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Molecular approaches for reveal a new three-way variant t(8;19;21) in a pediatric case of Acute Myeloid Leukemia with RUNX1-RUNX1T1

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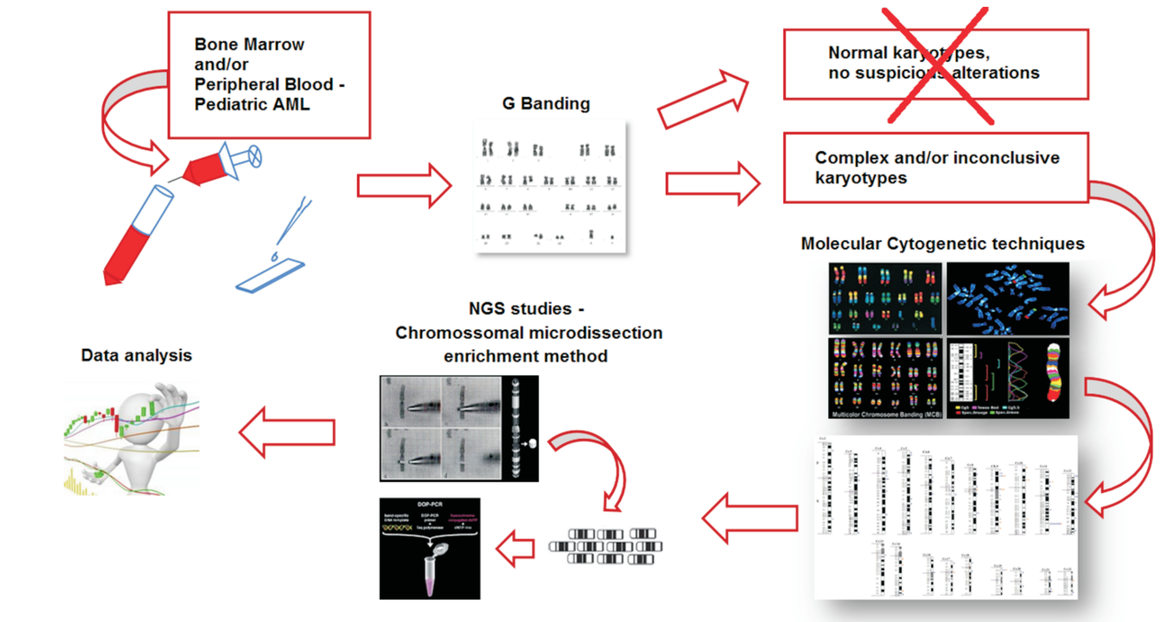
OBJECTIVE

The translocation t(8;21)(q22;q22)/*RUNX1-RUNX1T1* is one of the most common abnormality in childhood acute myeloid leukemia (AML) (~10–20%). Molecularly, it is defined by the involvement of the *RUNX1* gene on 21q22 chromosome (Chr) region and the *RUNX1T1* gene on 8q22, generating the *RUNX1-RUNX1T1* fusion gene. This fusion protein promotes alterations in cell differentiation, proliferation, and apoptosis. Also, the fusion product is a candidate as a driver of myeloid leukemogenesis for the AML-M2 subtype. Although t(8;21) is associated with a good prognosis, 3–4% of AML cases with the t(8;21)(q22;q22) also present complex karyotypes, and the impact of the complex t(8;21) variants remain controversial in the literature. Besides, these translocations are rare, so there is limited information on their prognostic impact. Thus, the clinical relevance and implications of t(8;21) variants in pediatric patients are yet to be determined. Herein we report a child with AML harboring a novel three-way cryptic variant t(8;19; 21), as revealed by detailed molecular studies.

MATERIAL AND METHODS



This project was approved by the research ethics committee of INCA (#088/07)

Patient

A 9-year-old girl that was admitted to IPPMG Hospital with a 1-month history of petechiae and ecchymosis, and 4 days of axillary adenomegaly with fever. At admission, her white blood cell count (WBC) was 12 x 109/l, platelet count was 36 x 109/l and hemoglobin 7,8 g/dl. Physical examination revealed lymphadenopathy involving the axillary region. Morphological examination of her bone marrow showed blasts suggesting morphology M2 (FAB classification). Flow cytometry revealed 20% of blasts with 3 different populations. The bigger one were positive for CD34, CD45, MPO, CD13, CD33, CD81, CD71, CD19 and CD123, thus suggestive the diagnosis of AML-M2.

The patient was stratified as being at standard risk and treated on AML-BFM-2012 protocol. After induction she didn't achieved remission, the MDR was 5,9%. After four courses of chemotherapy, before receiving the maintenance, she achieved remission 6 months after initial diagnosis, but her qualitative molecular biology remained positive up to now. The quantitative study isn´t possible. At this moment, the patient is under remission, with no indication for bone marrow transplantation.

Conventional Cytogenetics

Cytogenetic analysis was performed at diagnosis, before treatment administration, in bone marrow samples cultured for 24 hours according to the standard protocol.

Molecular Assays

Fluorescence *in situ* hybridization-based experiments were conducted using both, homemade and commercial probes, according to the manufacturers' instructions.

* The karyotypes were described according to the International System for Human Cytogenetic Nomenclature.

