Therapeutic Implications of Leukemia Stem Cell Development

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Abstract Acute myelogenous leukemias, and perhaps many other cancers, are maintained by a population of cancer stem cells that can regenerate themselves as well as give rise to more differentiated and less proliferative cells that constitute the bulk of the disease. Recent discoveries have shed light on both the nature of leukemia stem cells (LSC) and their cells of origin. Here, we review which hematopoietic cells could give rise to LSC, and the phenotype of fully developed LSC. The perturbed developmental pathways and cellular context of LSC development have implications for the development of new therapeutic approaches.

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Background

Stem cells are defined by their ability to generate more stem cells and differentiated cells committed to specific lineages. In this manner, stem cells serve to sustain and regenerate particular tissues by providing the cells necessary to perform specialized functions. This ability to divide and produce daughter cells that maintain proliferative and developmental potential is often referred to as self-renewal. The hematopoietic system is the most well understood tissue in terms of cellular hierarchy. Hematopoietic stem cells (HSC) are the only hematopoietic cells with significant self-renewal potential. HSC also give rise to committed progenitors that are incapable of self-renewal and are destined to differentiate into mature hematopoietic cells. Recent studies suggest that leukemias have a similar hierarchical organization with only a subset of cells possessing self-renewal capabilities. Such cells are frequently referred to as leukemia stem cells (LSC).

Areas of intense scrutiny in the field of leukemia biology include the cell of origin of specific leukemias, and the phenotype of the fully developed LSC. The oncogene products involved in leukemogenesis such as chromosomal translocation–associated fusion proteins, deregulated signaling molecules, and inactivated tumor suppressors are becoming increasingly well defined. An important next step will be to determine the cellular processes influenced by these oncogenes and the importance of cellular context. Recent studies in murine models indicate that certain fusion oncoproteins found in acute myelogenous leukemias (AML) can impart LSC properties on multiple different cell types including both committed hematopoietic progenitors and HSCs (1–3), whereas other oncogenes may impart their function only on normal HSCs (ref. 2; Fig. 1). These findings have important implications for the development of new therapeutic approaches.

Recent Advances

The cell of origin in AML. An important question in cancer biology is whether tumors arise from normal tissue stem cells or from more differentiated progenitors (4, 5), as the cell of origin may influence therapeutic efficacy. If, for example, the initial genetic event in myeloid leukemogenesis must occur in a normal HSC, the eradication of this preleukemic cell might be quite difficult without a significant detrimental effect on normal stem cells. If the initial event can occur in more committed hematopoietic progenitor cells, however, then eradication might be more easily attained. A detailed understanding of cellular phenotypes in normal hematopoietic development allowed for recent studies that have begun to directly address this issue. As normal HSC are long-lived and capable of extensive self-renewal, an initial genetic event (e.g., a translocation) could persist long enough such that the subsequent mutations necessary for the full development of leukemia could be acquired. Alternatively, the initial genetic event might occur in a developing hematopoietic progenitor that is normally not long-lived and doesn't possess self-renewal capability. In this scenario, however, the initial event would need to impart stem cell characteristics on the non–self-renewing hematopoietic progenitors such that subsequent mutations could be acquired (Fig. 1). The first direct evidence for leukemic transformation of a non-HSC was a mouse transplant model in which MLL-fusion proteins generated by AML-associated 11q23 translocations were able to transform not only HSCs, but also more differentiated committed common myeloid progenitors and granulocyte macrophage progenitors (GMP; ref. 1). These transformed cells showed properties of self-renewal *in vitro*, and induced AML when transplanted into mice.

