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Letter to the editor

Complex t(8;19;21)(q22;p13;q22) as a sole abnormality in a patient with de novo acute myeloid leukemia

Acute myeloid leukemia (AML) with t(8;21)(q22;q22) shows unique morphologic, molecular, cytogenetic, and clinical features in patients with acute leukemia. It is closely associated with French–American–British M2 subtype, and the common morphologic features include the presence of large blasts with abundant basophilic cytoplasm, prominent Auer rods, and eosinophilia [1,2]. In the molecular and cytogenetic aspects, t(8;21)(q22;q22) disrupts the *AML1* gene (alias *RUNX1*) on 21q22 and the *ETO* gene (alias *RUNX1T1*) on 8q22, results in the formation of the *AML1*/*ETO* chimeric fusion gene, and it is frequently accompanied by additional chromosomal changes such as loss of sex chromosome and del(9)(q22) [1]. In addition, AML with t(8;21) is usually associated with good response to chemotherapy and high complete remission rate with long-term disease-free survival when treated with high-dose cytarabine in the consolidation phase. Among those patients with *AML1*/*ETO* fusion, about 96% had

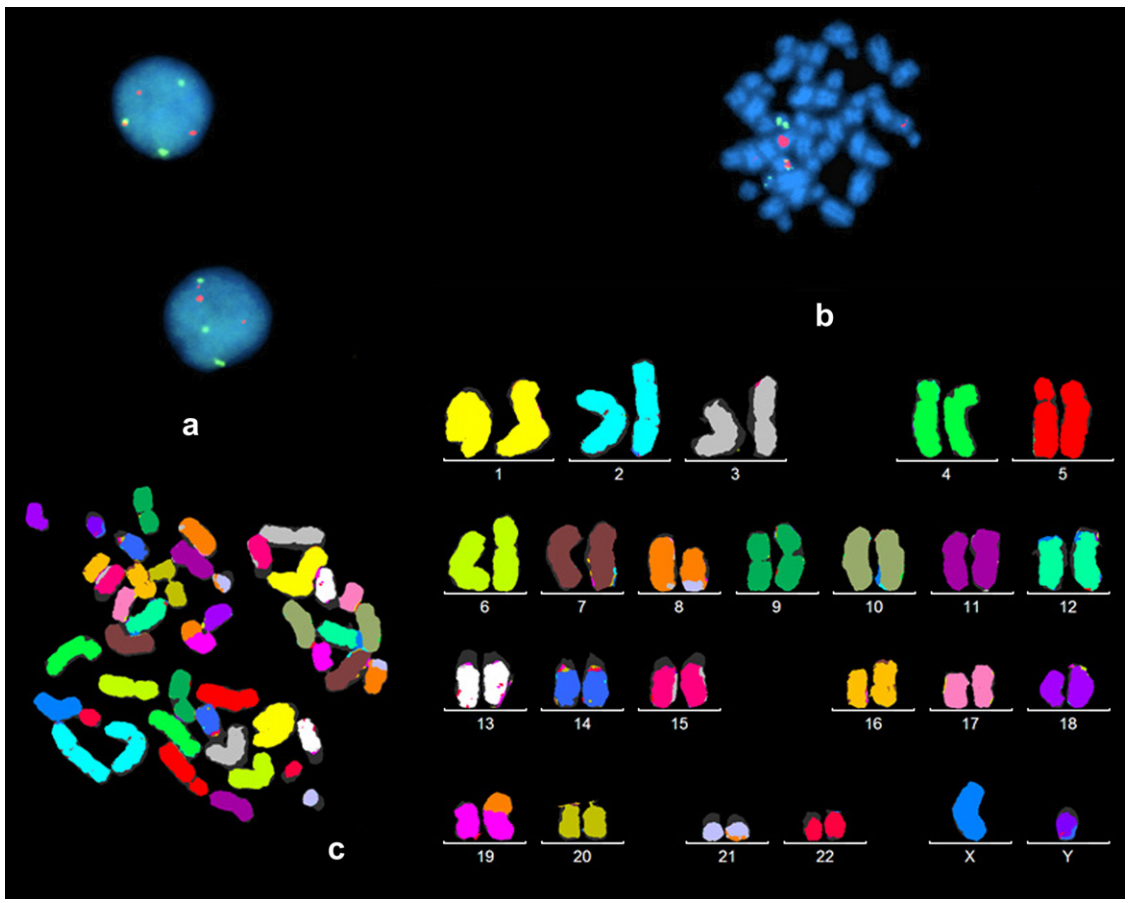

Figure 1. FISH analyses of the bone marrow cells using LSI *AML1*/*ETO* dual color, dual fusion translocation probe (Abbott Molecular/Vysis) and 24XCyte probes (mFISH probe; MetaSystems). (a) Interphase FISH shows the result of "nuc ish (*ETO* × 3),(*AML1* × 3),(*ETO* con *AML1* × 1)[116/300]," consistent with the abnormal pattern of 2O2G1Y (2 *AML1*, 2 *ETO*, 1 *AML1*/*ETO*). (b) Metaphase FISH with the *AML1*/*ETO* probe. (c) mFISH result shows an unexpected pattern of t(8;19;21) in this patient. It seems that a small fragment of chromosome 8 was translocated to chromosome 21, which was not in accordance with the results of chromosome and mBAND analyses.

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a classic t(8;21)(q22;q22) and about 4% had a simple or complex variant of t(8;21) in a report on 148 cases from the Groupe Français de Cytogénétique Hématologique [2]. To the best of our knowledge, about 100 cases with variant t(8;21) have been reported as three-way or four-way translocation, as well as inversion-associated translocation, in the literature [2–6]. Among these cases, only one case bearing a three-way variant (8;19;21)(q22;p13;q22) as a complex karyotype has been described [2], but a detailed clinical, laboratory, and cytogenetic characterization has never been attempted. In this study, we report the first case of t(8;19;21)(q22;p13;q22) as a sole abnormality in a patient with de novo AML.

