Mixed Lineage Leukemia Translocations and a Leukemia Stem Cell Program

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Abstract

Cancer stem cells (CSC) may provide the self-renewal capacity required to sustain a tumor. One possibility is that CSC arise from the stem cell counterparts in normal tissues. Alternatively, CSC may arise from more differentiated progenitor cells found in certain tissues. In support of this idea, we showed recently that mixed lineage leukemia fusion oncoproteins can convert committed hematopoietic progenitors into leukemias, which include leukemia stem cells expressing a self-renewal associated program in the context of a differentiated myeloid cell. The findings suggest a basis to understand the pathobiology of CSC and possible strategies to attack them to undermine the self-renewal capacity of a tumor. [Cancer Res 2007;67(18):8425–8]

Stem cell self-renewal is the process by which a stem cell divides and produces cells that possess identical developmental and proliferative potential. The loss of self-renewal capabilities during the transition between hematopoietic stem cells (HSC) and more differentiated cells is a hallmark of hematopoietic development that is likely shared by most tissues capable of regeneration. The tight controls on self-renewal may provide a safeguard against tumorigenesis as inappropriate self-renewal properties are found in leukemias and other cancers (1). Cancer cells capable of indefinite, dysregulated self-renewal and proliferation are often called cancer stem cells (CSC) or cancer-initiating cells because they can initiate and fully recapitulate all cellular characteristics of a primary cancer in recipient organisms, most frequently immunodeficient mice (2, 3). CSC represent a fraction of the total tumor and possess the ability to infinitely self-renew and generate more differentiated cells with limited proliferative capacity, features they share with normal tissue stem cells (2–6). As a result of these characteristics, CSC are crucial for tumor maintenance and regeneration, whereas the majority of the malignant cells may have only limited proliferative and self-renewal capacity. Cells with CSC properties were first identified in acute myelogenous leukemia (AML) through the seminal work of Dick et al. (4, 6). Subsequently, CSC have been identified in solid tumors (7–12).

Accumulating evidence suggests that the eradication of CSC such as leukemia stem cells (LSC) will be a prerequisite for the development of successful targeted therapeutic approaches and treatments that fail may do so because CSC escape treatment. Because normal stem cells and CSC most likely share features related to their self-renewal capability, identification of mechanisms important for CSC survival that differ from those responsible for their normal tissue counterparts may pinpoint programs or pathways that can be targeted with a high therapeutic index. To better understand the pathobiology of CSC formation, much recent effort has focused on the question if CSC always arise from normal tissue stem cells or if more differentiated progenitors can be induced to express the properties of self-renewal and multipotency (1). Because the LSCs identified in some human AMLs are phenotypically related to normal HSC in that they express several similar cell surface proteins (4), it is likely that some LSC originate from normal HSC that have undergone oncogenic transformation. Recent evidence suggests that this scenario is likely for some oncogenes. Transduction of committed progenitor populations with the BCR-ABL oncogene, which is found in chronic myelogenous leukemia (CML), did not impart self-renewal properties on these populations, indicating that initial transformation by the BCR-ABL oncogene in CML occurs in a multipotent cell such as an HSC (13).

However, other recent studies in murine transduction/transplantation models have also shown that leukemia can be initiated by at least some oncogenic fusion proteins found in human leukemias when expressed in more differentiated hematopoietic progenitor cells (13, 14). Transduction of committed hematopoietic progenitor populations with either the MLL-ENL or MOZ-TIF2 oncogenes leads to the reactivation of stem cell properties in these cells in vitro and generated AML with high penetrance when transplanted into mice (13, 14). These findings provided the first evidence that stem cell properties can be imparted on more differentiated committed hematopoietic progenitors to generate leukemia.

We showed recently that expression of MLL-Af9, another oncogenic fusion protein involving the mixed lineage leukemia (MLL) locus on chromosome 11q23, is capable of initiating leukemia in prospectively isolated granulocyte macrophage progenitors (GMP; ref. 15). Animals transplanted with MLL-Af9–transduced GMP developed AML, similar to other MLL fusion leukemia models (14, 16–18). Isolation of GMP-like leukemic cells (L-GMP) and transplantation into secondary recipient mice showed that this population is enriched for LSC as only four cells were necessary to transfer the disease. In contrast, propagation of L-GMP in liquid culture resulted in a loss of stem cell properties as indicated by the induction of a more differentiated immunophenotype and a significantly reduced number of leukemia-initiating cells (<1:5,000). These findings show that cells with a differentiated myeloid phenotype can possess LSC that both initiate leukemia and produce more differentiated progeny incapable of transferring the disease. To further characterize changes that occur during transition from GMP to GMP-derived...
LSC and to address the question if global reprogramming (or dedifferentiation) is required to generate a LSC from a committed progenitor, the gene expression profiles of normal HSC, common myeloid progenitors (CMP), normal GMP, and L-GMP were assessed. This showed that L-GMP have a unique gene expression program that is different from any of the HSC or progenitor populations but globally more similar to the GMP from which they arose than any other progenitor population. Further analysis revealed a group of 363 genes that are highly expressed in normal HSC, show decreased expression in committed progenitors, but are reactivated in L-GMP. High-level expression of this gene set is hence confined to populations with self-renewal potential (HSC and L-GMP) and was termed a "self-renewal associated signature" (Fig. 1). Because the identified "self-renewal associated signature" represented only a subset of the 1,137 genes highly expressed in normal HSC compared with committed progenitors, it seems that the activation of only a small fraction of a stem cell program is sufficient for the acquisition of leukemic self-renewal properties in L-GMP. Conversely, a portion of the self-renewal associated program was lost when LSC were forced to differentiate in liquid culture and lost their self-renewal capabilities. Another recent study analyzed the generation of LSC after introduction of MLL-Af9 into a population of cells that contained both stem and progenitor cells and showed that LSC were most similar to differentiated myeloid lineage cells, which have acquired partial properties of normal HSC (19). Taken together, these studies show that transformation of myeloid progenitors activates ectopic expression of a limited number of stem cell genes while still maintaining the global identity of differentiated myeloid cells, thus generating an abnormal hybrid cell that has characteristics of both stem cells and more differentiated cells (Fig. 1). Therefore, transformed progenitors do not fully dedifferentiate back to stem cells but rather acquire circumscribed stem cell–like features enabling them to self-renew and extensively proliferate.

