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may constitute a distinct subclass among ALK-negative ALCLs. Interestingly, ERBB4-positive cases frequently displayed an unusual Hodgkin-like morphology, but ERBB4-positive ALCL patients did not differ from other ALK-negative ALCL cases in terms of survival. Further studies are needed to determine whether ERBB4-expressing ALCLs overlap with other genetic subsets of ALK-negative ALCL and are also present in primary cutaneous ALCL. Whether ERBB4-positive ALCL patients may benefit from specific therapies remains to be explored. Indeed, the only partial effect of the kinase inhibition on the tumor growth in the preclinical model may indicate the need for combination therapies in relapsed or refractory ERBB4-positive ALCL patients. The identification of such patients in the clinical practice is another issue: in the absence of reliable ERBB4 antibodies applicable for immunohistochemistry in the clinical arenas, molecular tests would be needed unless the value of MMP9 expression as an alternative biomarker is further confirmed. Finally, the mechanisms leading to ERBB4 aberrant expression, especially whether epigenetic deregulation is involved, need to be clarified and better understood. This is of particular interest because various mutations affecting epigenetic modifiers have been recently described in PTCLs.

The article by Scarfò et al highlights how novel bioinformatics algorithms applied to a gene expression data set help identify novel molecular subsets within apparently homogeneous diseases. The recognition of this subclass of ERBB4 expressing ALK-negative ALCL, potentially targetable, is a new step toward a better understanding of ALCL pathogenesis. These findings add to the molecular landscape of ALK-negative ALCL which appears to include multiple subgroups driven by different genetic aberrations.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● MYELOID NEOPLASIA

Comment on Li et al, page 233

DNA binding modes of leukemia oncoproteins

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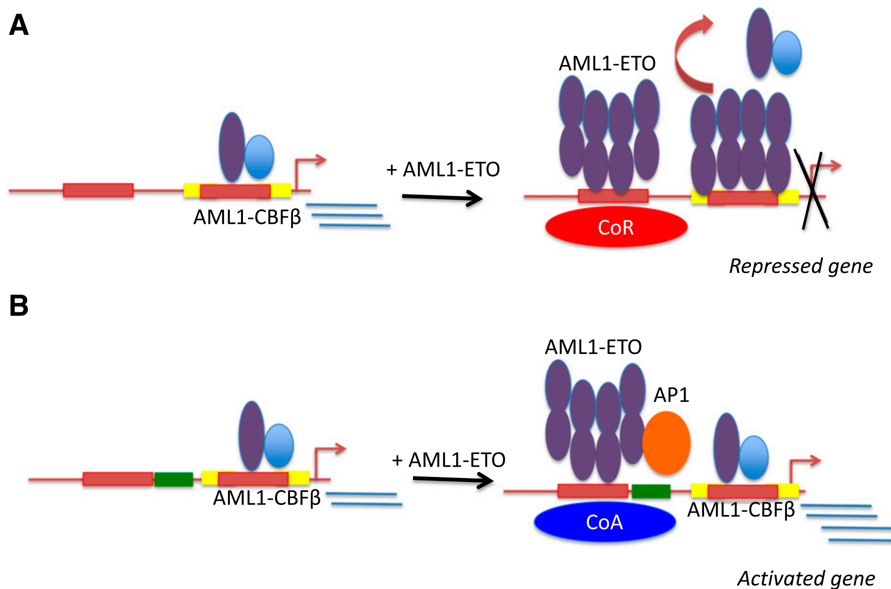
In this issue of *Blood*, Li et al expand our view on the mechanism of action of leukemia-associated oncoproteins and how they deregulate gene expression through altered modes of binding to DNA.[1]

Acute myeloid leukemias (AMLs) originate in many cases from chromosomal translocations that yield fusion proteins of transcription factors. It has been therefore assumed that altered regulation of transcription is a key mechanism of oncogenic transformation.[2] Indeed, several leukemia-associated oncoproteins (including AML1-ETO, also known as RUNX1-RUNX1T1, a fusion protein of the transcription factor AML1, a critical regulator of hematopoiesis) directly recruit transcriptional corepressor (CoR) complexes and silence genes expressed during myeloid maturation, triggering a block in differentiation.[3] This model, however, is too simplistic: gene expression profiles and genome-wide binding studies have shown that as many target genes are repressed by binding of various oncoproteins as there are genes which are upregulated.

