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Rethinking paraneoplastic eosinophilia
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In this issue, Dr. Xu and colleagues dissect the mechanism of eosinophilia that accompanies the ETV6 fusion ACSL6::ETV6. ETV6 is a transcription factor with predominantly inhibitory activity on its target genes (1). The ETV6 locus is involved in leukemia through a variety of different mechanisms (2, 3). The oldest recognized role is as a fusion partner in the t(12;21)(p13;q22) translocation, which results in the generation of ETV6::RUNX1 fusions (formerly known as TEL-AML) in acute lymphoblastic leukemia (ALL). ETV6::RUNX1 fusions are present in about 20% of ALL and enriched in patients with standard risk features (i.e. children between 1 and 10 years of age, low presenting white blood cell count). ETV6::RUNX1 fusion ALL has an excellent prognosis, particularly in the setting of additional low risk criteria (4). In addition to ETV6::RUNX1, there are multiple additional ETV6 fusions in ALL with a range of different fusion partners (5). These leukemias share transcriptomic features with ETV6::RUNX1 ALL, however, they are genetically more complex and outcomes for these patients are worse (6). Most breakpoints within ETV6 occur within the first 60 amino acids (AA) of the 450 AA long ETV6 protein. Most create a fusion gene with the 5’ part of ETV6, although detailed RNA and protein expression or functional data on these fusions is incomplete.

In AML, ETV6 is also found as part of fusions with a range of different fusion partners, including PDGFRβ, FGFR3, ABL1, FLT3, JAK2, MN1 and ACSL6(2, 7). Most kinase fusion (PDGFR, ABL1, FLT3, JAK2) fuse much of the kinase open reading frame to a small 5’ fragment of ETV6 (typically exon 5), resulting in expression of a fusion transcript and fusion protein with aberrant
kinase activity. A second type of ETV6 fusion involves transcriptional regulators such as MECOM (EVI1). MECOM is a hematopoietic stem cell transcription factor that is aberrantly expressed via translocation into other loci as well, most famously the GATA2 locus. Both in-frame and out-of-frame fusion that just results in MECOM expression have been reported. A third type of fusions involves 3’ ETV6 transcripts and regulatory regions. ETV6 has a large downstream super-enhancer. 5’ fusion partners can either fuse in frame to 3’ETV6 exons, or translocate out of frame with the ETV6 3’ enhancer driving aberrant expression of the fusion partner. One of the best studies examples of this type of fusion is the ETV6-MN1 fusion. Finally, several ETV6 translocations may or may not generate in frame fusions that lack transforming ability, but lead to overexpression of the entire reading frame of adjacent genes that do transform. Examples of such fusions include CHICK2::ETV6 and the ACSL6::ETV6 fusion that is the topic of this manuscript.

ACSL6::ETV6 is a rare but recurrent fusion in AML and – as in the patient described here – ALL (1). Pronounced eosinophilia is a hallmark of these leukemias. In this study, Xu and colleagues used comprehensive genomic analysis to better understand the biological effects of this fusion event. The ACSL6::ETV6 fusion is a reciprocal translocation (1). The genomic breakpoint in ETV6 on chromosome 12 and upstream of the ACSL6 coding frame (Figure 1). However, on an RNA-basis, ETV6 exon 1 is fused to ACSL6 exon 2. This results in a frameshift, premature stop codon, and no expression of an ASCL6-ETV6 fusion protein. The reciprocal derivative chromosome contains the majority of the ETV6 coding frame and 3’ super enhancer region translocated into the ACSL6 adjacent intergenic region, and no fusion RNA or protein are generated. Thus, the ACSL6::ETV6 translocation does not generate an oncogenic fusion protein, or aberrant expression of one of the direct fusion partners. Rather, it splits 5’ and 3’ ETV6 regulatory regions and perturbs the chromatin architecture of the breakpoint adjacent regions on the target chromosome 5. This results in increased expression of Interleukin 3, Interleukin 5, P4HA2 and SLC22A5, which translocated into the vicinity of the 3’ ETV6 super-
enhancer. Increased expression of IL3 and IL5 by the leukemia cells in turn result in the profound eosinophilia that accompanies ACSL6::ETV6 leukemias. The eosinophils themselves are not part of the leukemic clone.

Bromodomain inhibitors have been reported to predominantly affect transcription driven by super-enhancers, and Xu and colleagues were able to show that the bromodomain inhibitor tool compound JQ1 suppressed IL3 production of ACSL6::ETV6 leukemia cells (1). Bromodomain inhibitors were first reported to exert anti-leukemic activity in 2011, and despite multiple clinical trials their clinical efficacy as anti-cancer drugs is still not clear. However, ACSL6::ETV6 leukemia with eosinophilia could constitute a promising application.

While the elegant studies by Xu and colleagues explain the molecular reason for the paraneoplastic eosinophilia accompanying ACSL6::ETV6 fusions (1), the actual oncogenic mechanism remains unexplained. It is important to note that ETV6 inactivating mutations are common in hematopoietic malignancies, and germline inactivating mutations of ETV6 cause familial thrombocytopenia and a predisposition to ALL (8, 9). ETV6 haploinsufficiency therefore is likely to contribute to the mechanism of transformation of ETV6 translocations (2, 3).

Furthermore, the IgH-IL3 fusion (product of the t(5;14)(q31;q32) translocation) results in increased IL3 production, appears to be an initiating event in ALL and is also accompanied by massive eosinophilia (10). Future functional studies will need to clarify if IL3 (and IL5) overexpression in combination with ETV6 inactivation is sufficient to initiate malignant transformation, or whether other adjacent genes such as P4HA2 also play a role.
1. Xu W, Tian F, Tai X, et al. ETV6::ACSL6 translocation-driven super-enhancer activation leads to eosinophilia in acute lymphoblastic leukemia through IL-3 overexpression. Haematologica. xxx
**Figure 1: The ACSL6::ETV6 fusion.**  
**Top:** In the patient with an ACSL6::ETV6 fusion ALL described in by Xu ad colleagues, the ETV6 breakpoint is located within Intron 1. **Middle:** The 5’ portion of ETV6 if fused to the intergenic region 5’ to the ACSL6 gene. The fusion event results in transcription of a fusion RNA, whereby exon 1 of ACSL6 is skipped, and ETV6 exon 1 is fused to ACSL6 exon 2. This induces a frameshift and premature stop – no ETV6-ACSL6 fusion protein is expressed. **Bottom:** The reciprocal translocation places the large 3’ super enhancer of ETV6 in the vicinity of the IL5, SLC22A5, P4HA2 and IL3 genes, which are overexpressed as a result. ETV6 haploinsufficiency and IL3 overexpression likely cause or contribute to leukemic transformation. In parallel, the high levels of IL3 produced by the leukemia cells result in paraneoplastic eosinophilia.
ETV6 locus

ETV6-ACSL6

DNA

RNA

Reciprocal translocation

IL5  SLC22A5  P4HA2  IL3  ETV6 exon 2-8

ETV6 superenhancer

?  ?  ?

Leukemia cell growth  Eosinophilia