Molecular characterization of complex karyotypes in pediatric acute myeloid leukemia: Report of a rapidly progressive myeloid neoplasm harboring a cryptic *MECOM* rearrangement

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INTRODUCTION

Myeloid neoplasms are a group of heterogeneous hematological disorders that diverge in cell differentiation, proliferation pattern and clinical course. There are recurrent abnormalities related to this group of diseases which are known predictors of a poor outcome and among them, the ones involving the chromosome 7. Although rare, the translocation t(3;7)(q26;q21) may occur in the context of a complex karyotype, often harboring an *MECOM* gene rearrangement. *MECOM* is a transcription factor which plays an essential role in cell proliferation, maintenance and also epigenetic changes, whose aberrant expression levels are associated with adverse survival rates. Hence, its detailed characterization could provide new relevant biological and clinical information for helping patients with myeloid neoplasms.

OBJECTIVES

In this doctoral project, we aimed to detect and characterize complex karyotypes by combining conventional cytogenetic techniques and molecular approaches to better define and understand their biological role in pediatric AML.

In this context, we describe a unique karyotype, harboring three copies and *MECOM* gene overexpression, in a child with a myeloid neoplasm which presented a very poor outcome.

CASE REPORT

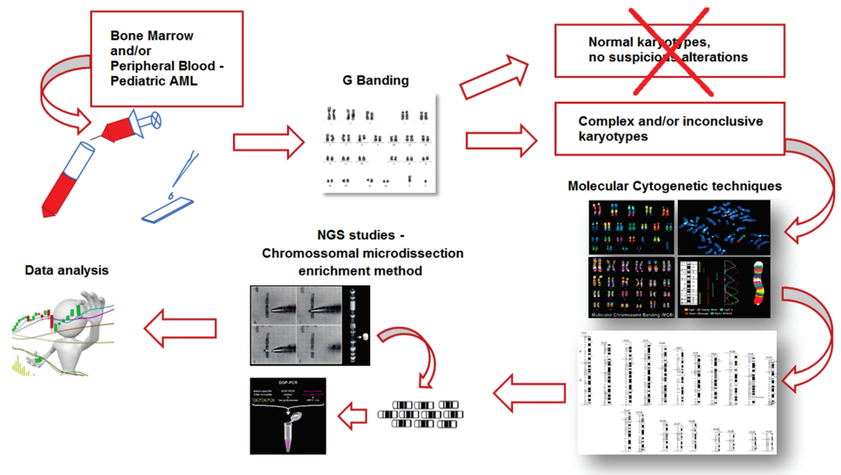
A 3-year-old girl was admitted to the Pediatric Oncohematology Service of Hospital Federal da Lagoa, Rio de Janeiro, Brazil. At physical examination, she presented with fever, pallor and a voluminous hepatosplenomegaly (10 cm below the left costal margin).

In the peripheral blood (PB), hemoglobin level was 7,5 g/dL, hematocrit was 13,8%, platelet count was 124 x 10^9/L and white blood cell count was 692 x 10^9/L, with blasts accounting for 28%, being 42% monocytes.

Bone marrow (BM) showed 23% of myeloid blast cells, with the following immunophenotype: CD34+, CD7+, CD45+lo, CD117+, HLADR++, CD13+, CD11b-, CD16-, CD10-, IREM2-, CD64-, CD14-, CD35-, CD33+, CD71-, CD105-, TdT-/+ (50%), CD56-, CD19-, CD123+, CD38+, CD15-/+ (25%), NG2-. BM showed monocytosis (28%), being 50% inflammatory monocytes (CD14+lo/ CD16+/ IREM2+) **(Fig. 1A)**.

The child was submitted to cytoreductive therapy with hydroxyurea, but she died 48 hours after admission due to brain hemorrhage originated from leukostasis.

EXPERIMENTAL DESIGN



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

METHODOLOGY & RESULTS

Conventional Cytogenetics

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

Molecular Cytogenetics

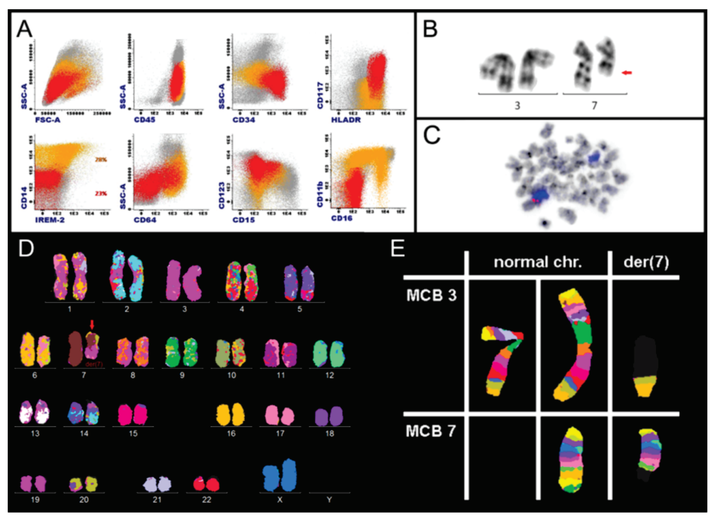
Fluorescence *in situ* hybridization-based experiments were conducted using both, homemade and commercial probes, according to the instructions of the respective manufacturers.

