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5' MLL Gene Deletion in a Case with Childhood Acute Lymphoblastic Leukemia

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Abstract

Myeloid/lymphoid leukemia (MLL) gene rearrangements are high risk cytogenetic characteristics of acute lymphoblastic leukemia (ALL). Translocations of this gene are well defined, and their impact on the patient's prognosis is well known, but deletions of the same region are rare, and little is known about their prognostic significance and the significance of their accompanying translocations. Here we present a case of childhood ALL with a deletion of the 5'region of the MLL gene detected by fluorescence in situ hybridization (FISH) analysis. This result also confirmed the sensitivity and efficiency of FISH analysis.

Myeloid/lymphoid leukemia (MLL) gene rearrangements are signs of a bad prognosis in childhood acute lymphoblastic leukemia (ALL).[1-3] This gene has an important role in developmental regulation of gene expression in normal hematopoiesis.[2] The MLL gene is located on the 11q23 chromosomal region. Translocations are the most common rearrangements detected, while deletions are rarely seen.[4] Some publications have reported that MLL gene deletions were predominantly at the 3′ end and were accompanied by a MLL translocation that had the major role in the designation of the prognosis.[5,6] However, they remarked it was not easy to predict the prognostic effect of the 3′ deletion of the MLL gene without a translocation.[5] Besides this report, no other publications have reported finding a 5′ deletion of the MLL gene.

Many techniques are systematically used for detecting MLL gene rearrangements. Conventional cytogenetics and FISH analysis are the first choices and are complementary to one another, but fluorescence in situ hybridization (FISH) is a more sensitive method for detecting chromosomal breakpoints.[1,6-8]

The case presented here is of a 5-year-old boy with ALL. Conventional cytogenetic analysis did not reveal any chromosomal aberrations, but FISH analysis revealed a deletion at the 5′ part of the MLL gene. The first aim in presenting this case is to demonstrate the new finding of a 5′ deletion of the MLL gene, and the second aim is to reemphasize the important role of FISH analysis, especially in detecting MLL gene rearrangements.

Case Presentation

A 5-year-old boy was admitted with fever, fatigue, and leg pain. A full blood count showed hemoglobin at 4.1 gr/dL, platelets at 16100/mm^3, and a white blood cell (WBC) count of 9180/mm^3 (80% blasts, 15% lymphocytes, 30% PNL, 1% basophiles, and 1% monocytes). A physical examination showed hepatosplenomegaly and multiple lymph nodes in the neck, axilla, and abdomen. Bone marrow and peripheral blood flow cytometria results were in agreement with pre-B ALL. Conventional cytogenetic analysis did not show any chromosomal abnormalities, but FISH analysis demonstrated the 5′ deletion of the MLL gene. Treatment began according to the standard chemotherapy protocols, but on the eighth day of chemotherapy, his WBC was 448/mm^3, and on the 15th day, the bone marrow biopsy analysis showed a late response to chemotherapy. The patient was therefore evaluated as high risk, and HRG chemotherapy was initiated. By the 33rd day of chemotherapy, the patient was in complete remission.

Laboratory Investigation

Conventional Cytogenetic Analysis

The bone marrow specimen was cultured in Chang Medium® BMC for 72 hours. Cultures were then treated with 0.1 mL (10 µg/mL) colcemid for 45 minutes, and a routine harvest procedure was performed. Prepared slides were kept in a 67°C incubator for 3 days before GTG banding. Karyotypes were defined according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).

FISH Analysis

Interphase FISH analysis was performed on slides prepared from fixed cell pellets using a dual color locus specific MLL probe (Vysis, Downers Grove, IL). The probe consisted of a 350 kb centromeric portion of the MLL gene breakpoint cluster region labeled in the green spectrum and an approximately 190 kb portion largely in the telomeric part of the breakpoint cluster region labeled in the orange spectrum (red image with red filter) (**Figure 1**). In addition to MLL gene analysis, BCR/ABL (Ph′) fusion gene locus analysis was performed by FISH using

locus-specific dual color probes (Vysis), and a mixed probe of centromere 11 and locus-specific 11q23 was performed to see if the centromere was intact. Metaphase FISH analysis could not be performed because of the low mitotic index and insufficient material.

