BRIEF COMMUNICATION

Molecular studies reveal a $\textit{MLL-MLLT3}$ gene fusion displaced in a case of childhood acute lymphoblastic leukemia with complex karyotype

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Rearrangement of the mixed-lineage-leukemia gene ($\textit{MLL-r}$) is common in hematological diseases and is generally associated with poor prognosis. The mixed-lineage leukemia gene translocated to $\textit{3 (MLLT3)}$ gene ($9p22$) is a frequent $\textit{MLL-r}$ partner (~18% of leukemias with $\textit{MLL}$ rearrangement) and is characterized by the translocation $t(9;11)$ ($p22;q23$), forming an $\textit{MLL-MLLT3}$ gene fusion. $\textit{MLL-r}$ are usually simple reciprocal translocations between two different chromosomes, although karyotypes with complex $\textit{MLL-r}$ have been observed. We present a rare case of a child with acute lymphoblastic leukemia with a complex karyotype in which the classical $t(9;11)$ ($p22;q23$) was cryptically relocated into a third chromosome in a balanced three-way translocation. At the genome level, however, the $\textit{MLL-MLLT3}$ three-way translocation still displayed both reciprocal fusion transcripts. This argues in favor for a model where a simple two-way $t(9;11)$ ($p22;q23$) was likely the first step that then evolved in to a more complex karyotype. Multicolor banding techniques can be used to greatly refine complex karyotypes and its chromosomal breakpoints. Also in the presence of putative new rearrangements, Long distance inverse-PCR is an important tool to identify which gene fusion is involved.

**Keywords** Acute lymphoblastic leukemia, $\textit{MLL}$ gene, complex karyotype, molecular cytogenetics, long-distance inverse polymerase chain reaction

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Rearrangements of the mixed-lineage leukemia ($\textit{MLL}$) gene (the $\textit{Drosophila}$ trithorax homologue) are important and generally adverse prognostic factors in hematological diseases (1). However, they appear to involve different biological mechanisms, as suggested by their diversity, their different distribution in acute lymphoblastic leukemia (ALL) versus acute myeloid leukemia (AML) as well as in infants versus children and adults, and the disparate outcomes in these groups (2). These rearrangements are among the most frequent genetic alterations in infant leukemia (approximately 70% of cases), but in noninfants, they are found in only 5–10% of AML cases and 2–5% of ALL cases (3).

The $\textit{MLLT3}$ gene (also known as $\textit{AF9}$) is present in approximately 18% of cases of acute leukemia with $\textit{MLL}$ rearrangement ($\textit{MLL-r}$); it is the second-most frequent of the 50-plus $\textit{MLL}$ translocation partners identified so far (4,5), but it is not often found in cases of ALL (1). Its overall frequency as a translocation partner in leukemia with $\textit{MLL-r}$ is approximately 11.9% in ALL (infant 16.5%, pediatric 18.0%, adult
1.8%) and approximately 28.8% in AML (infant 21.9%, pediatric 36.1%, adult 26.1%) (1). MLL3 encodes a nuclear protein that is part of the AF4/AF5 super elongation complexes steering the transcriptional elongation function. The MLL-MLLT3 fusion produces an in-frame chimeric protein with transcriptional function that acts within a complex, leading to deregulated activation of leukemic target HOX genes (4,5).

Generally, reciprocal MLL-r variants are not cryptic. Previous investigators have suggested that the three-way MLL variant results from a variant of a two-way-translocation (6), which is rarely observed in complex karyotypes (1). Here we describe a rare, complex rearrangement of the MLL-MLLT3 fusion that appears to have evolved from a simple two-way-chromosomal translocation. We investigated this case of pediatric ALL by molecular cytogenetic techniques and long-distance inverse (LDI)–PCR assays.

Materials and methods

Case report

A 4-year-old girl with a 4-month history of a right inguinal mass was admitted to Martagão Gesteira Institute of Pediatrics and Child Development for clinical evaluation and diagnosis. The patient’s medical history consisted of weight loss and pallor. Physical examination revealed a 4-centimeter mass in the right inguinal region but no other enlarged lymph nodes or visceromegaly. At the time of admission, her hemoglobin concentration was 86 g/L (13.5–18.0 g/L); white blood cell count, 12 × 10^9 cells/L (4 × 10^9 to 10 × 10^9 cells/L) with atypical mononuclear cells; platelet count, 64 × 10^9/L (150 × 10^9 to 400 × 10^9 cells/L), and serum lactate dehydrogenase activity, 1291 U/L (≤590 U/L). Morphological evaluation of bone marrow revealed moderate hypercellularity, with 100% of blast cells showing lymphoid characteristics. Flow cytometry analysis revealed a population of blast cells expressing CD19, clgM, cCD79a, CD22, CD20, and HLA-DR, compatible with a pre-B-cell ALL phenotype without CD10 expression (7).

The patient was assigned to the high risk arm of the IC-BFM2002 ALL protocol (8). Morphologic examination of the bone marrow on days 15 and 33 of induction chemotherapy revealed leukemic lymphoblasts comprising more than 25% (M3 bone marrow evaluation) and less than 5% (M1), respectively, of mononuclear cells. Because of the results of the M3 bone marrow evaluation at day 15, the patient underwent allogeneic stem cell transplantation as directed by the protocol. Treatment was initially well tolerated. However, the patient died of transplant-related complications 17 months after diagnosis.

