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Short communication

MLL insertion with *MLL-MLLT3* gene fusion in acute leukemia: case report and review of the literature

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Abstract A new chromosomal insertion involving the *MLL* gene was detected by fluorescence in situ hybridization in a patient with acute myeloblastic leukemia (AML) and a t(9;11)(p21;q13). Genomic polymerase chain reaction confirmed the *MLL-MLLT3* gene fusion. A review of the literature on *MLL* insertions shows that the opposite orientation of the genes involved in the fusion plays a role in the genesis of the rearrangement in most of the cases reported.

1. Introduction

Gene fusions by chromosomal rearrangements are genetic events possibly leading to hematologic malignancies. The majority of these chromosomal rearrangements are visible and easily diagnosed with cytogenetic banding techniques, but some of them may be difficult to detect because they are cryptic or complex [1]. This is the case for the cryptic t(12;21)(p13;q21) and t(5;14)(q35;q32) translocations (detected in children with B-acute lymphoblastic leukemia (ALL) and in T-ALL, respectively), as well as some complex and variant chromosomal aberrations. In these latter cases, fluorescence in situ hybridization (FISH), taking into account the clinical context, is an easy way to reveal a hidden recurrent gene fusion. In this article we describe an uncommon insertion of the *MLL* gene within the *MLLT3* locus leading to an *MLL-MLLT3* fusion. It gave us the opportunity to review the previously reported cases of insertions involving the *MLL* gene in hematologic malignancies to evaluate some mechanisms of *MLL*-partner fusion according to the respective 5′–3′ orientation of both genes and their chromosome arm localization.

2. Materials and methods

2.1. Case report

The patient, a 28-year-old woman without a previous history of malignancy was admitted in August 2007 to the Hematology Department of Hospital Necker-Enfants Malades (Paris, France) for acute leukaemia, which was discovered after tooth pain with gum hypertrophy. Hematologic data were as follows: in the peripheral blood, 157×10^9/L leukocytes with 99% blast cells, 21×10^9/L platelets, and 10.3g/dL hemoglobin. Bone marrow was hypercellular, with 99% blasts. The immunophenotype was CD13+, CD33+, CD123+, DR−, CD34−, CD117+, Myeloperoxydase+. The diagnosis was undifferentiated acute myeloblastic leukemia, classified as AML-M1 according to French–American–British nomenclature. Complete remission could be obtained with polychemotherapy.

3. Results

3.1. Cytogenetic and fluorescence in situ hybridization (FISH)

Conventional RHG-banded karyotype was performed on bone marrow cells after 24-h or 48-h in vitro cultures with synchronization with FrdU. The karyotype was 46,XX, t(9;11)(p21;q13)[24] (Fig. 1a).

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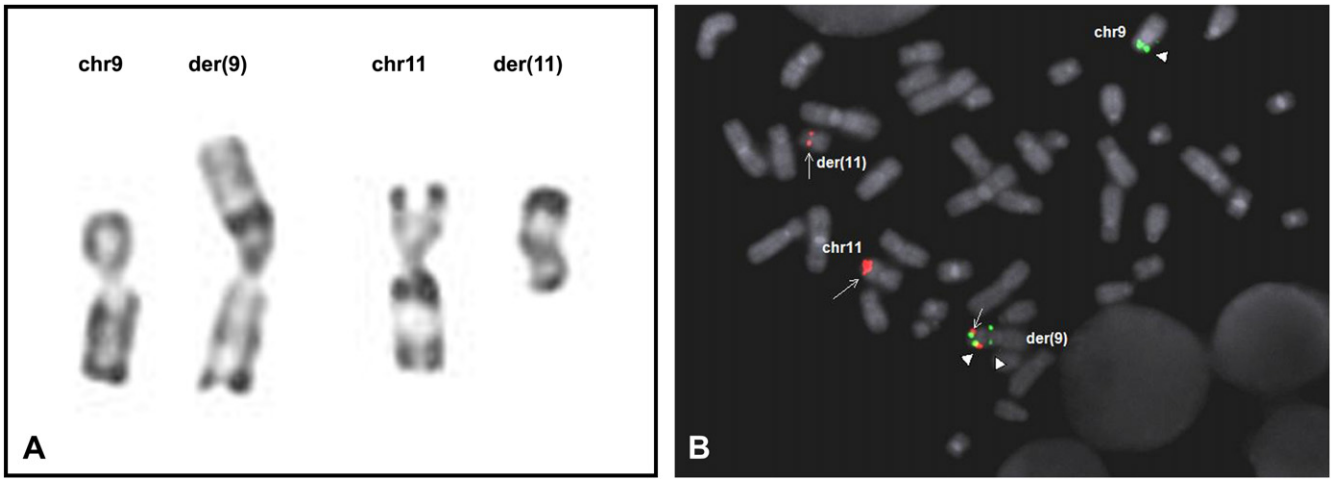