Here, Li et al perform a carefully designed set of genome-wide binding studies (chromatin immunoprecipitation sequencing [ChIP-seq]) of the oncoprotein AML1-ETO and of wild-type AML1 because they coexist in leukemic cells due to the presence of 1 nontranslocated, wild-type allele together with the allele involved in the chromosome translocation.[1]

Comparing the 2 binding distribution profiles, the authors conclude that AML1-ETO and AML1 colocalize on the large majority of genomic binding sites, and that AML1-ETO expression leads to a partial redistribution of AML1. As previously shown, AML1-ETO is found in association with other proteins of the AML1-ETO transcription factor complex, including other transcription factors with their own DNA binding specificity.[4] The co-occurrence of AML1-ETO and AML1 on chromatin, however, is not the consequence of overlapping binding sites, as previously thought (see Ptasinska et al[5] for a recent genome-wide study): in fact,



AML1-ETO works as a repressor or an activator of transcription. (A) Binding of AML1-ETO at sites associated with repressed genes leads to displacement of AML1 (that binds DNA as heterodimer with core-binding factor β [CBFβ] to sites with a slightly different motif) and recruitment of CoR complexes that lead to reduced transcription. (B) Binding of AML1-ETO at sites associated with activated genes occurs in combination with other transcription factors (AP1 characterized in this study) and recruitment of coactivator (CoA) complexes. In this case, AML1 is not displaced and stays at its own binding site, and the amount of AML1-ETO present is less than observed at repressed genes.

the 2 proteins tend to bind to adjacent sites within a short interval (around 100 bp). A slightly different binding site motif is found for AML1 and AML1-ETO, with the latter one showing a shorter, potentially more "relaxed" binding mode (see figure). The difference in binding was confirmed in vitro in DNA binding studies.

When these binding profiles were put in relationship to gene expression, an interesting correlation was observed between higher AML1-ETO occupancy accompanied by a reduction in AML1 binding on repressed genes, whereas the opposite correlation (ie, higher AML1 binding) was observed on upregulated genes. A potential mechanistical explanation of the repression on those target genes is therefore the partial displacement of AML1 due to binding of AML1-ETO and recruitment of negative cofactors: indeed, AML1-ETO depletion enhanced AML1 binding on those sites, and led to enhanced gene expression (see figure). The finding that AML1-ETO and AML1 may have distinct binding sites at target genes, and that the relative amount of binding correlates with transcriptional outcome, is offering a key to interpreting the interplay between the wild-type and fusion protein on chromatin. Of note, the role of the known ability of AML1-ETO (in contrast to wild-type AML1) to oligomerize, in mediating the intensity of binding observed at the different target sites, has not been explored in this study.[6]

How then is upregulation of AML1-ETO target genes achieved? Previously, it was shown that AML1-ETO is able to recruit transcriptional CoAs, though the molecular mechanisms regulating CoA vs CoR recruitment are not understood.[7] Here, analysis of AML1-ETO genome-wide binding sites revealed enrichment for AP1 binding sites at AML1-ETO upregulated target genes, confirmed by sequential ChIP studies (Re-ChIP, see figure). Inhibition of AP1 led to downregulation of AML1-ETO upregulated genes, indicating that AP1 recruitment indeed has functional consequences.

The fact that additional transcription factors (such as AP1, or components of the AML1-ETO transcription factor complex) are binding to their own binding sites to upregulate AML1-ETO targets suggests that the target gene primary DNA sequence context and the presence and specificity of additional transcription factor binding sites are critical determinants of the response to AML1-ETO binding (see figure). It remains to be seen whether particular chromatin features (distinct histone posttranslational modifications preexisting, or induced by AML1-ETO binding) are also playing a role because genome-wide profiles of histone marks were not analyzed in parallel.

Finally, one must consider that the generation of a fusion protein is only the first event in cellular transformation, and additional genetic/epigenetic events occur to reach the leukemic stage. Leukemic cell lines are, however, in this as in most cases, the model system chosen for the mechanistical studies. It would be of great interest to analyze the initial events triggered by AML1-ETO expression in normal cells, and to follow eventual changes in modality of binding, cofactor recruitment, and transcriptional responses during the steps to full transformation. Though we know more about DNA binding modes of AML1-ETO (potentially translatable to other fusion proteins), most remain unknown.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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