C/EBPα in Leukemogenesis: A Matter of Being in the Right Place with the Right Signals

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Leukemia-initiating cells can originate from hematopoietic progenitor cells that have acquired self-renewal capacity upon transformation with leukemic fusion genes. In this issue of *Cancer Cell*, Kirstetter and colleagues describe a mouse model for the frequent CEBPA mutations in human acute myeloid leukemia that result in the synthesis of only the 30kDa isoform, but not the 42kDa isoform of C/EBPα. This mutation uncouples C/EBPα's roles in myeloid differentiation and proliferation control. Furthermore, this mutation activates self-renewal in committed myeloid progenitor cells and induces myeloid malignancy with complete penetrance that is sustained by leukemia-initiating cells with a committed myeloid molecular signature.

Acute myeloid leukemia (AML) rises from the accumulation of genetic mutations that alter the regulation of proliferation, survival, and differentiation of hematopoietic progenitors. The hematopoietic system is a heterogeneous group of cells sustained by the hematopoietic stem cells (HSCs), a rare group of cells with the ability to self renew (i.e., the capacity to produce a daughter cell identical to the parental). Similarly, the leukemia tumor is a heterogeneous group of highly proliferative cells with partial differentiation capacity that is sustained by the "leukemia-initiating cells" (LIC or leukemia stem cells) (Passegue et al., 2003). The study of the origin and function of LICs is critical as they may be crucial targets in the treatment of leukemia.

The LICs can originate from HSCs that maintain self-renewal functions after transformation or from committed progenitors that have regained self-renewal capacity. Expression of the leukemia fusion protein BCR-ABL from a retroviral vector induces leukemia only when introduced in the HSCs, while MLL-ENL and MOZ-TIF2 can induce AML in committed granulocytic-macrophage progenitors (GMPs) (Cozzio et al., 2003; Huntly et al., 2004). It may therefore be a common theme that mutations found in AML may have the ability to reactivate self-renewal programs in short-lived progenitors.

The article published by Kirstetter and colleagues in this issue of *Cancer Cell* (Kirstetter et al., 2008) investigates alterations in hematopoietic differentiation and transformation in mice in the context of *Cebpa* mutations. The CCAAT-enhancer binding protein alpha (C/EBPα) transcription factor is a critical regulator of proliferation and differentiation in multiple cell types. In hematopoiesis, C/EBPα regulates myeloid differentiation at multiple levels, including the transition from common myeloid progenitors (CMPs) to the bilineage GMPs and granulopoiesis (Nerlov, 2004). The intronless *CEBPA* gene encodes an mRNA that is translated to a 42kDa (p42) and a 30kDa (p30) C/EBPα proteins from different translation start sites. C/EBPα modulates differentiation and proliferation by direct interaction with coactivators and repressors in a cell-type-specific manner. The N-terminal region unique to p42 contains transactivation elements TE-I and TE-II that interact with TBP/TF-IIB and CBP/p300. The common C-terminal region interacts with the SWI/SNF complex at TE-III and with E2F1, GATA-1, PU.1, RUNX1, and c-JUN at the basic region DNA-binding and leucine zipper dimerization domain (bZIP).

The expression of C/EBPα is frequently reduced in AML through different mechanisms (Nerlov, 2004). The expression of *CEBPA* mRNA can be repressed by the leukemia fusion protein AML1-ETO, and the expression of C/EBPα protein can be regulated by CBFβ-SMMHC and BCR-ABL. In addition, the function of C/EBPα can be inhibited by TRIB2. Mutations of *CEBPA* are present in approximately 9% of AML cases, commonly occurring in intermediate-risk karyotype and in the absence of *AML1-ETO* or *CBFB-MYH11*. *CEBPA* mutations frequently cluster in the 5′ region that specifically disrupts the synthesis of p42 while retaining the expression of p30. Another mutation hot spot is at the 3′ region that often results in creating dominant-negative proteins. In most cases, when *CEBPA* mutations occur in both alleles, at least one mutation is in the 5′ region. This observation strongly suggests that p30 may be essential to trigger AML. This is consistent with findings that *Cebpa* null mice remain leukemia free (Heath et al., 2004; Zhang et al., 2004) (Figure 1) and that BCR-ABL fails to produce AML in *Cebpa* null mice (Wagner et al., 2006).