Banding cytogenetics

At the time of diagnosis, standard cytogenetic G-banding analysis was performed on bone marrow cells, and the karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN 2013) (9).

Molecular cytogenetics

Molecular cytogenetic studies of fluorescence in situ hybridization (FISH) were performed on metaphase spreads, using the Cytocell Aquarius MLL dual-color, break-apart, locus-specific probe (Oxford Gene Technology, Begbroke, UK) according to the manufacturer’s instructions, and the karyotype was further delineated by performing multicolor FISH (10) using whole chromosome painting probes for chromosomes 2, 3, 9, and 11 and partial chromosome painting probes for the 11p and 11q arms. FISH was also performed with multicolor banding (MCB) to identify the breakpoints on chromosomes 3, 9, and 11. MCB was unnecessary for chromosome 2 because the breakpoint could be identified by using G-banding, whole chromosome painting probe 2, and inverted 4′, 6-diamidino-2-phenylindole (DAPI)-banding (11).

Molecular genetics

Long-distance inverse PCR (LDI-PCR) assays were performed to identify the MLL transcription partner genes and their respective breakpoints. Briefly, 1 μg of genomic DNA was digested, and the resulting DNA fragments were self-ligated. This re-ligated DNA was used for the subsequent LDI-PCR analysis. PCR amplifiers were purified from the gel and subsequently sequenced to obtain chromosomal breakpoint details (12).

Results

Cytogenetic G-banding analysis revealed an abnormal karyotype with a derivative chromosome 2, monosomy of chromosome 3, an 11q23 chromosome region that appeared to be deleted, and a marker chromosome (Figure 1A). Partial chromosome painting of the 11p and 11q arms identified chromosome 11p material on a chromosome 2 derivative and 11q material on chromosome 3 and 9 derivatives (Figure 1B). Results of the FISH with a dual-color MLL break-apart probe showed one normal copy of the MLL gene; the 5′ portion of the other MLL gene homologue appeared on the marker chromosome and its 3′ portion on a derivative chromosome 9 (Figure 1C). MCB analysis also identified the breakpoints of the derivative chromosomes, redefining the karyotype as 46,XX,der(2)(2;11) (p23;p14), der(3) (3p21→3q29:11q13→11q23:9p21→22→3pter), der(9)(9;11) (p21→22;q23), der(11) (2p1ter→2p23:3p21→3p14:11p14→11q13:3p14→3pter) (Figures 1D–1F).

Because the 5′ MLL signal had an unusual rearrangement, LDI-PCR was performed to identify the MLL translocation partner. This analysis revealed the recurrent gene fusion MLL-MLLT3 and its reciprocal, MLLT3-MLL. The genomic breakpoint on the derivative (11) chromosome was mapped to MLL exon 11 and MLLT3 intron 5 (Figure 1G), and the reciprocal fusion on the derivative (9) chromosome was mapped to MLLT3 intron 5 and MLL intron 10 (Figure 1H).

Discussion

FISH analysis using locus-specific probes is a very useful complement to conventional cytogenetic analysis when used to screen MLL abnormalities, especially in cases in which
unusual or new rearrangements are present (13). In particular, MCB of marker chromosomes can greatly refine complex karyotypes by identifying breakpoints more precisely. LDI-PCR is also an important tool for identifying which gene fusions are involved in putative new rearrangements. MLL-MLLT3 fusion has frequently been found in association with AML cases (2,6,14). Some investigators have observed that patients harboring a t(9;11) may exhibit different epidemiologic factors, such as a higher frequency of AML phenotype, predominance of male gender (1,15), and diverging outcomes (16,17). The world distribution of the t(9;11) also looks very discrepant. A multicentric study of MLL+ observed that the great majority of patients were Europeans, and few were Americans or Asians (251, 16, and 24 patients, respectively) (1). Little is known about the t(9;11) in childhood ALL, and even less is known about its complex variants. In a previously reported study, our group observed two cases of childhood ALL that presented with an MLL-MLLT3 fusion; in one of those cases, the gene fusion was cryptically inserted into a t(11;22) (q23;q?), and LDI studies described the cryptic three-way MLL-MLLT3-CYP2DP1 fusion gene (18). Furthermore, a collaborative MLL recombinome study comprising a great number of institutions worldwide showed that 8.5% of cases presenting MLL-MLLT3 fusions had complex karyotypes. Moreover, the majority of karyotypes bearing a t(9;11) did not present three-way gene fusions, which could point to differences in leukemic transformation mechanisms. The study’s findings
also suggest that ALL cases harboring the MLL-MLLT3 fusion are more frequent than previously reported (1). We must consider that, generally, BCP-ALL blast cells have poor chromosomal morphology (19,20), which could lead to underestimation of the presence of the t(9;11) (p22;q23) variants by conventional cytogenetic techniques. Such technical difficulties reinforce the importance of molecular approaches, such as screening for MLL-r by using split-signal probes and the complementary use of MCB with LDI-PCR to elucidate MLL-r within complex karyotypes. In addition, an RT-PCR screening assay for MLL-MLLT3 fusion transcripts would provide an invaluable clinical tool with which to guide treatment approaches.

In conclusion, although metaphase split-signal FISH analysis is a crucial screening process for detection of MLL-r, other molecular cytogenetic techniques, such as MCB, can elucidate the complexity of this karyotype. Additional studies are needed to verify the gene fusions implicated by other breakpoints observed in this case and their biological relation to leukemia.

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