem cells (HSC) or myeloid progenitors (CMP or GMP), giving rise to preleukemic populations (denoted by asterisk). Subsequent development of preleukemic cells may be arrested at the stage of the original mutation or progress partially to the various populations shown. Finally, after some interval of growth, additional mutations occur to generate the fully transformed AML stem cell.

On the surface these studies appear to conflict with the work of Somervaille and Cleary, in that the immunophenotype of AML-SCs is quite different. However, it is actually not possible to directly compare the reports due to substantial differences in methodology. The two groups employed different populations of normal cells for the original transduction with MLL-AF9, different strategies for functional characterization of AML-SCs, and different methods to estimate the frequency of AML-SCs. Taken together, these factors could certainly lead to varying interpretations and conclusions, despite the common feature of studying MLL-AF9-induced leukemias.

Considered more broadly, the reports of Somervaille and Cleary (2006) and Krivtsov et al. (2006) serve to emphasize an intriguing point—the basic properties of AML-SCs may be relatively heterogeneous and may vary as a function of genetics and developmental origin. Figure 1 illustrates some of the hypothetical steps leading to the generation of AML-SCs. When one considers the specific type of initial mutation (step 1), the originating target cell (step 2), subsequent differentiation or lack thereof (step 3), and the types of subsequent mutations that may occur (step 4), the number of possible permutations in the genesis of AML-SCs is quite large. Furthermore, given the inherently unstable genome of most malignant cells and possible changes evoked by challenge with various drug therapies, the resulting phenotype of human AML-SCs is potentially even more complex.

Given the issues above, several questions regarding the evolution and properties of AML-SCs should be considered. Perhaps most importantly, while the elegant studies in mouse models have indicated differing paths by which leukemia stem cells may arise, are any of these scenarios prevalent in primary human disease? To date, the only direct studies indicating a GMP-like origin for an acute form of myeloid disease is the report by Jamieson et. al. that describes studies of blast crisis CML (chronic myeloid leukemia) (Jamieson et al., 2004). CML is unique among myeloid leukemias in that it displays an overt and well-defined pathology at each stage of progression, and thereby permits the isolation and analysis of stem cells from early (chronic) and late (blast crisis) forms of disease. However, aside from CML, in five of the other eight major subtypes of AML (designated as FAB types M0, M1, M2, M4, and M5), the only AML-SCs characterized to date are both rare and phenotypically similar to HSCs (Bonnet and Dick, 1997). Therefore, while the data from mouse models are compelling, their direct relevance to human disease remains a largely unanswered question. Indeed, from a genetic perspective, evidence suggests that transformation of human cells is more complex than murine cells (Rangarajan and Weinberg, 2003); thus, findings from one species should be validated in the other. Going forward, it will be important to identify and isolate human counterparts to the entities described thus far from murine studies. Such efforts may come from the analysis of primary specimens, as described by Jamieson et al., or may also derive from the generation of better experimental models. In this regard, the studies cited above using gene transfer into normal murine progenitor cells have likely established an important precedent for future efforts using primary human cells.

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AKT and cancer—Is it all mTOR?

AKT, a key regulator of cell proliferation and survival, is commonly dysregulated in human cancers. Activated AKT kinase is oncogenic and required for tumorigenesis in PTEN-deficient animals. However, the importance of AKT in mediating transformation by other oncogenes and which of its targets are necessary for this process are poorly understood. In this issue of *Cancer Cell*, Skeen et al. show that AKT is required for transformation by mutant H-Ras and for experimental skin carcinogenesis. Moreover, the effects of AKT are mediated predominantly or solely via mTORC1. This suggests that AKT or mTOR inhibitors will be useful treatments for many cancers.

The PI3K/AKT kinase pathway is a central regulator of cell metabolism, proliferation, and survival and is dysregulated by oncogenic events in a substantial fraction of tumors. Constitutive activation of growth factor receptors, mutation of PI3K, and inactivation of the PTEN phosphatase cause the activation of PI3K signaling in the majority of glioblastomas and breast, endometrial, and prostate cancers, among others. Furthermore, PI3K is an effector of RAS function and