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Mutant CEBPA: Priming Stem Cells for Myeloid Leukemogenesis

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In a recent study published in *Cancer Cell*, Bereshchenko and colleagues (2009) report a knockin mouse model that represents the most frequently occurring biallelic combination of *CEBPA* mutations found in human acute myeloid leukemia.

Acute myeloid leukemia (AML), like all malignancies, is considered to result from the sequential acquisition of multiple genetic lesions in a normal, likely long-lived cell. This paradigm presents a conundrum, however, since emerging evidence increasingly indicates that the leukemia stem cells (LSCs) responsible for sustaining AML exhibit features of downstream myeloid progenitors, cells which under normal circumstances are irreversibly committed to becoming terminally differentiated within days to weeks (Somervaille and Cleary, 2006; Krivtsov et al., 2006; Taussig et al., 2008). A critical component of the model is the presumed requirement for self-renewal, a type of cell division occurring in stem cells which results in at least one daughter cell with essentially the same extensive proliferative potential and differentiation status as its parent. One possibility is that key leukemia-initiating mutations occur in hematopoietic stem cells (HSCs), the only normal blood cells that undergo self-renewal, resulting in a population of preleukemic HSCs with a proliferative and/or survival advantage over their non-mutated counterparts. The accumulation of further mutations in these initiated cells over time results in full-fledged leukemia, associated with aberrant self-renewal in downstream myeloid progenitors. An alternative possibility is that leukemia-initiating mutations may directly confer the capacity of inappropriate self-renewal on downstream cells. A new study (Bereshchenko et al., 2009) provides insight into this issue by demonstrating in a knockin mouse model that *CEBPA* mutations can prime HSCs to generate downstream myeloid leukemia stem cells.

CEBPA (CCAAT/enhancer binding protein alpha) is a leucine zipper transcription factor (expressed as two isoforms of either 30 kD or 42 kD) that regulates myeloid differentiation in hematopoiesis, as well as the differentiation of other tissue-specific cell types in liver, fat, and lung. It functions in part through repression of E2F and MYC activity, and its complete absence in hematopoietic cells induces pleiotropic effects, including a block in myeloid differentiation accompanied by profound reductions in mature myeloid cell populations, an accumulation of myeloblasts in the bone marrow without progression to AML, and an enhanced self-renewal capacity of HSCs (Koschmieder et al., 2009).

CEBPA mutations are present in 7%–10% of human AML and are mostly associated with a normal karyotype. The mutation patterns observed are generally biallelic (~70% of cases) and almost always combine an N-terminal frameshift mutation on one allele with a C-terminal in-frame insertion/deletion mutation on the other allele (Pabst and Mueller, 2007). N-terminal mutations prohibit expression of the 42 kD protein isoform, whereas C-terminal mutations disrupt DNA binding and dimerization by both isoforms (Figure 1). The molecular mechanisms through which *CEBPA* mutations promote AML are not entirely clear, although the 30 kD isoform binds and transactivates target genes with lower efficiency than the 42 kD isoform, suggesting that hypomorphic mutant alleles have an impaired capacity to couple cell-cycle arrest to terminal myeloid lineage differentiation.

Bereshchenko et al. generated two knockin mutations of the mouse CEBPA locus that correspond to each of the two common alterations observed in human AML samples. The "K allele" (a K313KK mutation) generated a C-terminal mutant, while the "L allele" (a nonsense codon in the p42-specific region of CEBPA) mimicked the human N-terminal mutation (Figure 1A). To study the cell-intrinsic potential of these *CEBPA* mutations to initiate AML, the authors transplanted fetal liver cells from K/L knockin mice that expressed one of each of the two mutant alleles or cells from L/L mice or K/K mice into irradiated, wild-type recipients. Recipients in all three cohorts succumbed to AML with a median latency of 40–50 weeks. Four weeks following transplantation and prior to overt leukemia development, mice that received K/K or K/L cells (but not those that received L/L cells) exhibited a modest relative expansion of the immunophenotypic HSC compartment, and a much more substantial expansion of multipotent progenitors (MPPs) downstream of HSCs. Both preleukemic HSCs and MPPs were less quiescent. Thus, the presence of the K mutation promotes proliferation and expansion of HSCs and MPPs in the early stages of leukemogenesis, although it is unclear if the mutant MPPs at this preleukemic stage have already acquired unlimited self-renewal potential.