Figure 1. Cytogenetic and FISH studies. (A) Partial RHG karyotype. (B) FISH analysis of patient bone marrow cell using *MLL* probes RP11-278O8, RP11-30G1, RP11-59N1, and RP11-112I9 in gray (arrows) and *MLLT3* probe RP11-477L10 in white (arrowhead) showing fusion of one *MLL* signal and one *MLLT3* signal on chromosome der(9). *(B)* FISH analysis of patient bone marrow cell using *MLL* probes RP11-278O8, RP11-30G1, RP11-59N1, and RP11-112I9 in red (arrows) and *MLLT3* probe RP11-477L10 in green (arrowhead) showing fusion of one *MLL* signal and one *MLLT3* signal on chromosome der(9).

Because of the rearrangement of chromosomes 9 and 11 in AML, and despite the unusual chromosomal breakpoint, dual-color FISH was performed on the patient's metaphases with probes surrounding the *MLL* locus on chromosome 11 (RP11-278O8 and RP11-30G1 on the 5′ side, and RP11-59N1 and RP11-112I9 on the 3′ side) as well as a probe covering the *MLLT3* locus on chromosome 9 (RP11-477L10). The *MLL* and *MLLT3* probes were nick translated with dUTP-FITC (green fluorescence) and dUTP-rhodamine (red fluorescence), respectively, as described previously [2].

After hybridization, two green signals of the *MLL* probes were observed — one on the nonrearranged chromosome 11 and one on the der(11)t(9;11). Two red signals of the *MLLT3* probes were observed — one on the nonrearranged chromosome 9 and one on the der(9)t(9;11). A yellow signal generated by the coalescent of a third green and a third red signal on the tip of the short arm of the der(9)t(9;11) was also observed (Fig. 1b). This result suggested a *MLL-MLLT3* fusion by virtue of insertion of the 5′ part of *MLL* within the *MLLT3* locus. The revised karyotype was 46,XX,t(9;11)(p21;q13)[24].ish ins(9;11)(p21;q13q23)(MLLT3+,MLLT3conMLL+;MLL+) [10].

3.2. Genomic breakpoint analysis

The *MLL-MLLT3* fusion was confirmed on the patient's genomic DNA by an asymmetric multiplex LD-PCR for t(9;11) at the DCAL/Frankfurt/M [3] with the following primers: MLL-F1A, 5′-gcagcctccaccaccagaatcaggtgagtg, located in exon 7 of *MLL*; and MLLT3-R25, 5′-aagcccactttcactatgattgggagaga, located in intron 8 of *MLLT3*. Sequence analysis of the polymerase chain reaction (PCR) product showed a fusion between intron 7 of *MLL* and intron 6 of *MLLT3* (Fig. 2). This result predicts an in-frame fusion of exon 7 of *MLL* with exon 7 of *MLLT3*. To our knowledge, this is the first case in which the intron 7 of the *MLL* gene is fused to intron 6 of the *MLLT3* gene.

4. Discussion

An uncommon chromosomal translocation t(9;11) occurring in a patient with AML-M1 and rearrangements of 9p and 11q suggested the possible presence of *MLL-MLLT3* fusion. FISH analysis showed a cryptic insertion of the *MLL* locus in the *MLLT3* gene on chromosome 9 short arm, and the asymmetric multiplex LD-PCR for t(9;11)

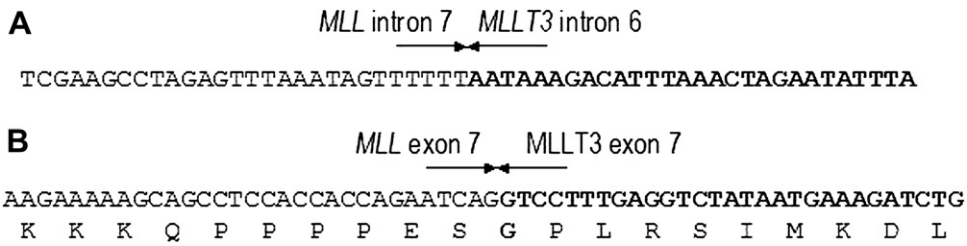

Figure 2. Molecular analysis of the *MLL-MLLT3* fusion. (A) PCR amplification of the *MLL-MLLT3* fusion gene from the patient's sample. (B) Predictive sequence of the *MLL-MLLT3* fusion transcript. The exon 7 of *MLL* is fused in frame to *MLLT3* exon 7 (bold). The amino acid translation spanning the fusion is shown under the sequence.