It seems, however, that not all leukemia-inducing fusion proteins are transforming in all cell types. When transduced into common myeloid progenitors or GMPs, a MOZ-TIF2 fusion protein generates a myeloid leukemia in mice, whereas the BCR-ABL fusion protein does not (2). The investigators

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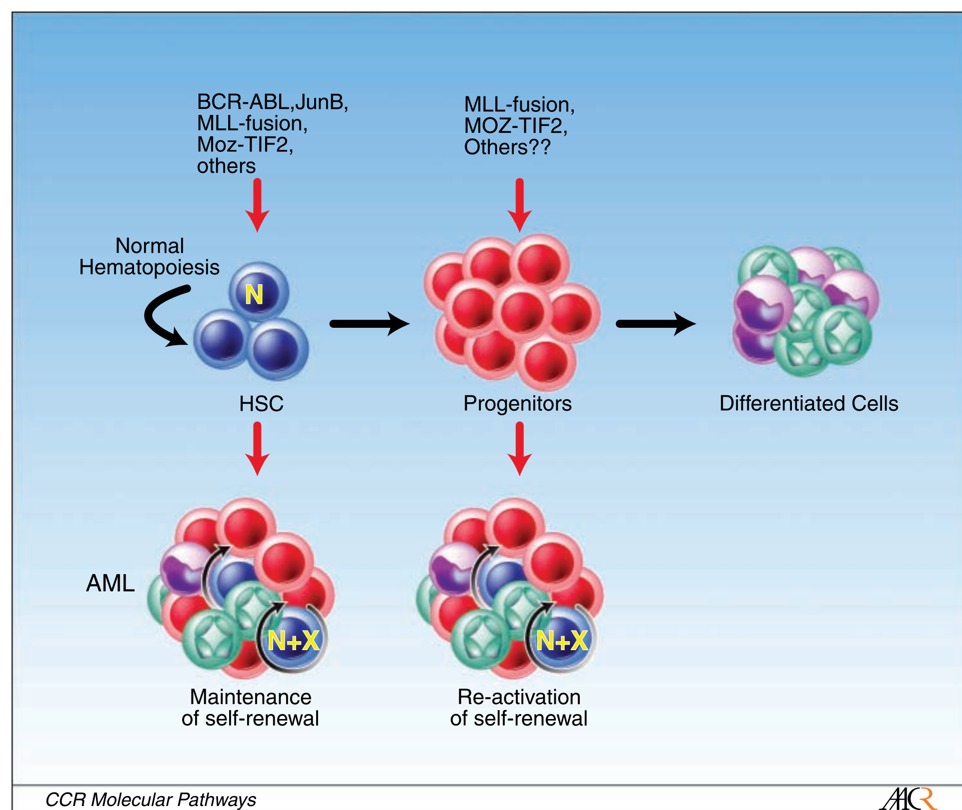

Fig. 1. Leukemia oncogenes can directly transform different hematopoietic cells. Normal hematopoietic development proceeds from HSC through committed progenitor cells to specialized fully differentiated cells. Which cells can be transformed into LSCs may depend on the initial genetic event (*N*). Certain initial mutations may require the properties of the normal HSC, i.e., extended life span and self-renewal, such that subsequent mutations (*X*) can be subsequently acquired. However, fusion proteins such as MLL-AF9 possess the ability to induce sufficient self-renewal in committed progenitors and therefore subsequent mutations can be acquired. In HSC-derived AML, a reservoir of mutant HSC would likely make eradication more difficult, whereas in progenitor-derived AML, such a reservoir would not exist.

suggest that the differing transforming abilities of these oncoproteins could be due to differing abilities to induce self-renewal in progenitor cells. Whereas BCR-ABL may allow increased proliferation and survival, MOZ-TIF2 could also induce stem cell–associated properties like self-renewal, thus leading to an acute leukemia. Another example of an HSC-specific effect is found in the JunB-associated murine myeloproliferative disease. Deletion of *JunB* in hematopoietic cells leads to the development of a myeloproliferative disease that can be transferred to secondary recipient mice by mutant HSC, but not by mutant progenitors. This shows that *JunB* loss has its myeloproliferative disease–associated effect specifically in HSC (6, 7). Thus, it seems, at least in model systems, that certain, but not all, oncogenes could induce leukemia from either stem cells or committed progenitor cells. Studies are under way to determine if progenitor cells could be the target cells for the initial genetic event in *de novo* human acute myeloid leukemias. Initial studies suggest that this may be the case. When $CD34^+$,$CD33^-$ early progenitor cells were obtained from AML samples bearing specific cytogenetic abnormalities including monosomy 7, t(8;21), or t(15;17), only $CD34^+$,$CD33^-$ cells from monosomy 7 AML cells consistently possessed the cytogenetic abnormality. This suggests that at least the t(15;17) AMLs may have arisen from a more committed progenitor cell. Interestingly, the leukemias that possessed immature $CD34^+$, $CD33^-$ cells harboring the monosomy 7 abnormality did not respond well to induction therapy (8). Future detailed studies should determine the extent of committed progenitor cells as the cell of origin in human AML, and if the cell of origin influences outcome.