RESULTS

Conventional cytogenetics showed 46,XX,t(8;19;21)(q22;?p;q22) (**Figure 1**). FISH analysis confirmed a cryptic fusion *RUNX1-RUNX1T1* on derivative chromosome 8 with a *RUNXIT1* signal on derivative chromosome 19 (**Figure 2**).

In order to clarify the breakpoints of this complex rearrangement, we conducted WCP studies, defining the final karyotype as: 46,XX,t(8;19;21) (q21.3;q13.43;q22.12) (**Figure 3**).

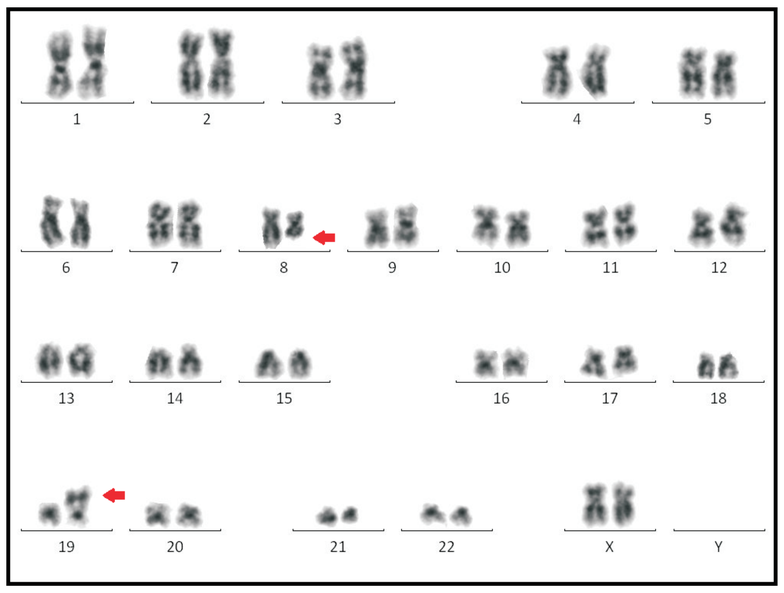


Figure 1: G-banded karyotype. In the patient's karyotype the red arrows point a loss of chromosome material in chromosome 8 and a gain in chromosome 19. Chromosome 21 appears to be normal by G-banded analyses.

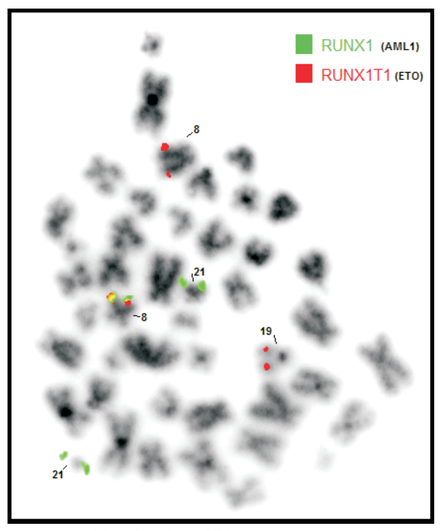


Figure 2: FISH assay. A FISH experiment using the Locus specific (LSI) AML1-ETO dual color, dual fusion commercial probe revealed the translocation t(8;21) by showing the RUNX1-RUNX1T1 fusion on derivative chromosome 8. It also showed a RUNX1T1 splitted signal on der chromosome 19.

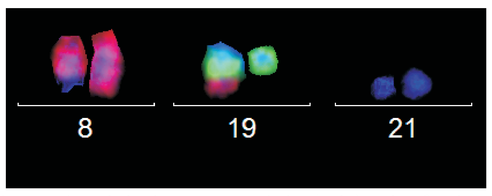


Figure 3: Molecular cytogenetics. FISH experiment using whole chromosome painting (WCP) probes for chromosomes 8 (pink), 19 (green) and 21 (blue), revealed the three-way translocation t(8;19;21).

DISCUSSION AND CONCLUSION

The t(8;21)(q22;q22)/RUNX1-RUNX1T1 is related to a good prognosis, however, on rare occasions, the involvement of a third chromosome occurs in this translocation, which may confer an adverse prognosis to the patient, so the prognostic value of the RUNX1-RUNX1T1 fusion has been widely discussed in the literature. Therefore, the relevance and clinical implications of variant translocations t(8;21) in pediatric AML patients still need to be established.

The combination of conventional and molecular cytogenetic techniques proved to be very efficient, because in contrast to other cases in the literature, the variant t(8;19;21) (q21.3;q13.43;q22.12) described in this study, presented a new breakpoint, being, therefore, a new finding.

Of importance, in this and other cases from our cohort which involve a variant t(8;21), part of the RUNX1T1 gene always translocates to the third chromosome involved in the translocation. Thus, the observation of this similarity in the complex variants t(8;21) formation mechanism, reinforces the importance of a precise characterization and a continuous study of these complex cases.

It is important that cases with complex karyotypes and a variant RUNX1-RUNX1T1, involving $\geq$ 3 chromosomes, are analyzed by a combination of molecular assays. This approach can provide further knowledge about the heterogeneity of the RUNX1-RUNX1T1 fusion gene and a possible association with a so far controversial prognosis.

Acknowledgements

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