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Molecular approaches identify a cryptic *MECOM* rearrangement in a child with a rapidly progressive myeloid neoplasm

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Myeloid neoplasms are a heterogeneous group of hematologic disorders with divergent patterns of cell differentiation and proliferation, as well as divergent clinical courses. Rare recurrent genetic abnormalities related to this group of cancers are associated with poor outcomes. One such abnormality is the *MECOM* gene rearrangement that typically occurs in cases with chromosome 7 abnormalities. *MECOM* encodes a transcription factor that plays an essential role in cell proliferation and maintenance and also in epigenetic regulation. Aberrant expression of this gene is associated with reduced survival. Hence, its detailed characterization provides biological and clinical information relevant to the management of pediatric myeloid neoplasms. In this work, we describe a rare karyotype harboring three copies of *MECOM* with overexpression of the gene in a child with a very aggressive myeloid neoplasm. Cytogenetic studies defined the karyotype as 46,XX,der(7)t(3;7)(q26.2;q21.2). Array comparative genomic hybridization (aCGH) revealed a gain of 26.04 Mb in the 3q26.2–3qter region and a loss of 66.6 Mb in the 7q21.2–7qter region. RT-qPCR analysis detected elevated expression of the *MECOM* and *CDK6* genes (458.5-fold and 35.2-fold, respectively). Overall, we show the importance of performing detailed molecular cytogenetic analysis of *MECOM* to enable appropriate management of high-risk pediatric myeloid neoplasms.

Keywords Molecular cytogenetics, myeloid neoplasm, *MECOM* gene, array-CGH, RTqPCR, chromosome 7, cryptic abnormalities

Introduction

Precise diagnosis and the use of therapy adapted to specific genetic changes have resulted in improved outcomes in patients with hematologic disorders. The World Health Organization (WHO) classification of hematologic neoplasms has

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contributed to better stratification of patients in diverse risk categories (1). The expansion of molecular findings in myeloid neoplasms will probably increase the clinical relevance of this information with respect to the diagnosis, classification, and treatment of these tumors.

Myeloid neoplasms are a group of heterogeneous hematologic disorders with divergent patterns of cell differentiation and proliferation, as well as divergent clinical courses (2). Recurrent abnormalities related to this group of cancers, especially those abnormalities involving chromosome 7, are predictors of poor outcomes (1).

Myeloproliferative neoplasms harboring the translocation t(3;7)(q26;q21) are exceedingly rare. In this chromosomal rearrangement, the *MECOM* gene (at the 3q26.2 locus), a dominant oncogene associated with myeloid leukemias, is juxtaposed with the cyclin-dependent kinase 6 (*CDK6*) gene (at the 7q21 locus), an oncogene that is reportedly deregulated and disrupted in many hematologic malignant neoplasms (3,4).

MECOM encodes a transcription factor that plays an essential role in cell proliferation and the maintenance of hematopoietic stem cells and also actively induces epigenetic changes (4,5). The gene is normally expressed at very low levels in healthy individuals; its increased expression is associated with an adverse prognosis for both adult and pediatric myeloproliferative neoplasms (6,7). *MECOM* rearrangements are usually cryptic in the context of a complex karyotype, comprising a vast heterogeneity of chromosomal aberrations. Thus, such rearrangements may be difficult to recognize by conventional cytogenetic techniques. *MECOM* expression is elevated in most patients harboring cryptic *MECOM* rearrangements and is associated with reduced survival rates (4,5). Hence, analyzing *MECOM* gene rearrangement and expression with a combination of molecular and cytogenetic approaches could provide biological and clinical information that is relevant to patients with myeloid neoplasms.

We report the clinical and molecular data for a child with a rapidly progressive and ultimately fatal myeloid neoplasm. Our study revealed a cryptic chromosomal translocation in a rare karyotype that harbored three copies of *MECOM* with overexpression of the gene.

