Acute Myeloid Leukemia

Assessment of submicroscopic genetic lesions by single nucleotide polymorphism arrays in a child with acute myeloid leukemia and FLT3-internal tandem duplication

The same FLT3-internal tandem duplication (ITD) positive clone was detected at diagnosis and relapse, but not at birth, in a child with M1 acute myeloid leukemia. Single nucleotide polymorphism arrays demonstrated that chromosome 13 acquired uniparental disomy, in association with del(9q), represented a progressive event in the course of the disease, and it was responsible for the homozygous FLT3-ITD at relapse.

We studied the clonal evolution from birth to diagnosis and relapse in a child with FLT3-internal tandem duplication (ITD)-positive acute myeloid leukemia (AML) who experienced two early relapses and for whom we fortuitously had a cord blood sample. FLT3-ITD was used as a minimal residual disease marker, and to backtrack the leukemic cells into the cord blood.

The patient was a female diagnosed at 6 years of age with AML-M1. At diagnosis, the patient’s bone marrow karyotype was normal; the molecular screening showed FLT3-ITD mutation, a wild type NPM1 sequence and negativity for CBFB-MYH11 and CBFA-ETO fusion genes. She underwent autologous bone marrow transplantation in complete disease remission following induction therapy...
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(AIEOP-02 AML protocol). However, 3.5 months later the patient relapsed. Four months after an allogeneic bone marrow transplant, she had a second relapse and died of disease progression.

FLT3-ITD monitoring from diagnosis to relapse was performed by a patient-specific highly sensitive (10⁻⁴) real-time quantitative (RQ)-polymerase chain reaction (PCR), showing a progressive decrease of minimal residual disease (Figure 1, panel D). The same FLT3-ITD clone re-emerged at relapse, suggesting that the leukemic clone responsible for the first diagnosis was stable and could be used for backtracking the leukemia into the cord blood.

When tested by highly sensitive RQ-PCR, the cord blood DNA resulted negative for the FLT3-ITD mutation, consistent with the current hypothesis that FLT3-ITD is a secondary event in leukemogenesis. This is the first direct report on post-natal occurrence of FLT3-ITD.

We then applied a 10K single nucleotide polymorphism (SNP) array approach (Affymetrix, Inc., Santa Clara, CA, USA) to search for genome-wide loss of heterozygosity and/or copy number changes of genes involved in tumor evolution, comparing cord blood, diagnostic and relapse samples. SNP arrays on the relapse sample showed chromosome 13 uniparental disomy (UPD) (Figure 1, panel A), confirmed by fluorescent in situ hybridization (FISH) with a 13q14.3 LSI D13S319 SpectrumOrange probe (Vysis, Abbott GmbH, Germany) (Figure 1, panel B). We assume that, through the evolution of the disease from diagnosis to relapse, chromosome 13 harboring the FLT3-ITD had undergone non-disjunction. The leukemic cells with chromosome 13 UPD and FLT3-ITD homozygosity may have acquired a clonal advantage over those with the wild type FLT3 allele. This finding was also confirmed by qualitative PCR, as shown in Figure 1, panel C. Agarose gel electrophoresis of FLT3-ITD PCR products indicated the increase of the ITD PCR amplification over the wild type band. Acquired UPD has been recently demonstrated in approximately 20% of AML and UPD on chromosome 13 was detected by SNP arrays in three out of five relapse samples from FLT3-ITD positive patients. In addition to UPD13, an interstitial deletion was found by SNP arrays on chromosome 9q21.32-q33.2 (Figure 2, panel A), confirmed by FISH with BAC-derived probe RP11-402M4 located on 9q31.1 (Invitrogen, Paisley, UK) (Figure 2, panel B). Unfortunately, no information about the karyotype was available on the relapse sample to exclude the presence of structural chromosomal rearrangements. Del(9q) has been reported in about 2% of AML cases, frequently associated with t(8;21) and rarely with t(15;17). The presence of tumor suppressor genes potentially involved in disease progression on the 9q deleted chromosomal fragment must be further explored.

We hypothesize that a still unknown primary event, such as a mutation in a proto-oncogene, hit the patient's

Figure 2. Analysis of chromosome 9 deletion on a relapse sample. A. A schematic diagram of SNP calls on chromosome 9. Copy number ratio was calculated for each SNP using a ratio of the signals from the germ line (cord blood) and leukemia. For each SNP a mean number of copies in a window of 10 adjacent SNP was used. Green lines represent SNP calls retained in the germ line and the leukemia, with light green representing heterozygous and dark green homozygous SNP. Red lines represent loss of call compared with the germ line. In the relapse sample, there is complete LOH with decrease in the copy number on chromosome 9q21.32-q33.2, suggesting interstitial deletion. B. FISH analysis with BAC probe RP11-402M4 shows only one signal, validating SNP array results on 9q deletion.
leukemia stem cell and that the FLT3-ITD occurred as a further step, affecting the same stem cell population. Subsequently, the cell harboring FLT3-ITD accumulated both chromosome 13 UPD and 9q deletion, giving rise to the subsequent relapses.

In summary, by using a combined SNP array and RQ-PCR approach we have been able to indicate the post-natal origin of the FLT3-ITD mutation observed at diagnosis in this AML patient, and to demonstrate the occurrence of loss of heterozygosity as a mechanism of disease progression, which may be associated with both copy number changes -del(9q)- or UPD (FLT3-ITD homozygosis). We are aware that the primary event(s) giving rise to leukemia in association with FLT3-ITD mutation may be represented by aberrations not detected by SNP array analysis, and affect genes involved in differentiation pathways, as expected from the model of co-operative mutations. Other methods must be applied in order to find these class II putative mutations.

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References


