



Identification of Mixed Lineage Leukemia Gene (*MLL*)/*MLLT10* Fusion Transcripts by Reverse Transcription-PCR and Sequencing in a Case of AML With a FISH-Negative Cryptic *MLL* Rearrangement

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Dear Editor

The mixed lineage leukemia gene (*MLL*) on chromosome 11q23 is a frequent target of chromosomal translocations and rearrangements in childhood leukemia and adult therapy-related leukemia [1]. By using a commercially available dual-color FISH break-apart probe, almost all *MLL* rearrangements can be detected, including variant and cryptic forms [2-4]. We report a unique case of cryptic *MLL* rearrangement, in which the patient exhibited a karyotype that did not include t(10;11)(p12;q23) and FISH results did not reveal *MLL* break-apart signals; however, molecular analysis by reverse transcription (RT)-PCR revealed a 388-bp amplicon corresponding to the *MLL/MLLT10* fusion transcript.

A 17-yr-old male was admitted to our hospital for evaluation and management of multiple palpable neck lymphadenopathies. Blasts accounted for approximately 94% of cells on peripheral blood smear. On bone marrow (BM) aspiration smear, 90.5% of nucleated cells were blasts.

Chromosomes were analyzed via GTG banding. Complex

structural abnormalities were observed, and the karyotype was 46,XY,der(10)t(10;21)(p12;q21),der(12)del(12)(p11.2)add(12)(q24.2)[17]/46,XY[3] (Fig. 1). FISH analysis was performed according to the manufacturer's instructions, including *MLL* (LSI Dual-Color, Break Apart Rearrangement Probe, Vysis, Downers Grove, IL, USA). No break-apart signals were observed for the *MLL* probe (Fig. 2). Multiplex RT-PCR was performed with HemaVision (DNA Technology, Aarhus, Denmark), according to the manufacturer's instructions. Molecular analysis revealed an amplicon corresponding to the *MLL/MLLT10* fusion transcript. RT-PCR analysis was performed with a combination of 5' *MLL* and 3' *MLLT10* primers: *MLL* Forward 5'-GGAAGTCAAGCAAGCAGGTC-3' and *MLLT10* Reverse 5'-GCTGCCATTGATGAATTTG-3'. PCR products were subjected to direct sequencing by using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) on ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). RT-PCR revealed a 386-bp product ranging from the exon 8 region of the *MLL* gene to the exon 16 region of the *MLLT10* gene, linked

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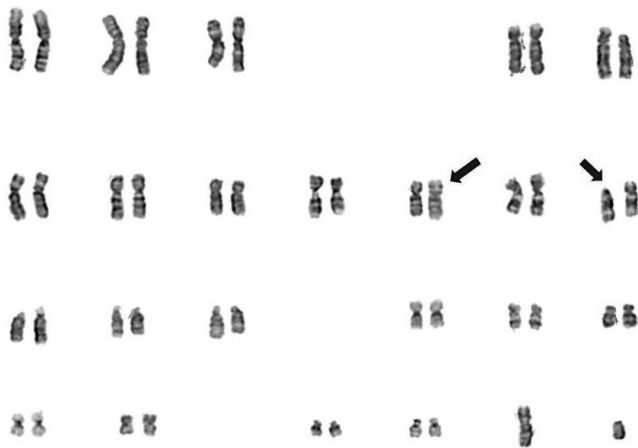


Fig. 1. Bone marrow karyotype showing derivative chromosomes 10 and 12 with an apparently normal chromosome 11. Karyotype: 46,XY,der(10)t(10;21)(p12;q21),der(12)del(12)(p11.2)add(12)(q24.2)[17]/46,XY[3].

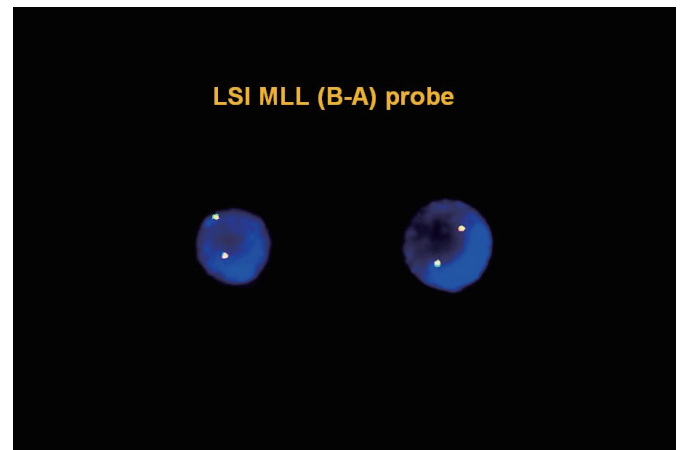


Fig. 2. Two fusion signals (2F) with no *MLL* break-apart signals. LSI *MLL* (B-A) probe: LSI *MLL* dual-color, break-apart rearrangement probe.
Abbreviation: *MLL*, mixed lineage leukemia gene.