Case report

A 3-year-old girl was admitted to the Pediatric Oncohematology Service of the Hospital Federal da Lagoa, Rio de Janeiro, Brazil, 48 h after receiving initial emergency care at the Adão Pereira Nunes hospital in Rio de Janeiro. At her physical examination, the patient presented with fever, pallor, and a voluminous hepatosplenomegaly (10 cm below the left costal margin). In her peripheral blood, the hemoglobin level was 7.5 g/dl, the hematocrit was 13.8%, the platelet count was 124×10^9/l, and the white blood cell count was 692×10^9/l, with blasts accounting for 28% of the cells. The bone marrow contained 23% myeloid blast cells, which had the following immunophenotypes: $CD34^+$, $CD7^+$, $CD45^{+lo}$, $CD117^+$, $HLADR^{++}$, $CD13^+$, $CD11b^-$, $CD16^-$, $CD10^-$, $IREM2^-$, $CD64^-$, $CD14^-$, $CD35^-$, $CD33^+$, $CD71^-$, $CD105^-$, $TdT^{-/+\ (50\%)}$, $CD56^-$, $CD19^-$, $CD123^+$, $CD38^+$, $CD15^{-/+\ (25\%)}$, $NG2^-$. The patient's bone marrow showed monocytosis; 28% of the cells were monocytes, with 50% of them being inflammatory monocytes ($CD14^{+lo}$/$CD16^+$/$IREM2^+$) (Figure 1A). The child was subjected to cytoreductive therapy with hydroxyurea, but she died 48 h after admission as a result of a brain hemorrhage that was probably caused by leukostasis.

Methods

Cytogenetic analysis of the patient's peripheral blood was performed in the Cytogenetics Department of the National Cancer Institute (INCA), Rio de Janeiro, Brazil. The karyotype was classified in accordance with the International System for Human Cytogenetic Nomenclature (ISCN) 2016 (8). We used commercial probes for subtelomeric 7q (Subtel7q) (Vysis, Abbott Laboratories, Abbott Park, IL) and whole-chromosome painting for chromosome 7 (WCP7). Homemade multicolor chromosome banding (MCB) probe sets specific for chromosomes 3 and 7 were used as previously described (9).

Flow cytometry with a FACSCanto II system (Becton Dickinson, San José, CA) was used to characterize the immunophenotype in accordance with EuroFlow standardized procedures and panels (10,11).

Array comparative genomic hybridization (aCHG) was performed as follows. First, genomic DNA was extracted using a Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN). The DNA concentration was determined with a NanoDrop spectrophotometer (Thermo Scientific), and the DNA quality was checked using agarose gel electrophoresis. DNA samples from two healthy male subjects and two healthy female subjects were used as reference samples. Next, aCGH was performed using a SurePrint G3 Human Genome Microarray (180 K) (Agilent Technologies, Santa Clara, CA). This is an oligonucleotide microarray containing approximately 180,000 60-mer probes with an average probe spacing of 17 kb. Each patient's genomic DNA was cohybridized with a male control DNA (Agilent Technologies). Labeling was performed with the Agilent Genomic DNA Enzymatic Labeling Kit in accordance with the manufacturers' instructions. After hybridization, the aCGH slide was scanned on an Agilent scanner and processed with Feature Extraction software (v10.7), and the results were analyzed using CytoGenomics software (v2.9.1.3) running ADM-2 as the aberration algorithm (12).

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA). For each sample, 2 μg of RNA was treated with a DNAse Amplification Grade I Kit (Invitrogen), and the sample was reverse transcribed into cDNA by using SuperScript II reverse transcriptase (Invitrogen). The sequences of the oligonucleotides used are shown in Supplementary Table S1. Reverse transcription polymerase chain reaction (RT-PCR) was used to evaluate the expression of wild-type isoforms of *MECOM* (*MECOM*.E13.F2 and *MECOM*.E14.R1) and *CDK6* (*CDK6*.E3.F1 and *CDK6*.E4.R2). The mRNA expression levels of *MECOM* and *CDK6* were evaluated by quantitative realtime PCR (RT-qPCR). RT-qPCR was performed with SYBR Green Master Mix (Invitrogen) in a Rotor-Gene Q thermocycler (Qiagen, Hilden, Germany). *MECOM* was amplified using the primers MECOM FW and MECOM RV (13). *CDK6* was amplified using the primers CDK6 FW (spanning exons 2 and 3) and CDK6 RV (for exon 3). The mRNA levels were normalized to the average Ct of the housekeeping genes *B2M*