enhancer positively influences, but is not required for, CEBPA expression in multiple cell types and the second enhancer (referred to as the +42-kb enhancer) is only active in myeloid cells. Avellino et al subsequently showed that the +42-kb enhancer contains transcription factor–binding sites that are occupied by several critical myeloid transcription factors, including runt-related transcription factor 1 (RUNX1), the Spi-1 proto-oncogene protein product PU.1, and myeloid–clustered regularly-interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) genome-editing system to remove the proposed 1.1-kb–wide myeloid–specific enhancer in the mouse germ line.6 The authors found that removal of this enhancer significantly decreased CEBPA expression specifically in total bone marrow. In addition, unlike previous models that deleted the entire CEBPA gene, removal of the +42-kb enhancer produced viable animals with an HSC self-renewal defect and abnormal expansion of the multipotent progenitor population characteristic of a preleukemic state.2,7,8

The clinical significance of altered CEBPA expression in hematopoiesis has largely been limited to cases involving mutations in the coding region of the single-exon CEBPA gene itself. Although leukemias with CEBPA mutations are often associated with favorable prognoses, an individual’s risk and prognosis can vary by both the type of CEBPA mutation as well as mutations in other genes or chromosomal abnormalities that occur concurrently.3,5 In particular, the RUNX1–RUNX1 translocated to 1 (RUNX1-RUNXIT1, or AML1-ETO) fusion protein that results from the t(8;21) AML translocation constitutes an entire AML subgroup in which CEBPA expression is suppressed due to the mislocalization of the RUNX1 transcription factor.4

The epigenetic regulation of CEBPA by RUNX1 is not itself a novel finding. The mouse enhancer region homologous to the +42-kb enhancer used by Avellino et al was first reported by Guo and colleagues in 2012.9 In that study, 4 occupied RUNX1–binding sites were identified in the enhancer that, when mutated, greatly reduce CEBPA expression in mouse cell lines. However, whereas Guo and colleagues investigated the effect of an inducible in vivo RUNX1 deletion on the HSC compartment, Avellino and colleagues specifically characterize the significance of the CEBPA enhancer on myelopoiesis and bone marrow engraftment competency (see figure).

In summary, Avellino and colleagues have identified a single enhancer required for myeloid priming in HSCs, and their study provides a critical link between enhancer activation, transcription factor binding, lineage commitment, and gene expression. The proof that deletion of a 1.1-kb enhancer can lead to the unchecked expansion of the multipotent progenitor cell population provides new insight into the role of epigenetics and the preleukemic state.

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Lymphoid Neoplasia

Comment on Aguilar-Hernandez et al, page 3015

IL-4, IgM, and resistance to BTKi and PI3Kδi in CLL

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In this issue of Blood, Aguilar-Hernandez et al1 refine our understanding of the mechanism of action of interleukin 4 (IL-4) in promoting B-cell receptor (BCR)-mediated signaling in primary human chronic lymphocytic leukemia (CLL) and resistance to a Bruton tyrosine kinase inhibitor (BTKi), ibrutinib, and a phosphatidylinositol 3-kinase δ inhibitor (PI3Kδi), idelisib. The tumor microenvironment is firmly established as providing stimuli to cause CLL cells residing in the lymph nodes or bone marrow to proliferate, survive, and resist chemotherapy as demonstrated by animal models, which suggests a requirement for T cells2 and stromal cells3 whereas immunohistochemistry has shown close connections between leukemic B cells and T cells in lymph node proliferation centers. As well as direct cell:cell contacts, growth factors, including IL-4, and chemokines are also important modes of communication between the microenvironment and the leukemic cell. Many of these soluble factors are produced in T cells, and the CD4+ T-cell subset, the follicular helper T-cell subset, and T112 cells may be particularly important in producing IL-4.
IL-4 is an important factor driving normal B-cell proliferation and class switching.\(^4\) Work on the mechanism of action of IL-4 has shown that it modifies BCR signaling: in the absence of IL-4, BCR signals phosphorylate ERK in a PI3K, phospholipase C, and protein kinase C\(\beta\)–dependent manner,\(^3\) these enzymes constituting essential components of the signalosome. However, the presence of IL-4 alters BCR signaling in such a way that ERK phosphorylation is no longer dependent on the signalosome elements.\(^5\) In addition, although the BCR pathway activates NF-\(\kappa\)B through the signalosome, the IL-4 pathway leads to ERK activation without affecting NF-\(\kappa\)B. Thus a model has emerged, mainly from studies of mouse B cells, of parallel BCR pathways—one arm mediated by the BCR in the absence of IL-4 and dependent on the signalosome and the other, in the presence of IL-4, leading to ERK phosphorylation without requiring the signalosome.