Results

Conventional cytogenetic analysis did not reveal any chromosomal aberrations. The FISH analysis showed neither structural nor numerical aberrations for chromosomes 9 and 22 in addition to the results of the intact centromeres of chromosome 11. However, the results of MLL gene probe analysis showed a deletion at the 5′ part of the MLL gene, which was observed as 1 fusion (yellow) and 1 red (in the original, it is labeled in the orange spectrum, but the image is red due to the red filter) signal in 52% of cells (**Image 1**). In these cells, no green signal was observed. This deletion involved the entire 350 kb portion and minimal 190 kb portion of the MLL gene (**Figure 1**). [In normal cells, there are 2 fusion (yellow) signals due to the colocalization of 2 probes labeled in green and orange/red spectrum. In the presence of a translocation 1 of the fusion signals splits and 3 signals (1 red, 1 green, 1 yellow) are seen.]

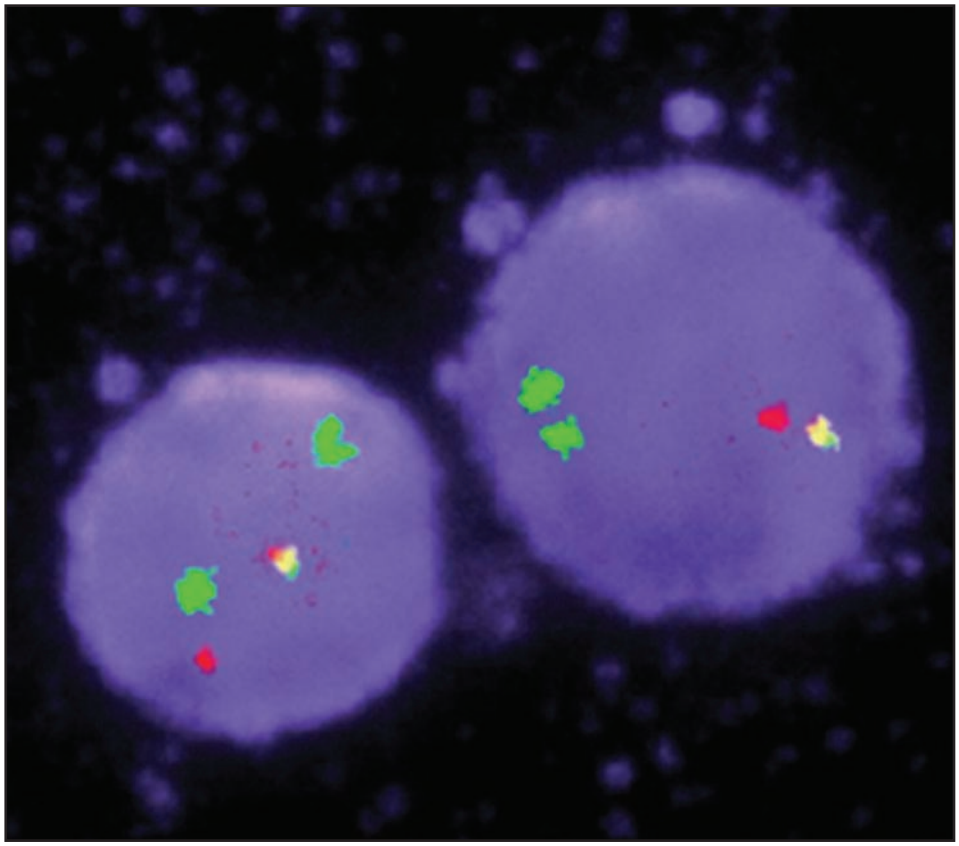

Image 1_The 5′ deletion of the MLL gene in the ALL patient. Green signals show disomic chromosome 11 centromeric regions, the yellow (red and green 1:1 mixture) signal shows the intact 11q23/MLL gene locus, and the presence of a red signal without the green signal shows the 5′ deletion of 11q23/MLL gene locus.

Discussion

We have presented a case of childhood ALL with no cytogenetic evidence of 11q23 rearrangement as well as an abnormal signal pattern of 1 fusion signal and 1 orange signal using the Vysis MLL probe. The absence of the green signal is consistent with the deletion of the 5′ region of the MLL gene.

In previous studies, rearrangements of the MLL gene locus were reported in approximately 1-2% of childhood and 60-80% of infant ALL cases.[1,2] Cases with MLL rearrangement usually had a pro-B immunephenotype in addition to WBC, organomegaly, CNS involvement, and a poor outcome. The most common rearrangements of the MLL gene are reciprocal chromosomal translocations, and their prognostic relevance is well established. Deletions of the same locus are rare, and more cases than those that have been reported are needed to evaluate their impact on prognosis. Most of the reported MLL deletions in ALL cases were at the 3′ end of the gene and were usually accompanied by a simultaneous translocation, which determined the clinical progression.[1,5,7]

