Abstract

Childhood acute leukemias are genetically complex disorders, with recurrent or random aberrations found in most patients. Their proper functional characterization is crucial for understanding the role they play in the process of leukemogenesis. We aimed to identify and characterize the genetic background of two leukemic entities.

The transient myeloproliferative disorder (TMD) is a preleukemic condition that occurs in 10% of newborns with Down syndrome. Trisomy 21 together with in-utero gained mutations in the GATA1 gene are essential in TMD and represent an ideal "multi-hit" model to study leukemogenesis. We investigated an alternative pathogenic mechanism enabling TMD development in a confirmed absence of trisomy 21. Novel deletions in the GATA1 and JAK1 genes were described as potential drivers of this TMD. The deletion D65_C228 in GATA1 results in the expression of an aberrant isoform, which is predicted to lose transactivation potential and, more importantly, to partially lose the ability of recognizing physiological DNA binding sites, possibly triggering TMD alone. Our thorough characterization of JAK1 F636del questions its role in TMD development. Analysis of JAK/STAT signaling suggested decrease of kinase activity upon F636 loss. Cells harboring the aberrant JAK1 did not obtain cytokine-independent growth when assessed in the Ba/F3 assay. Moreover, JAK1 F636del had no impact on cell proliferation and maturation when studied in a "prenatal" environment represented by fetal hematopoietic stem and progenitor cells expressing mutated GATA1. Combined, we described the molecular events in the first case of trisomy 21-independent GATA1-mutated TMD.

The ETV6-ABL1 fusion gene represents a rare recurrent event in acute lymphoblastic leukemia (ALL). We characterized a single chromosomal rearrangement leading to the formation of ETV6-ABL1 together with two novel fusion genes: ABL1-AIF1L and AIF1L-ETV6. The production of three in-frame fusion genes from a single rearrangement is a rare event. Moreover, we report, to the best of our knowledge, the first disruption of the AIF1L gene in leukemias. Chimeric protein analysis in HEK293T cells showed that AIF1L-ETV6 is expressed and localized in the nucleus, where it may bind to DNA via its ETV6 domain. We demonstrated the prenatal origin of the observed rearrangement by detecting the patient-specific ETV6-ABL1 fusion gene breakpoint sequence in the patient's Guthrie card by PCR, therefore

confirming that all three fusion genes are insufficient to cause overt leukemia. Additional "second" hit mutations were required, in this case probably represented by deletions in the IKZF1 and/or CDKN2A/B genes.

Our findings regarding trisomy 21-independent TMD shed new light on the pathogenesis of this intensely investigated leukemia-like condition. Furthermore, our thorough characterization of a unique chromosomal rearrangement resulting in the prenatal production of multiple in-frame fusion genes expands our knowledge regarding ETV6-ABL1-positive ALL.