More recent work using normal mouse B cells has further defined the mechanism of action by showing that IL-4 induces the proteins Ig\(a\) and Ig\(\beta\) that form a sheath around the transmembrane portion of surface IgM, thereby increasing overall surface IgM expression and increasing B-cell activation and proliferation.\(^6\)

Aguilar-Hernandez et al add to this data by demonstrating apparently similar mechanisms in primary human CLL: they show that IL-4 increases the surface expression of IgM together with that of the human sheath proteins, CD79A and CD79B especially on CLL with unmutated immunoglobulin genes (U-CLL). Interestingly, this alteration might be expected to enhance both the IL-4–dependent and –independent components of BCR signaling in U-CLL, thus providing an additional boost to pathways that are already overactive compared with mutated CLL (M-CLL). In an additional line of work, these authors show that IL-4 reduced expression of the chemokine receptor CXCR4 on the surface of the human leukemic cells. CXCR4 engagement by its ligand CXCL12 produces prosurvival effects in human CLL,\(^7\) and more recently, abrogating BCR signaling with a BTK inhibitor in a mouse model has been shown to reduce expression and function of CXCR4 to lead to reduced retention of leukemic cells in tissue.\(^8\) Therefore, CXCR4 has been implicated in survival and trafficking of CLL. It will clearly take more work to arrive at an overall view of the effects of IL-4 in CLL but Aguilar-Hernandez et al demonstrate how this growth factor modifies signaling in multiple pathways (see figure).

What are the implications of this work for the clinical use of small-molecule inhibitors in CLL? Inhibitors for BTK and PI3K\(\delta\) are available and in clinical use. BTKi, in particular, are well-tolerated and their effects appear to be sustained over several years. Whether the addition of other small-molecule inhibitors to a BTKi, to deepen responses, would be clinically beneficial if it could be accomplished without an increase in toxicity, remains an open question. Aguilar-Hernandez et al demonstrate that IL-4 suppresses the effects of a BTKi and a PI3K\(\delta\)i on BCR signaling and protects against apoptosis. The effects of IL-4 on CXCR4, however, would appear to suggest that it might reduce the prosurvival effects of CXCL12 signaling. Small-molecule inhibitors and blocking antibodies against CXCR4 are available, and others have raised the possibility that CXCR4 might be a therapeutic target in CLL.\(^10\)

Intriguingly, the results of Aguilar-Hernandez et al might suggest that the combination of interrupting BCR signaling and CXCR4 signaling could be a useful therapeutic route.

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Comment on Lavallée et al, page 3054, and Maxson et al, page 3094

JAKed up phenotype of CEBPA-mutant AML

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In this issue of Blood, studies by Lavallée et al and Maxson et al provide a powerful example of functional and genomic data integration to reveal unexpected correlations between genotype and phenotype in acute myeloid leukemia (AML).1,2

The landscape of recurrent genetic events in AML has been elegantly unraveled in recent years3; however, for most disease subsets this has not yet led to facile deployment of therapeutics targeting aberrant genetic events. The resulting gap in our ability to decipher genetic drivers versus our capacity to harness this knowledge for therapeutic advantage will require the integration of genotype information with transcriptomic, proteomic, and drug response data. The studies by Lavallée et al and Maxson et al conclude that AML patients with biallelic mutation of CCAAT enhancer binding protein α (CEBPA) exhibit dysregulation of JAK-STAT pathways and dependence upon Janus kinase–signal transducer and activator of transcription (JAK–STAT) signaling.

In the first study, Lavallée et al show that JAK–STAT pathways are dysregulated more frequently than average in biallelic CEBPA (CEBPAββ)–mutated AML, which correlates with sensitivity to JAK kinase inhibitors. Both this study by Lavallée et al and another study by Maxson et al show that CEBPA mutations also frequently coincide with secondary mutations in JAK–STAT pathway regulators, especially granulocyte colony-stimulating factor 3 receptor (CSF3R). These findings yield the exciting conclusion that JAK kinase inhibitors could be an effective component of improved therapeutic regimens for AML patients with CEBPAββ mutation, a hypothesis that can be tested with prospective clinical trials targeted to this patient population.

CEBPA is a transcription factor that is crucial for regulating myeloid lineage development. In particular, it has been shown to regulate expression of CSF3R, thereby promoting neutrophil differentiation.4 Mutations in CEBPA are observed in 5% to 15% of AML patients3,5,6 and those mutations can be classified into the following 3 categories: (1) monoallelic mutation, (2) typical CEBPAβi AML (ie, biallelic mutation with one allele harboring an N-terminal frameshift or nonsense mutation and the other allele having an inframe insertion or deletion of the C terminus), and (3) atypical CEBPAβi AML (ie, biallelic mutation with other configurations of mutations on each allele [eg, point mutations, two N-terminal mutations]).7 Prior studies have shown that CEBPAβi AML patients exhibit distinct gene expression patterns compared with those who have wild-type (WT) or monoallelic CEBPA AML, and only CEBPAβi mutation confers improved prognosis.7 However, the significance of these CEBPAβi AML gene expression signatures for disease pathogenesis or design of targeted therapies has remained unclear.

CSF3R is a cell surface receptor that binds the ligand CSF3 and activates downstream signaling cascades, most notably JAK–STAT.
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