The reported frequency of 3′ deletions in MLL translocations ranges from 8%-28%.[1] Although there is no reported frequency of 5′ deletions or mutations of the MLL gene, some studies mention the majority of MLL rearrangements involve breaks within an 8.3 kb breakpoint cluster region between exons 5 and 11 in the 5′ region, especially in de novo and adult leukemia and in the 3′ region of infant leukemia patients.[8,9] In a study by Felix et al, 40 childhood cases of de novo leukemia were examined to assess the translocation breakpoint distribution within the MLL genomic breakpoint cluster region. In 38 children who had MLL rearrangements, MLL genomic breakpoints were localized to the 5′ portion of the breakpoint cluster region (BCR) in 14 cases and in the 3′ portion in 18 cases.[10]

According to some investigators, 3′ deletions of the MLL gene are not always the result of concurrent translocation and deletion events. Barber et al presented 12 ALL patients who had unusual FISH signal patterns seen as the presence of 5′ green and the loss of 3′ red and 1/2 fusion signals. In these cases, they confirmed an 11q23 / MLL translocation in 8 of the 12 cases by conventional cytogenetic and metaphase FISH analysis. In 1 case, del (11) (q23) was observed only in G-Banded analysis. In 3 cases, an MLL rearrangement was excluded by FISH and Southern blotting analysis, and the loss of the red signal was explained as an interstitial deletion in chromosome band 11q23.[1]

Quigley and Wolff presented the case of a 12-year-old T-cell ALL patient who had t (11,19) as

a rearrangement of 1 MLL locus and deletion of the 3′ region of the other MLL locus. Although pediatric T-cell ALL is seen as a high-risk disease, t(11;19), which gives rise to MLL/ENL fusion, is associated with a favorable outcome in the T-lineage. The investigators mentioned it was not easy to predict the prognostic effect of the 3′ MLL deletion in that case because of the possibility of a cryptic translocation. The patient was in clinical and cytogenetic remission at 14 months post diagnosis.[5]

Woo et al observed the complete deletion of the MLL gene in 2 of their 65 childhood ALL patients. These patients had 1 fusion signal in 19% and 41% of 200 consecutive interphase cells as seen by FISH analysis. These cases had a relatively longer survival in concordance with the report by Harbott showing the relationship between good prognosis and MLL deletion.[11,12]

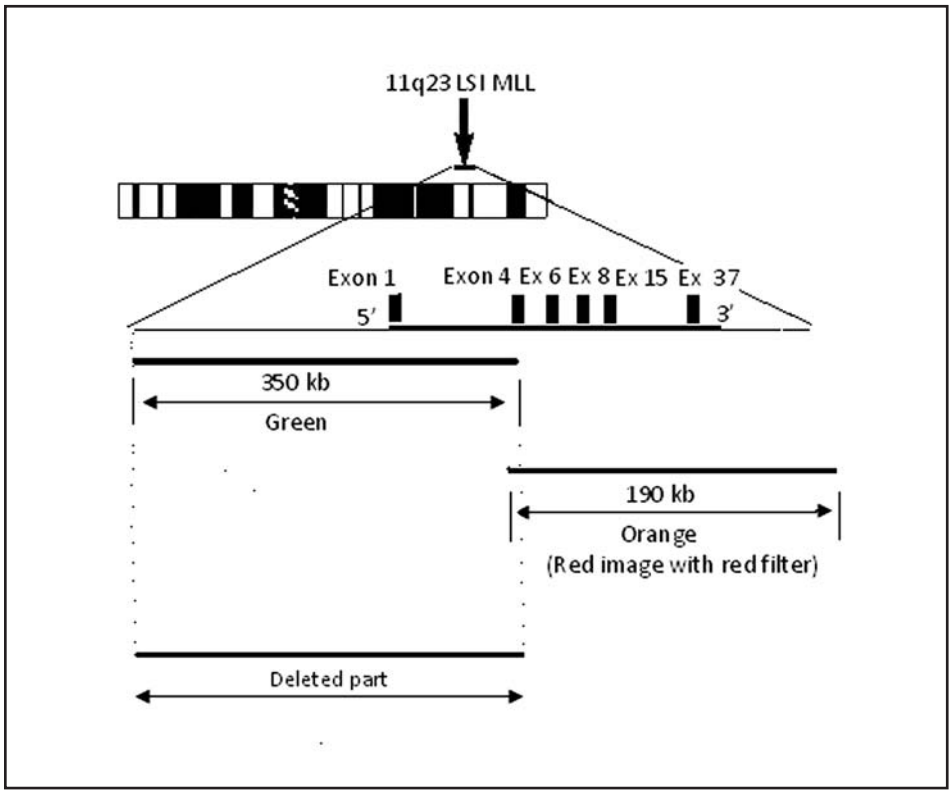

Figure 1_The LSI MLL dual color probe map and the deleted 5′ MLL region in this case.

In the study of Pais et al, 213 newly diagnosed ALL (infants/pediatric/young adults) patients were investigated to identify various MLL gene aberrations leading to MLL gene mutation. Partial deletion of the MLL gene was found in 2 cases which had the 11q23 deletion in conventional cytogenetic analysis. One of these cases had early pre-B ALL immunephenotype, and interphase FISH analysis revealed 1 yellow/1 green signal pattern in 22% of cells. The other case had a pre-B immunephenotype, and the 2 yellow/2 green signal pattern was identified in 5% of interphase cells by FISH. According to the authors, the cause of partial deletion might be the MLL translocation followed by the loss of 3′ sequences, or the partial deletion could be caused by MLL breaks leading to the partial loss of MLL. In the second case, 2 fusion signals, 2 extra green signals, and the absence of the red signals were interpreted as a result of partial duplication in MLL followed by multiple breaks in the 5′ region of MLL and self-fusion of the 3′ region of aberrant MLL. In another case, a monoallelic loss of MLL was determined in the 30% interphase clone by FISH analysis and was interpreted as a possible double mutational mechanism in MLL due to MLL rearrangement in 1 allele followed by the loss of a homolog. This case also had an 11q23.[2]

In our case, we observed 1 fusion and 1 orange signal with the absence of a green signal in 52% of cells. G banding results revealed no involvement of chromosome 11. These findings are consistent with an interstitial deletion of the 5′ region of the MLL gene. What is the impact of this deletion on prognosis, and is there an accompanying cryptic translocation? We do not know the answers to these questions; however, some features are clear from this case.

Our case is consistent with the clinico-hematopathological features of MLL gene rearrangement except for WBC, hepatosplenomegaly, and lymphadenopathy, as in the study of Pais et al, in which organomegaly and WBC were not found to be associated with MLL aberrations.[2] Our patient has a deletion in the MLL gene, and Harbott and Woo's study indicated that MLL deletion was associated with good prognosis.[11,12] In the study of Takeuchi, loss of heterozygosity (LOH) of chromosome arm 11q was found in 18 of 113 primary childhood ALL samples. In detailed examination, 3 distinct deletion regions on the long arm of chromosome 11 were identified, and 2 of them contained the MLL gene region. All the cases with LOH at MLL locus lacked detectable MLL gene rearrangements. The same study revealed the children with LOH of 11q tended to have a lower incidence of relapse during the 8 years of follow-up (22%) as compared to the cohort without the 11q LOH (29%).[13] Our patient is 5 years old, and it is known that infants younger than 1 year with 11q23/MLL gene rearrangement have an extremely poor prognosis, although older children have a better prognosis.[14] t(9;22) is 1 of the cytogenetic high risk characteristics and was not observed in our patient.[15]

Some studies mention that in MLL rearrangements, cells exposed to pro-apoptotic signals undergo site specific cleavage at 11q23, and these apoptotic activators trigger site specific cleavage adjacent to exon 12 within the MLL breakpoint cluster region.[16,17] In our case, the deletion involved exons 1 through 4.

In leukemic rearrangements the results of sequence data analysis from each of the breakpoint regions revealed that large submicroscopic deletions occur in Alu segment repeat regions.[18] The 5′ half of the 8.3 kb MLL BCR contains a series of Alu repeat sequences, and most of the breakpoints map to the centromere 5′ half of this region in leukemias.[19] Analysis of 11q23/MLL breakpoints in rearrangements show that der (11) encodes the critical 5′ MLL/3′ partner gene transcript in leukemias and

the fusion products encoded by the 5′ portion of MLL represent the most biologically relevant oncogenic proteins. The role of der (non-11) bearing the 5′ partner/3′ MLL fusion is less certain and genomic imbalance rather than overexpression of non-critical 5′ partner/3′ MLL is likely to be the important outcome.[20,21] Loss of normal function of the MLL gene may be a critical event in leukomogenesis. In addition, the submicroscopic deletions in leukemia-associated genomic rearrangements and the associated haploinsufficiency may result in the differences in clinical behaviors.[18]

Our study confirmed FISH as the most suitable assay for detecting MLL rearrangements because of its sensitivity and speed, and because it is complementary to conventional cytogenetic analysis.[1,6,15]

Characterization and evaluation of MLL gene abnormalities other than translocations may be very important in understanding the leukemogenic process, and more cases are needed to extend the knowledge about MLL gene aberration mechanisms. LM

Keywords: MLL gene deletion, Acute Lymphoblastic Leukemia, FISH analysis

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