Transformation from normal hematopoietic cell to LSC. If we are to specifically target LSC, a critical question is the extent to which leukemia self-renewal/proliferation relies on similar pathways as does normal HSC self-renewal. An elegant study showing that some pathways are shared assessed the Polycomb group family member *Bmi-1*. *Bmi-1* is necessary for self-renewal in mouse HSCs, but which downstream pathways mediate this function remains incompletely described. Repression of the $p16^{Ink4a}/p19^{Arf}$ tumor suppressor locus by *Bmi-1* allows for self-renewal and maintenance of neural stem cells (9). *Bmi-1* is not required for the generation of LSCs, but is necessary for extensive leukemia self-renewal and proliferation (10). Mice transplanted with *HoxA9/Meis1*–transduced *Bmi-1*$^{-/-}$ fetal liver cells develop myeloid leukemia with a latency similar to mice transplanted with *HoxA9/Meis1*–transduced *Bmi-1*$^{+/+}$ fetal liver cells. Unlike the *Bmi-1*–expressing leukemia, however, the *Bmi-1*$^{-/-}$ leukemias cannot be transferred to secondary recipient mice. *Bmi-1* was subsequently implicated in human AML when it was found to be up-regulated in cord blood cells transformed *in vitro* (11). These studies suggest that some pathways associated with normal HSC self-renewal are necessary for the extensive proliferation and self-renewal found in leukemia.

Detailed gene expression studies in a murine model of MLL-fusion–associated AML further highlight the notion that at least part of a stem cell or self-renewal–associated program is active in LSC. This study showed that LSC derived from committed myeloid progenitors transduced with an MLL-fusion protein retains an overall progenitor-like expression program, but also expresses a group of genes normally highly expressed only in HSC. Within this signature are many previously described stem cell–associated genes, including much of the *HoxA* cluster (*HoxA5-HoxA10*) and the Hox-binding protein gene *Meis1*, as well as several genes less identifiable with HSC

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self-renewal (e.g., *Mef2C, Runx2*, and *Itf-2*; ref. 3). These data suggest that leukemic transformation of hematopoietic progenitors proceeds by activation of stem cell (or self-renewal)–associated programs in an abnormal context, and provide an opportunity to begin to dissect such a program.

The phenotype of LSC. Recent studies have begun to characterize the immunophenotype and gene expression profiles of LSC. Initial studies in human AML have shown $CD34^+$,$CD38^-$ cells contained the LSC fraction, whereas the majority of the leukemia cells possessed a more differentiated immunophenotype (12). Follow-up experiments have elegantly shown a hierarchy of cells in leukemia and characterized the properties of human LSC in nonobese diabetic/severe combined immunodeficiency mice (13). As normal human HSCs possess a $CD34^+$,$CD38^-$ phenotype, these data suggest that in some AMLs LSC may be quite similar to normal HSC.

The phenotypic markers of a particular LSC, however, may not be constrained to HSC markers. Human LSC antigens that differ from HSC antigens include CD90 and c-Kit (both expressed in HSC but not LSC), and IL3R (expressed in LSC, but not HSC; ref. 14). In a recent study, a GMP-like population was isolated from patients with chronic myeloid leukemia in blast crisis, and β-catenin levels were found increased in the GMP-like chronic myeloid leukemia cells as compared with normal GMPs (15). Furthermore, chronic myeloid leukemia–associated leukemic GMPs showed *in vitro* self-renewal properties. Another example indicating a difference between LSC and HSC is a mouse model of *CALM-AF10*–derived AML, in which LSCs were found to express B220 (a lymphoid-specific marker) and no myeloid antigens (16). Of interest, this $B220^+$ population was also found in human *CALM-AF10*–associated AML. Also, *MLL-GAS7*–induced murine leukemias could arise from methylcellulose-cultured cells that possess similarities to multipotent progenitors (17), indicating that the surface antigens on various LSCs may include markers normally seen on more mature hematopoietic cells. This point is clearly made in a murine study in which LSCs in a murine model of AML were found to express antigens associated with differentiated myeloid cells (18). The demonstration that LSC could express genes associated with more differentiated cellular phenotypes provides hope that LSC can be specifically targeted.