The myeloid differentiation block observed in the K/K AMLs, a mutation combination not found in human AML, appeared more profound than in the K/L and L/L AMLs, resulting in some cases in erythroleukemia rather than AML, a phenotype reminiscent of that observed in *CEBPA* null blood cells. Thus, there

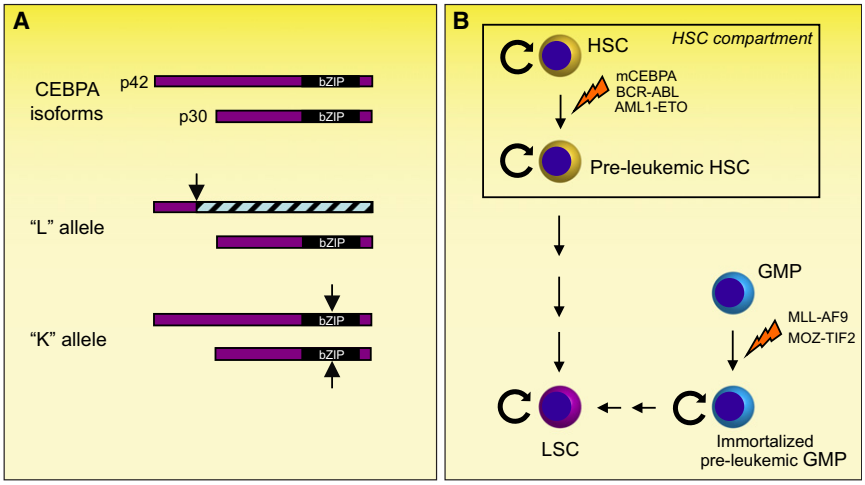

Figure 1. CEBPA, Hematopoietic Stem Cells, and Acute Myeloid Leukemogenesis.
(A) CEBPA is produced as two isoforms that differ at their amino termini. Leukemia-associated mutations (arrows) may prohibit production of the p42 isoform due to an upstream frameshift mutation (modeled by the L allele in this study) or disrupt dimerization and DNA binding of both isoforms through in-frame insertions or deletions (modeled by the K allele in this study).
(B) Two potential pathways to acute myeloid leukemogenesis. Some AML initiating mutations may accumulate within and alter the properties of self-renewing hematopoietic stem cells (HSCs), whereas others may directly confer self-renewal potential on committed downstream progenitors (e.g., granulocyte-macrophage progenitors, GMPs). In both scenarios, the LSCs are downstream aberrantly self-renewing progenitor cells but the cellular targets for initiating mutations are different. Secondary genetic or epigenetic changes (dashed arrows) are required in both cases to produce fully functional leukemia stem cells (LSCs). Circular black arrows indicate presence of self-renewal capacity.

may be a critical hypomorphic range of *CEBPA* activity that is leukemogenic in humans, with either too little or too much being insufficient to induce disease, explaining the prevalent mutation combination observed in *CEBPA* mutated AML patients.

Despite preleukemic expansion and elevated cycling activity of the HSC and MPP populations, the LSCs responsible for sustaining AML did not exhibit features of HSCs or MPPs. Rather, LSCs had a downstream myeloid precursor immunophenotype ($CD117^+$, $Sca1^-$, $Mac1^+$) similar to that reported previously in mice with leukemias initiated by MLL fusion oncogenes (Somervaille et al., 2009). Thus, the preleukemic HSCs appear to spawn downstream myeloid cells that are predisposed to complete LSC conversion by acquisition of secondary genetic or epigenetic changes that remain undefined.

In addition to their proliferative expansion, mutant HSCs also displayed alterations in their differentiation programming. Gene-expression profiling of K/L mutant HSCs revealed that granulocyte-macrophage gene sets were significantly downregulated, whereas erythroid lineage gene sets were significantly upregulated, suggesting both the presence of skewed lineage priming and that CEBPA actively regulates expression of multiple myeloid lineage-specific genes even in uncommitted HSCs. This finding also suggests that in *CEBPA* mutant HSCs, the prevailing transcriptional state predicts the subsequent block in myeloid differentiation in a downstream cell. Thus, mutant HSCs appear primed for leukemogenesis, ready for the acquisition of further changes that contribute to the generation of inappropriately self-renewing downstream LSCs.

Although it is not currently clear what potential selective advantage a single *CEBPA* mutation might confer on an HSC, this study provides support for the model in which certain leukemia initiating mutations have the capacity to convert HSCs to proliferative, developmentally skewed, preleukemic HSCs, whose downstream progeny subsequently acquire the requisite secondary genetic or epigenetic changes necessary to become full-fledged AML LSCs (Figure 1B). This model is somewhat reminiscent of acute blast crisis of chronic myeloid leukemia, where BCR-ABL-positive HSCs in the chronic phase of the disease eventually generate granulocyte-macrophage progenitors (GMPs) with enhanced in vitro proliferative potential in the acute phase (Jamieson et al., 2004), and also *AML1-ETO*-associated AML, where the fusion oncogene may be found expressed in the HSC compartment for years following apparent cure of the disease (Miyamoto et al., 2000). However, this priming model does not appear to be generalized for AML pathogenesis or necessary, since it is not a feature of several other mouse models of AML. For example, powerful oncogenes such as MLL-AF9 or MOZ-TIF2 (Huntly et al., 2004) have the capacity to confer self-renewal directly on downstream myeloid progenitors such as GMPs as an alternative pathway for AML initiation. It remains to be determined how accurately the different mouse models reflect human AML. A future challenge will be to identify and isolate preleukemic and leukemic clones from patients with AML, aiming to compare the mutational status of these functionally distinct populations.

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