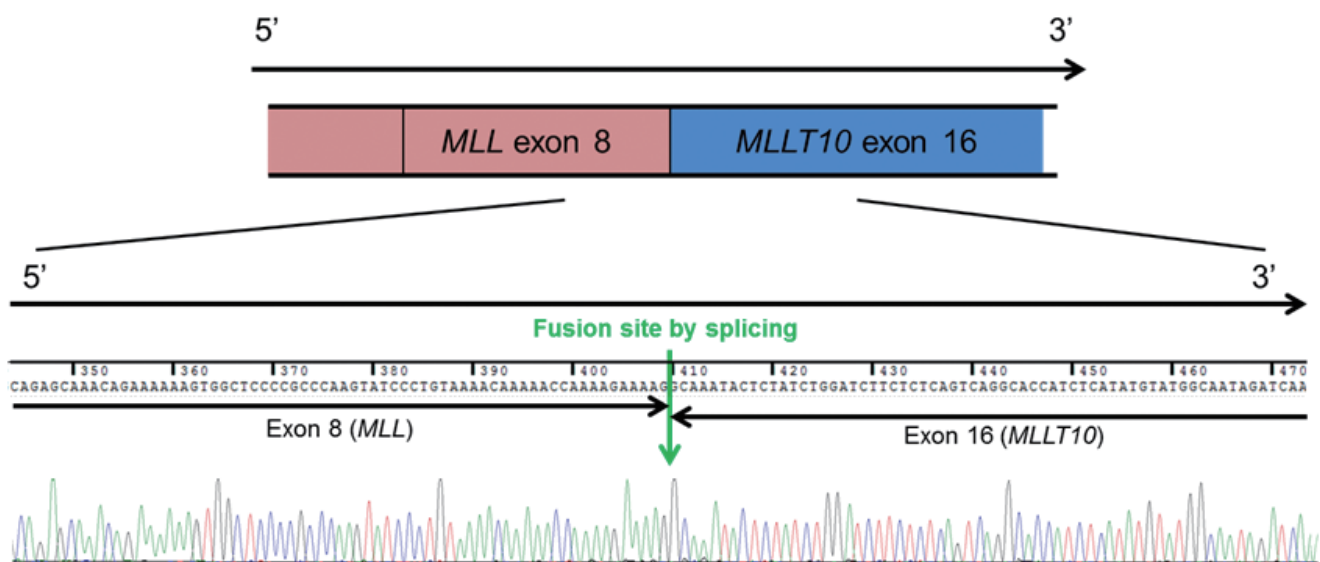


Fig. 3. Identification of the *MLL/MLLT10* fusion transcript in acute myeloid leukemia. Sequencing analyses confirmed that the transcript was from *MLL* (exon 8)-*MLLT10* (exon 16) rearrangement.
Abbreviation: *MLL*, mixed lineage leukemia gene.

via two orphan nucleotides (Fig. 3).

A diagnosis of “variant *MLL* translocation in acute leukemia” was made. The patient was treated with induction chemotherapy, including cytarabine and idarubicin. Complete hematologic remission was achieved 24 days later.

Conventional cytogenetic analysis can miss *MLL* rearrangements, possibly owing to poor metaphase division of leukemic cells and complex or cryptic chromosomal abnormalities. FISH and RT-PCR are advantageous because they are fast and target specific genes [5]. However, most commonly employed multi-

plex RT-PCR kits detect a limited number of rearrangements, since only the most common regions are included [5-7]. *MLL* rearrangement cases outside of the breakpoint cluster region can be detected by FISH despite being RT-PCR negative [8]. Currently, long-distance inverse PCR (LDI-PCR) is the most powerful, verified method for detecting known and unknown partner genes in *MLL* rearrangements [8, 9]. In our case, a genetic aberration, an *MLL* (exon 8)-*MLLT10* (exon 16) rearrangement, was confirmed by sequencing. However, we were not able to perform additional testing such as chromosome microar-

rays or re-testing with different FISH probes (i.e., from a different manufacturer) or LDI-PCR because of insufficient specimens.

The *MLLT10/AF10* gene was found to be involved in “chromosomal fragment insertions,” a complex mechanism that occurs when either a fragment of chromosome 11 (including portions of the *MLL* gene) is inserted into another chromosome, or a fragment of another chromosome (including portions of a translocation partner gene) is inserted into the breakpoint cluster region of the *MLL* gene, and is most frequently involved in the latter mechanism [9]. The *MLLT10/AF10* gene was also found to be one of the 20 genes that are involved in complex rearrangements, with three- or four-way translocations resulting in more than two fusion alleles [9]. While the 3′-portions of the translocation partner genes were regularly fused to the 5′-portion of the *MLL* gene, the genes involved in complex rearrangements, including the *MLLT10/AF10* gene, were fused to the 3′ portion of the *MLL* gene [9]. There is a high likelihood that our case also involved a complex rearrangement, considering that the 3′ portion of the *MLL* gene was fused to the 5′ portion of the *MLLT10* gene.

To the best of our knowledge, this is the first case report of a cryptic *MLL* rearrangement detected by RT-PCR only, despite testing negative for FISH. However, this case may not represent a truly cryptic *MLL* rearrangement, considering that the chromosome results revealed a complex karyotype. While FISH assays are recognized for their usefulness in detecting cryptic *MLL* rearrangements, this case report emphasizes that RT-PCR can be useful for detecting FISH-negative cryptic *MLL* rearrangements. It would be useful to document other similar cases so that a strategy for diagnosis can be developed.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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