Mimicking the 5′ region mutations found in human AML, Kirstetter and colleagues created a *Cebpa* "*L*" allele in mice that expressed p30, but not p42, by introducing a nonsense codon between the two translation-start sites. Careful characterization of $Cebpa^{L/L}$ hematopoietic progenitors that preceded leukemic transformation revealed a differentiation block at the myeloblast stage and neutropenia that progressed to granulocyte hyperproliferation. The GMPs and committed myeloid progenitors (cMP) were hyperproliferative with signs of self-renewal (Figure 1). Interestingly, serial replating of purified progenitors confirmed the

enhanced self-renewal in cMPs and not in HSCs. Although multiple modulators of cell-cycle progression and differentiation are known to interact with C/EBPα, the mechanism of action is still unclear, and competing models have been proposed (Nerlov, 2004). C/EBPα binds to E2F1 via the bZIP domain to repress c-Myc, thereby inhibiting proliferation and promoting differentiation. The *Cebpa*$^{BRM2/BRM2}$ mice, carrying a point mutation in the bZIP domain that impairs E2F1 interaction, show similar preleukemic features as *Cebpa*$^{L/L}$ but with the significant difference that *Cebpa*$^{BRM2/BRM2}$ mice do not develop myeloid leukemia (Porse et al., 2005). Considering that p30 maintains an intact bZIP domain but lacks the ability to repress E2F activity, E2F/c-Myc is probably a critical mediator of C/EBPα modulation of proliferation and self-renewal programs. The rescue of myeloid differentiation by p30 shows an uncoupling of proliferation and differentiation capacities and suggests that other partners that interact with p30 may be involved.

A critical finding of this pivotal study is that, unlike *Cebpa*$^{BRM2/BRM2}$ mice, *Cebpa*$^{L/L}$ mice succumb to myeloid leukemia after 9 to 14 months. Transplantation assays of immunofractionated leukemia cells demonstrated that the *Cebpa*$^{L/L}$-LICs reside in the cMP compartment. The expression profile of *Cebpa*$^{L/L}$-LICs and its clustering with the expression profile of MLL-AF9-transformed leukemia-GMPs (Krivtsov et al., 2006) revealed that LICs may share common and initiator-mutation-specific expression signatures that drive leukemia development. The p30-specific gene set showed the dysregulation of genes encoding members of the C/ebp complex, with upregulation of the C/ebp repressor *Ddit3* and repression of *Cebpb*. Further dissection of these programs may provide valuable therapeutic targets.

This study demonstrates that a basal *CEBPA* function provided by p30, and not the loss of expression, is required for leukemia transformation. Considering the variety of partners known to associate with and to modulate (or be modulated by) C/EBPα, p30 may not just provide a basal C/EBPα function but rather a unique combination of domains that allow transformation. A precedent of this model in leukemia is GATA1, which is also involved in regulation of myeloid differentiation. The *GATA1* exon-2 mutations found in down syndrome-associated acute megakaryoblastic leukemia maintain the expression of the short GATA1s protein that lacks a transactivation domain but abrogates the expression of the full-length GATA1 factor (Wechsler et al., 2002). Therefore, the increase in proliferation and self-renewal within *Cebpa* null CMPs may present an unfavorable environment for transformation. Instead, p30-mediated differentiation to GMPs may permit a permissive cell environment for leukemia development. These cases suggest that reduction, but not loss, of transcription factor function may be a common theme in myeloid leukemia.

The *CEBPA* mutations are probably early events that occur in the HSCs. The *Cebpa*$^{L/L}$ mice are the first *Cebpa* mouse model of leukemia and represent a powerful tool to further dissect leukemogenesis. For example, how does p30 affect HSC function in an otherwise normal environment? What mutations cooperate with p30 expression in transformation of the cMPs? Is p30-expression just permitting differentiation to MPs, or does it play a role in the acquisition of additional mutations?

The elegant study reported by Kirstetter and colleagues emphasize the importance of understanding the specific effect of human-leukemia mutations in the etiology of leukemia. This model validates the causality of *CEBPA* mutations as observed in human AML and has brought together proliferation and self-renewal to the GMP compartment to trigger transformation. The identification of the committed myeloid LIC will also be valuable in the test for efficient drug therapy.

