Molecular Basis of ETV6-Mediated Predisposition to Childhood Acute Lymphoblastic Leukemia

Lab meeting journal club
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Background

- GWAS analyses have identified common polymorphisms associated with ALL risk affecting: IKZF1, ARID5B, CEBPE, CDKN2A, BMI1-PIP4K2A, and TP63
- Most recently, germline variants in ETV6 have been identified
  - As a transcription factor, ETV6 primarily functions to repress the expression of a wide spectrum of target genes, many of which are highly regulated during hematopoiesis.

A repressor may get in the way of the basal transcription factors or RNA polymerase, making it so they can’t bind to the promoter or begin transcription.

A screen of 23 families with autosomal dominant thrombocytopenia, high red cell mean corpuscular volume (MCV) and occurrences of B-cell precursor acute lymphoblastic leukemia found two with ETV6 mutations.

Noetzli et al. *Nat Genet.* 2015
Targeted sequencing of ETV6 in 4,405 childhood ALL cases discovered 31 exonic variants (4 nonsense, 21 missense, 1 splice site, and 5 frame shift variants) that are potentially related to ALL risk in 35 cases.
31 B-ALL and 2 AML samples with germline ETV6 variants from 32 unique patients at St. Jude Children’s Research Hospital, Dana-Farber Cancer Institute or the Children’s Oncology Group.

**Diagnostic** bone marrow or Peripheral blood:
- Total RNA
- Tumor DNA
- Whole genome seq of matched germline and tumor DNA (n = 30/32)
- Only tumor samples were sequenced (n = 2)
- Whole exome seq was applied on one paired sample

**Clinical remission** bone marrow or Peripheral blood:
- Germline DNA
- Whole transcriptome seq (n = 22/33)

231 children with ALL from the Ma-Spore ALL clinical trial, and 30 ETV6-RUNX1 ALL cases from St. Jude Children’s Research Hospital.

- Whole transcriptome seq data
- Whole genome seq data

13 ALL cases with somatic ETV6 variants from St. Jude Children’s Research Hospital.

- Somatic variant data
- Germline variant data
- Transcriptome seq data
Results

- measured transcription repressor activity with a luciferase reporter assay
- 22 of the 34 variants tested (65%) exhibited significant impairment of transcription repression
  - 22 damaging variants
  - 12 WT like variants
- 22 damaging variants exhibited a significant decrease in ability to bind to an ETS DNA consensus sequence
- 22 damaging variants exhibited a significant loss of nuclear localization
A summary of the functional classification for each variant as either damaging or WT-like
- Most of the damaging variants are in the ETS binding domain
- Damaging variants also include more nonsense, frameshift and splicing variants compared to WT-like variants
• Assessed luciferase activity following co-transfection of constructs encoding WT and increasing amounts of variant ETV6.
• Consistently observed a dose-dependent inhibition of WT ETV6 activity, pointing to a potential dominant-negative mechanism of action.

• All selected variant ETV6 proteins exhibited robust dimerization with WT ETV6, Using a co-immunoprecipitation assay
• These variants caused a general reduction in the ratio of nuclear to cytoplasmic WT ETV6
Key points

- Leukemia predisposition variants in ETV6 lead to dramatic loss of transcription repressor activity, mainly by disrupting DNA-binding.
- Germline ETV6 variants influence ALL transcriptional profile with a striking resemblance of ETV6-RUNX1 ALL, but unique somatic mutations.