An 18-year-old Korean boy with a chief complaint of gum bleeding for 7 days was admitted to the Severance Hospital of Yonsei University. Complete blood count showed a hemoglobin level of 4.9 g/dL, a platelet count of 16,000 /μL, and a white blood cell count of 28,770 /μL with 20% segmental neutrophils, 17% lymphocytes, 3% monocytes, 6% band neutrophils, 4% myelocytes, 3% metamyelocytes, 2% atypical lymphocytes, and 45% blasts. Initial bone marrow biopsy revealed a hypercellular marrow with marked myeloblasts with maturation, consistent with AML-M2 morphology. The initial and follow-up results of karyotype in this patient was 46,XY,t(8;19;21)(q22;p13;q22) in all 34 cells analyzed. Fluorescence in situ hybridization (FISH) signals from *PML/RARA*, *CBFB/MYH11*, *MLL*, and *BCR/ABL* probes (Abbott Molecular/Vysis, Des Plains, IL) were within reference ranges, whereas *AML1/ETO* FISH showed the result of "nuc ish (*ETO* × 3),(*AML1* × 3),(*ETO* con *AML1* × 1)[116/300]," consistent with the abnormal pattern of 2O2G1Y (2 *AML1*, 2 *ETO*, and 1 *AML1/ETO*) in 38.7 % of the nuclei examined. Metaphase FISH with the *AML1/ETO* probe (Abbott Molecular/Vysis) revealed an orange signal on a normal chromosome 8, a green signal on a normal chromosome 21, a fusion signal on a derivative chromosome 8, a small orange signal on a derivative chromosome 19, and a small green signal on a derivative chromosome 21 (Fig. 1). Multicolor banding (mBAND; MetaSystems, Altlussheim, Germany) results were consistent with the karyotype of this patient, whereas the multicolor FISH (mFISH; MetaSystems) result showed a discrepancy in one of the long arms of chromosome 21 (Figs. 1 and 2). Flow cytometry showed the blasts to be positive for CD7, CD10, CD13, CD19, CD45, HLA-DR, and MPO and negative for CD3, CD14, CD20, cCD22, CD33, CD79a, and TdT. In addition, the results of the gene rearrangement test for *AML1/ETO* were repeatedly positive, but all other rearrangement tests were negative in the patient's marrow specimen. The patient was treated with chemotherapy (daunorubicin and cytosine arabinoside), and the follow-up bone marrow examination showed hypocellular marrow without residual leukemic blasts.