Results

At diagnosis, G-banding defined the karyotype as 46,XX,del(7)(q31?), in 50 metaphases **(Fig. 1B)**. FISH with Subtel7q and WCP7 probes confirmed a loss of chromosome material on derivative 7 **(Fig. 1C)** and suggested that a portion of an unknown chromosome material was present in this same derivative chromosome. M-FISH revealed a duplication on chromosome 3 and the cryptic translocation between chromosomes 3 and 7 **(Fig. 1D)**. MCB technique was performed, overall, refining the complex and cryptic alteration between chromosomes 3 and 7 **(Fig. 1E)**. The final karyotype was characterized as: **46,XX,der(3)t(3;7)(q26;q21.1~21.2),del(7)(q21.1~21.2)**

aCGH revealed two large genomic imbalances: A gain of 26.04 Mb in the region of 3q26.2 to 3qter, between positions 168,986,287 and 198,022,430, and a loss of 66.6 Mb in the region of 7p21.2 to 7qter, between positions 92,453,443 and 159,138,663 **(Fig. 2A)**.

RT-PCR confirmed the presence of the wild-type *MECOM* and *CDK6* isoforms. *MECOM* transcription level analysis in the BM revealed a significant high-level expression of *MECOM* (458,5-fold) and also a greater expression of *CDK6* gene (35,2-fold) **(Fig. 2B)**.



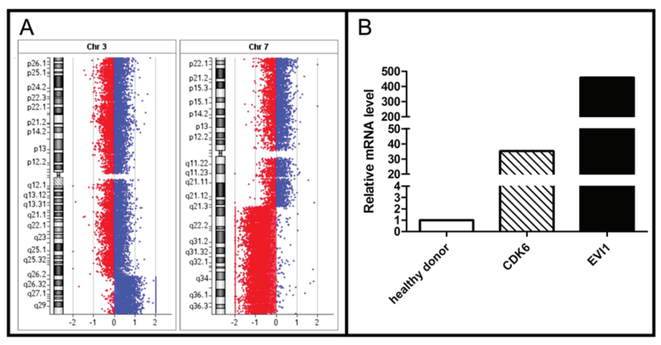


Figure 1. **(A)** BM immunophenotype showing 23% of blasts (red dots) and 28% of monocytes (orange dots) being 50% inflammatory monocytes. **(B)** Partial G-Band Karyotype showing chromosomes 3 and 7. The red arrow points the del(7q31). **(C)** FISH with Subtel7q (Red signal) and WCP7 (blue) probes showing a loss of chromosome material on derivative 7. **(D)** M-FISH Karyotype showing the translocation t(3;7). The red arrow points the derivative chromosome 7. **(E)** MCB panel for chromosomes 3 and 7, showing the derivative chromosome 7 with material translocated from chromosome 3. Figure 2. **(A)** aCGH characterized a gain in 3q26.2 to 3qter and a loss in 7p21.2 to 7qter. **(B)** Relative levels of mRNA of *CDK6* and *MECOM* genes. The fold-change was calculated in relation to average expression of two healthy donors.

DISCUSSION

The present case had intense monocytosis in PB and BM, splenomegaly, the absence of Philadelphia chromosome/t(9;22) and del(7q), thus, raising a suspicion of a juvenile myelomonocytic leukemia (JMML) diagnosis undergoing a blast crisis phase. We sequenced (Sanger) the exons 2 and 3 from *NRAS* and *KRAS* genes, and also the exons 3 and 13 from *PTPN11* gene, JMML biomarkers. Although the sequencing results showed no mutations in these regions, less common mutations, in genic regions not analyzed in our study, could be associated with this malignancy's phenotype.

Besides the translocation t(3;7)(q26;q21), she also presented a chromosomal duplication in the 3q26 region, producing an extra copy of *MECOM*, associated with a deletion in the long arm of chromosome 7. *MECOM* disruption impairs cell differentiation, upregulates cell proliferation, induces cell transformation, and furthermore, its overexpression is associated with minimal treatment response and poor outcome. However, the mechanism which leads to this abnormality remains to be elucidated.

Some groups report that, by means of **chromosome translocation**, gene fusion and juxtaposition of enhancers or promoters near the *MECOM* gene may induce its overexpression. Others hypothesize that *MECOM* overexpression could be triggered by **haploinsufficiency** of *MECOM* transcriptional regulators on a chromosome. Thus, the high *MECOM* expression may have been triggered by any, if not both, of the above-mentioned phenomena. Particularly in this case, the extra copy of the 3q26 region, by itself, may explain such overexpression.

We also observed an increase of 35-fold in *CDK6* expression, a gene related to G1 phase progression, which its aberrant expression has been related to several hematological malignancies. Taken together, *MECOM* and *CDK6* overexpression could have been responsible for the poor outcome observed in our patient.

CONCLUSIONS

In conclusion, we contributed to the registry and biology of complex karyotypes in children with myeloid neoplasms:

We demonstrated, for the first time, a child with a rapidly progressive myeloid neoplasm presenting a cryptic translocation t(3;7)(q26;q21), harboring a 3q26 region duplication and *MECOM* gene overexpression;

We reinforced the importance of molecular cytogenetic tests in selecting cases for further genomic investigations, searching for *MECOM* targets and its interaction with other partners in order to open new perspectives for therapeutic approaches.

ACKNOWLEDGEMENTS

The authors acknowledge with gratitude the help of Cláudia Diniz S. de Atayde, Amanda Faria de Figueiredo and Alice Theophilo T. de Matos. We are indebted to all of the physicians from the many pediatric oncology centers throughout Brazil, and from our institution, who supplied us with samples from their patients.

This work was supported by PROBRAL (DAAD No. 419/14), and FAPERJ (project No. E-26/110: 868/2013 and E-26/200.50/2016), INCT Para o Controle do Câncer, the St. Jude Children's Research Hospital (Memphis, Tenn., USA) and Center of Excellence Grant, Tenn., USA.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA