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AML1 Amplification and 17q25 Deletion in a Case of Childhood Acute Lymphoblastic Leukemia

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We report a case of childhood acute lymphoblastic leukemia (ALL) with both acute myeloid leukemia 1 (*AML1*) amplification and 17q25 deletion. *AML1* gene is located on 21q22 and encodes a transcription factor. *AML1* amplification is a common finding in childhood ALL, and itis observed as an increase in gene copy number by the FISH analysis. The mechanism of *AML1* amplification is not associated with *AML1* gene mutations. The 17q25 is a gene-rich chromosomal location and distinct abnormalities of this region have been observed in previous cases of different kinds of leukemia. Deletion of the 17q25 region has been reported in two leukemia patients. Septin 9 (*SEPT9*) and survivin genes are located on 17q25. High expression of these genes and *AML1* amplification are regarded as markers in tumorigenesis and disease progression; however, more data are needed for accurate prognostic evaluation. J. Clin. Lab. Anal. 23:368–371, 2009. © 2009 Wiley-Liss, Inc.

Key words: *AML1* amplification; *SEPT9*; acute lymphoblastic leukemia; 17q25; aberration

INTRODUCTION

In childhood acute lymphoblastic leukemia (ALL), cytogenetic findings are very important for the diagnosis, prognosis, and planning of medical care. Conventional cytogenetic analysis, supported by fluorescence in situ hybridization (FISH) analysis, is highly effective in characterizing cytogenetic aberrations. In ALL cases, it is common to see new, unknown genetic aberrations as well as the well-known diagnostic genetic findings that have a known influence on prognosis (1). The presented case is the first patient with childhood ALL who had both a 17q25 deletion and acute myeloid leukemia 1 (*AML1*) amplification. The *AML1* gene is located on 21q22, and encodes a transcription factor that participates in the core binding factor (CBF) complex. This protein plays a role in the regulation of granulocyte macrophage colony stimulating factor, t-cell antigen receptor, myeloperoxidase, and interleukein-3 genes. It has been suggested that expression of *AML1-a*, which is an isoform of *AML1*, increases in cases that have acquired chromosomal aberrations and that this expedites the leukemic process (2). Various types of *AML1* rearrangements have been reported in various types of leukemias (3). Amplification of the *AML1* gene, which is not rare in childhood ALL, is observed as an increase in gene copy number by the FISH analysis.

The 17q25 is a gene-rich chromosomal location, and various aberrations of this region have been reported in leukemias (4). Both mixed lineage leukemia (*MLL*) septin like fusion (*MSF*) Septin 9 (*SEPT9*) and survivin (BIRC5) (baculoviral IAP repeat-containing 5 [*BIRC5*]) genes are located on 17q25. *MSF* (*SEPT9*) has a role in cytokinesis. Survivin expression is high in many cancers, including leukemias. In recent publications, a high expression of survivin was associated with a poor prognosis. Therefore, the role of 17q25 region in the initiation and progression of leukemias should be clarified (5,6).

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CASE

A 17-year-old female patient, whose early complaints were limited to cough and sputum production, had a blood count that revealed acute leukocytosis. In addition, her hemoglobin was 5.6 g/dl and her platelet count was 56,000 /mm^3. Her physical examination was remarkable for pallor, icteric conjunctivae, 1–2 cm cervical lymphadenopathy, and 2 cm splenomegaly. She had 48% L1 blasts in her peripheral blood and 90% L1 blasts in her bone marrow. The flow cytometry results were consistent with preB-ALL. Conventional cytogenetic analysis were applied as a routine procedure and the results revealed a karyotype of 46, XX (16)/46, XX, del(17)(q25) (4). No rearrangements were observed in the *MLL* () gene or *BCR*/*ABL*, *TEL*/*AML1* fusion genes by concurrent FISH analysis, but *AML1* amplification was noted in 70% of 200 cells analyzed. The ALL-BFM-95 HRG protocol was initiated. After one week, FISH analysis showed that *AML1* amplification had decreased to 18%. Later analysis revealed a 46, XX [15]/46, XX, del(17)(q25) [5] karyotype and 52% amplification. When the analysis was repeated after 20 days, neither 17q25 deletion nor *AML1* amplification was observed in any of the cells.

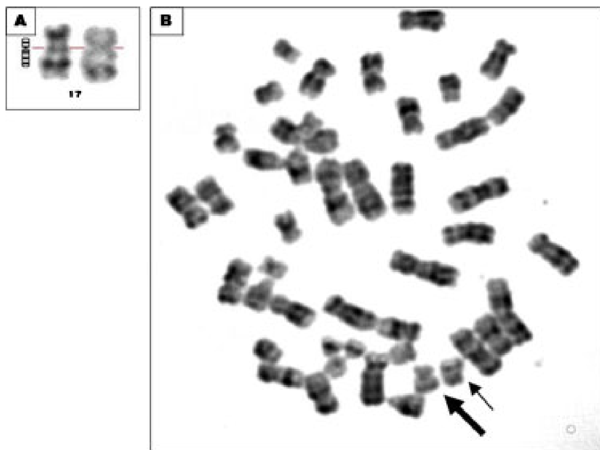

Fig. 1. (**A**) Partial karyotype of a metaphase showing the del (17) (q25) on the right side and normal chromosome 17 on the left and (**B**) GTG banded metaphase showing del (17) (q25) (thick arrow) and normal chromosome 17 (thin arrow).

MATERIALS AND METHODS

Cytogenetic Analysis

The bone marrow specimen was cultured in Chang Medium® BMC for 48–72 h. Cultures were then treated with 0.1 ml (10 µg/ml) colcemid for 45 min and a routine harvest procedure was performed. Prepared slides were kept in a 67°C incubator for three days before trypsin-giemsa (GTG) banding. Karyotypes were defined according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).

FISH Analysis

FISH was performed with the dual color probes of t(9,22) *BCR*/*ABL*, 11q23 *MLL* and t(12,21) *TEL*/*AML1* fusion genes on nuclei from the cytogenetic pellet. After painting with 4′-6-diamidino-2-phenylindole (DAPI), cells were analyzed with Nikon E 600 fluorescence microscope of Quips Imaging System that has red, green, dapi, aqua, and gold filters. The findings were photographed.

DISCUSSION

In the present case, we observed *AML1* gene amplification by FISH analysis (Fig. 2A, B). According to previous studies, these amplifications are usually observed in childhood ALL and their mechanism is not associated with *AML1* gene mutation (7).

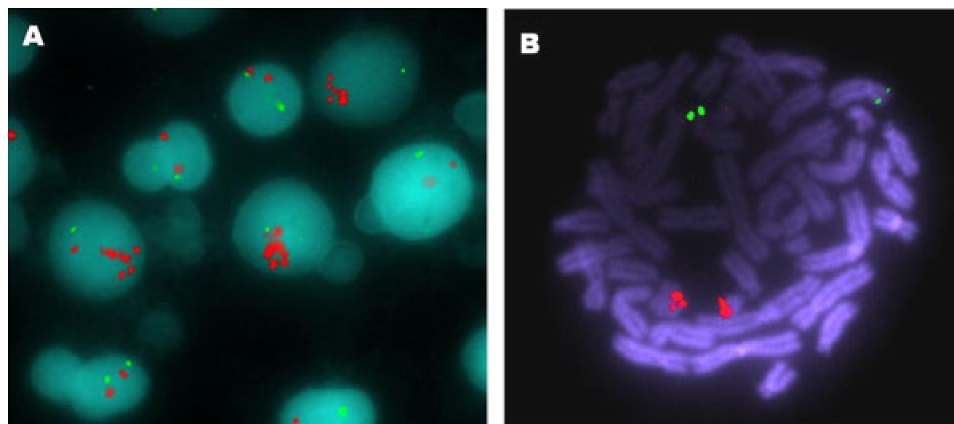

Fig. 2. FISH results of *AML1* amplification in interphase (**A**) and metaphase (**B**) cells. Red signals show *AML1* gene, which is located on chromosome 21 and green signals show TEL gene, which is located on chromosome 12.

Polysomy 21, marker chromosomes, or intrachromosomal amplification may be the cause of extra *AML1* signals (8). Usually, gene amplification is observed as double minutes (dm) or homogeneously staining regions (hsrs) using conventional cytogenetic techniques; however, in complex rearrangements originating from irregular duplications of distinct regions of 21q, gene amplification may not be visible using conventional cytogenetic techniques (3,9). In these cases, *AML1* amplification has been observed as an increase in gene copy number with 4–15 fold signal amplification on a marker or derivative chromosome (10).

Le Coniat et al. have found four copies of *AML1*, amplified in the interphase nuclei of a case with a normal karyotype (11). Similarly, two additional cases with a normal diploid DNA index and *AML1* amplification were reported by Casado et al. In these cases, cells with amplified *AML1* were present at rates of 84 and 89%, respectively (12). In that case, in addition to the normal karyotype, *AML1* amplification with 4–9 copies was observed in three consecutive FISH analyses in which the rates were 70, 18, and 56%.

The relationship between disease prognosis and FISH findings of *AML* and *TEL* loci in childhood ALL was described by Martinez-Ramirez et al. According to this study, when the FISH findings are negative in cases with a complex karyotype, the prognosis is poor, and if the FISH findings are positive, but the karyotype is not complex or has only numerical abnormalities, the prognosis is relatively good (8).

Distinct abnormalities of 17q25 region can be observed in different types of leukemias. t(11;17)(q23,q25) has been described as one of the most common abnormalities and t(13;17)(q14;q25) is another abnormality that was seen in a patient with *AML-M4* (4). In another patient, who had a transformation from essential thrombocytopenia to acute myeloid leukemia, t(2,17)(p22;q25) was reported (13). Deletion of the 17q25 region has been reported in two leukemia patients. One of these patients had hairy cell leukemia, and the other had chronic lymphocytic leukemia with a cryptic deletion (14,15).

The present case is the first ALL patient with a 17q25 deletion (Fig. 1A, B). Another patient with ALL had trisomy 13 early in the disease, but after remission, in his relapse period, had two new subclones one of which had an insertion in the 17q25 region. The karyotypes of these two clones were: 47,XY,+13,ins(17;7)(q25;q22q32) and 47,XY,del(6)(q21q23),+13 (16).

The *MSF* (*SEPT 9*) gene, which is located on 17q25, is a fusion partner of the *MLL* gene, and has a potential role in tumorigenesis. Elevated expression of this gene has been observed in many tumors, including leukemias (5). In 2007, Troeger et al. described the effect of elevated survivin expression on the risk of early relapse in pediatric acute B-cell precursor lymphoblastic leukemia (6).

In 2008, another investigation confirmed high survivin expression as an unfavorable prognostic factor in acute lymphoblastic leukemias (17).

In the present case, the karyotype was not complex, and *AML1* amplification was observed by FISH analysis. One month after starting therapy, abnormal genetic findings, including *AML1* amplification and 17q25 deletion, had disappeared. This disappearance may be the result of good response to chemotherapy, and may indicate a good prognosis related to the cessation of dysregulation on survivin and *AML1* gene products. The case has been followed up for three years and she is in complete remission. However, further investigation is necessary for accurate evaluation of the prognostic significance of related regions and genes in childhood ALL. Therefore, at the time of first diagnosis, planning the conventional cytogenetic and FISH analysis as a usual procedure may be helpful to see new, unknown genetic aberrations as well as the well-known genetic findings.

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