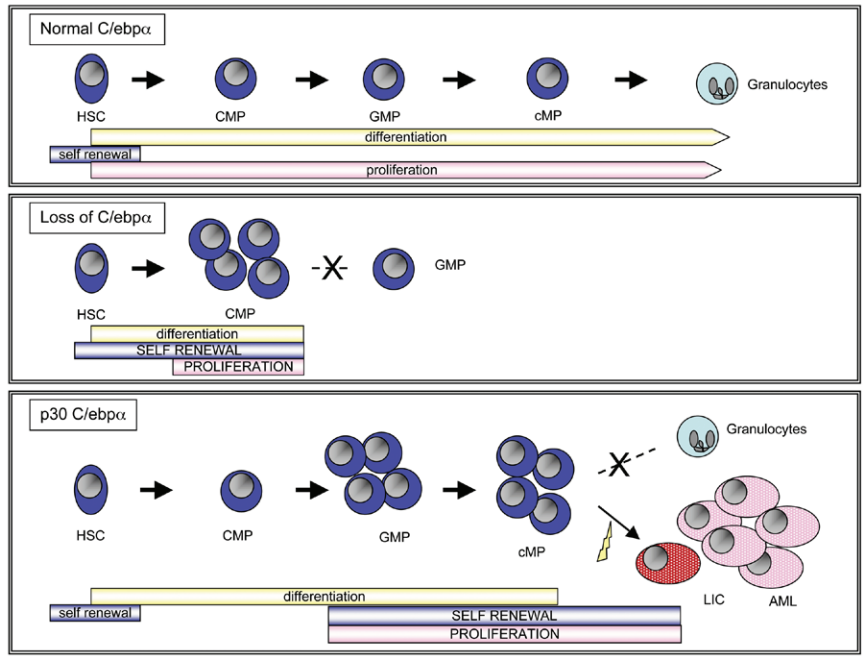

Figure 1. Role of p30 Cebpα Protein in Myeloid Leukemia Development
Top: normal myeloid differentiation follows a hierarchy of progenitors that include hematopoietic stem cells (HSCs) with self-renewal capacity (blue) that progressively differentiate (yellow) to common myeloid progenitors (CMPs), granulocytic-macrophage progenitors (GMPs), and committed myeloid progenitors (cMP), which mature to granulocytes. Middle: hematopoiesis in *Cebpa* null mice show enhanced (capital letters) self-renewal capacity in HSCs and CMPs, hyperproliferation in CMPs, and differentiation block of CMPs. Bottom: hematopoiesis in *Cebpa*$^{L/L}$ mice, which express p30, but not p42, has GMPs and cMPs with enhanced self-renewal and proliferation capacities. These abnormal progenitors acquire additional mutations (yellow lightning bolt) to develop myeloid leukemia (pink cells). The leukemia-initiating cells (red cell) are immunophenotypically a cMP.

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A Molecular View of Anti-ErbB Monoclonal Antibody Therapy

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Abnormal activation of the epidermal growth factor receptor (EGFR) and its homolog HER2 (Neu/ErbB2) has been associated with many human cancers, and monoclonal antibodies targeting EGFR and HER2 are effective anticancer therapies. Structural studies of these receptors and antibodies have revealed much about how they function. In this issue of *Cancer Cell*, Schmiedel et al. report structural and functional studies of the anti-EGFR monoclonal antibody Matuzumab. They show that Matuzumab binds and inhibits EGFR in a manner distinctive from that of other therapeutic anti-EGFR antibodies and suggest that combination therapies with Matuzumab and other antibodies may prove beneficial.

The epidermal growth factor receptor (EGFR/ErbB1/HER1) consists of an extracellular ligand binding region followed by a single membrane-spanning helix, a cytoplasmic tyrosine kinase domain, and a C-terminal tail of ~230 amino acids (Burgess et al., 2003). Ligand binding to the extracellular region promotes receptor dimerization, which in turn leads to activation of the cytoplasmic tyrosine kinase (Holbro and Hynes, 2004). When activated, the EGFR kinase phosphorylates several tyrosines in the EGFR C-terminal tail that then serve as docking sites for downstream signaling effectors that initiate signaling cascades and stimulate cell growth and differentiation (Holbro and Hynes, 2004). Three EGFR homologs, HER2 (Neu/ErbB2), HER3 (ErbB3), and HER4 (ErbB4) are found in humans and, together with EGFR, make up the EGFR/ErbB family of receptors. HER2 is an atypical member of this family in that it is not directly activated by ligand but rather serves as a universal heterodimeric partner for each of the other ErbB family members (Holbro and Hynes, 2004).

EGFR was the first cell-surface receptor to be associated with cancer, and abnormal EGFR or HER2 function has subsequently been found to contribute to the severity of many human tumors (Hynes and Lane, 2005). For this reason, agents targeting EGFR or HER2 have been actively pursued as cancer therapies. These agents fall into two general classes: monoclonal antibodies, which bind to receptor extracellular regions and will be discussed here, and small-molecule kinase inhibitors that target the cytoplasmic kinase activity. To date, two monoclonal antibodies against EGFR, Cetuximab (Erbitux) and Panitumumab (Vectibix), have been approved by the FDA for treatment of colorectal and/or head-and-neck cancer, and two EGFR kinase inhibitors, erlotinib (Tarceva) and gefitinib (Iressa), have been approved for the treatment of lung cancer. A monoclonal antibody targeting HER2, Trastuzumab (Herceptin), and a pan-ErbB kinase inhibitor, lapatinib (Tykerb), have also been approved for treatment of HER2-overexpressing breast cancers. Many other ErbB-targeted therapies are under development.

Beginning ~5 years ago, X-ray crystallographic studies of the extracellular regions of ErbB family members uncovered the basic mechanism by which ligand binding