As far as we know, only two cases of three-way variants of t(8;21) involving chromosome 19 have been reported in AML as a complex karyotype [2,3,5]. Two different

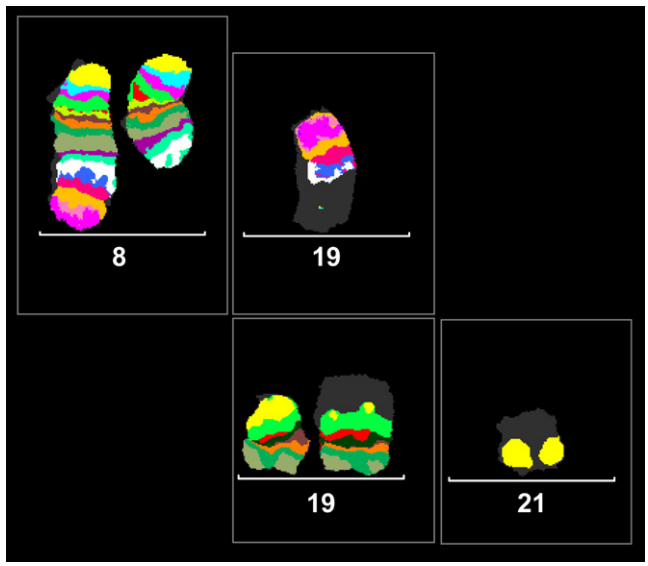

Figure 2. The chromosomes 8 and 19 specific mBAND probe kits, XCyte 8 (top image), and XCyte 19 (bottom image; MetaSystems) were hybridized to metaphase cells of bone marrow. The above image (chromosomes 8 and 19) that represents part of chromosome 8 was translocated to the short arm of chromosome 19. The bottom image (chromosomes 19 and 21) shows that the part of chromosome 19 was translocated to the distal part of the long arm of chromosome 21. This mBAND analysis confirmed the three-way t(8;21) variant, consistent with the result of cytogenetics of t(8;19;21)(q22;p13;q22).

chromosome regions, 19p13 and 19q13 (not definitely determined), were described to be involved in t(8;19;21) variants. Interestingly, the case presented here would be misinterpreted as a straightforward t(8;19)(q22;p13), a simple variant of t(8;21), if the reverse transcriptase-polymerase chain reaction result for *AML1/ETO* rearrangement were not available in advance. Such a simple variant should be suspected in a patient who subsequently is negative for the *AML1/ETO* fusion transcript [7]. In addition, we encountered an unexpected mFISH result showing small fragment of chromosome 8 translocated to chromosome 21, which was not in accordance with the results of cytogenetics and mBAND analyses. The XCyte mFISH and mBAND from MetaSystems uses five different fluochromes. Four of them [diethylamino-coumarin (DEAC), FITC, SpectrumOrange, and TexasRed] are coupled directly to the probes, and the other labeling is carried out using Biotin as a reporter molecule, which has to be detected by Streptavidin-Cy5. All three chromosomes (nos. 8, 19, and 21) involved in this translocation have the TexasRed and Cy5 in common. The distinct color fluorochromes of chromosome 19 and 21 are DEAC and SpectrumOrange, respectively. In our hybridization experiment, however, the small fragment of chromosome 19 translocated to chromosome 21 showed a weak DEAC signal, indicated by the diminished peak of the fluorescence intensity profile for the DEAC channel of the der(21) (Fig. 3). Dr. Chudoba, who carried out the mFISH experiment, suggested two possibilities as to why this signal (DEAC) was so weak and only the signals from color