Translational Implications

Data are accumulating that suggests that eradication of LSCs will be a necessary part of any therapeutic approach. Current successful therapies likely attack both the LSC and the more differentiated blast cells along with normal hematopoietic stem and progenitor cells. An alternative approach might target only the LSC and not the more differentiated leukemia cells. Such an approach would likely be less toxic and more effective than those used now if LSC could be specifically targeted in a manner that spares normal HSC. As described previously, studies are now beginning to delineate the various populations of cells that possess LSC activity in specific leukemias, therefore testable hypotheses to target these cells can now be developed.

One approach would target specific self-renewal or survival pathways necessary for LSC but not normal HSC. Whether such pathways that differentiate LSC and HSC exist is just beginning to be assessed, however, proof-of-principle experiments in human and murine leukemias suggest that this approach may be possible. As described previously, a highly characterized mouse model of *MLL-AF9* leukemias possesses LSC that express a subset of stem cell–associated genes in the context of a more differentiated myeloid cell (3, 18). This abnormal gene expression program thus produces a partial stem cell with aberrant self-renewal and proliferative capacity. If this can be shown to be the case in human leukemias, it may be possible that the aberrant LSC is more dependent on specific aspects of the stem cell program than would be a normal HSC that might possess redundant mechanisms. A proof-of-principle experiment in a murine model of *Pten*-deficient leukemia showed activity of the mTOR inhibitor rapamycin against LSC while sparing the HSC (19). Furthermore, initial experiments in human leukemias suggest that compounds do exist that have more activity against LSC than HSC. The sesquiterpine lactone parthenolide seems to be one such compound. $CD34^+$,$CD38^-$ cells from AML patients and normal patients were compared for parthenolide sensitivity both *in vitro* and *in vivo*. In both cases, parthenolide induced apoptosis in the AML cells, but not in the normal cells (20). Future studies will further assess the viability of such an approach, but early studies provide hope that there are significant differences between HSC and LSC that can be leveraged for therapeutic benefit.

Another viable option for specific targeting of LSC would take advantage of cell surface antigens expressed on LSC. Identification of the gene expression programs expressed in highly enriched populations of LSC provides an opportunity to identify antigens highly expressed on LSC. One such antigen, CD44, is highly expressed in normal HSC and LSC (3). Remarkably, recent studies using human and murine leukemias suggests that antibody-based targeting of CD44 has activity against LSC both in murine systems and in human leukemias (21, 22). A recent breakthrough that could further enhance the antibody-based approach is the ability to engineer monoclonal antibodies to carry specific small interfering RNAs as a payload to be delivered directly to a cell of interest (23). Such an approach would be similar to antibodies engineered to deliver payloads, such as anti–CD33-Calicheamicin (gemtuzumab ozogamicin); however, because the attached small interfering RNA should possess much less systemic toxicity than general toxins, such as calicheamicin, the therapeutic window of such an approach might be better. Antibody-based targeting of LSC with or without attached small interfering RNA, although in its infancy, provides an avenue with significant potential.

In summary, the cells of origin, pathways for transformation, and phenotype of cancer stem cells in leukemia are beginning to be unraveled. Studies in murine models suggest that the phenotypes of LSCs are variable and may be dependent on the genetic event that initiates the leukemia. Future studies will define if similar heterogeneity exists in human leukemias. Growing evidence that LSC and HSC possess significant differences, however, provides a potential opportunity to specifically target the subset of leukemia cells with extensive proliferative and self-renewal properties while leaving the HSC intact. A detailed understanding of these processes should provide a better understanding of the successes and failures of our current therapeutic approaches, and more importantly, the opportunity to develop more effective, less toxic alternatives.

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