The activation of a "self-renewal associated signature" in committed myeloid progenitors prompts the question about the nature of development of such a program. Is it immediately fully activated by the translocation product or does it accumulate over time suggesting a hierarchical organization and multistep development? Time course gene expression analysis after MLL-Af9 transduction showed that only a small subset of the signature genes including multiple Homeobox A cluster genes is immediately

Figure 1. Model for development of LSC from granulocyte-macrophage progenitors (GMP) through introduction of an Mll-Af9 fusion protein. Within 48 h of Mll-Af9 expression, an “immediate gene expression signature” is induced, which represents a subset of the complete “self-renewal associated signature” present in fully developed L-GMP/LSC. Despite the acquisition of stem cell properties, the LSC retain a phenotype and global expression identity that is most similar to more differentiated myeloid cells. Development of therapeutic strategies with activity against LSC through specific targeting of this ectopically expressed gene expression program is an approach that warrants further exploration.
up-regulated after introduction of the *MLL-AF9* fusion (an “immediate signature”), whereas the full signature evolves over time probably as a summation of activation of various different pathways or accumulation of subsequent genetic/epigenetic events (Fig. 1). This supports a view that the architecture of the *MLL* fusion-induced transcriptional program is hierarchical in nature where certain *HOX* genes might play an important role as direct proximate *MLL* fusion targets and important regulators of *MLL* fusion-induced leukemogenesis.

Having identified a self-renewal associated signature after introduction of *MLL-AF9* in the murine model system, a key question is whether the acquisition of stem cell properties in committed progenitors and the accompanying expression of a self-renewal associated signature is relevant and reproducible in human disease. First evidence for the acquisition of stem cell-like properties in human committed progenitors was provided by a recent report assessing CML blast crisis where cells with a phenotype similar to GMP were identified as LSC candidates and transcriptional targets of β-catenin as potential mediators of self-renewal (5). In our study, we showed significant overlap between the murine self-renewal associated signature and a previously defined human *MLL*-rearranged AML signature (20). This further supports the relevance of the identified signature and suggests that the mechanisms underlying leukemia formation seem to at least partially overlap in murine leukemia model systems and primary human disease. The finding that LSC do not necessarily have a similar phenotype/gene expression program compared with normal HSC but rather activate a limited self-renewal program in the context of a committed myeloid cell has important implications for understanding how CSC develop and may aid in the successful design of new therapeutic approaches.

Further evidence that somatic cells can be reprogrammed to a pluripotent state through a few genetic manipulations comes from a series of elegant studies, which have shown that fibroblasts engineered to express only four transgenes (*Klf4, Sox2, Oct4, and c-Myc*) acquire properties that are comparable with pluripotent embryonic stem (ES) cells. These “inducible pluripotent stem” cells resemble ES cells in morphology, proliferation, and teratocarcinoma formation including differentiated cell types of all three germ layers and chimaera formation in adult mice when injected into wild-type mouse blastocysts. Gene expression analysis revealed activation of a transcriptional program that is similar but not identical to multipotent ES cells (21–24). These important studies provide further evidence that a limited number of genetic alterations can induce reprogramming of a somatic genome to a stem cell state.

However, many important questions remain. The temporal and spatial architecture of the LSC self-renewal associated program needs to be defined in more detail such that rational approaches for reversal agents can be developed. Although the activation of a limited subset of genes immediately after introduction of the *MLL-AF9* fusion implicates a hierarchy for the overall self-renewal signature, the detailed structure of this hierarchy as well as the interactions and regulatory interdependences of discrete signature members remain to be uncovered. Future analysis will include characterization of genes primarily involved in self-renewal as opposed to others more broadly relevant for immortalization and the extent to which these processes overlap. Data suggest that deregulated expression of certain *HOX* gene family members contribute to the molecular control of aberrant self-renewal properties in hematopoietic cells. It will be of interest to evaluate to which degree other signaling pathways previously linked to self-renewal, such as *Bmi1* and/or other polycomb genes, the WNT-β-catenin pathway, or the Notch and SHH signaling pathways, contribute to the global transcriptional network underlying this self-renewal associated signature.

Another important next step will be the recapitulation and validation of the applied strategy in primary human cells. Currently, it still remains to be fully established if *MLL* fusion genes can directly transform human progenitor cells in addition to HSC. It will be of considerable interest if a similar “self-renewal associated signature” can be identified in human model systems of acute leukemia. The fact that a significant subset of the murine self-renewal signature is found enriched in human *MLL*-rearranged AML suggests that at least parts of the signature might be found in both murine LSC and human LSC. Studying self-renewal associated gene expression changes induced by other leukemia-associated fusion genes, in other nonhematopoietic cancers and fibroblast reprogramming, will ultimately allow determination if parts of the “self-renewal associated signature” represent a universal program important not only for human leukemias but also for other human malignancies, and the extent to which these programs are activated in other types of cellular reprogramming.

**Acknowledgments**


**References**


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Cancer Res 2007; 67: (18). September 15, 2007 8428 www.aacrjournals.org
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