Complex Chromosome 8;21 Translocation With Associated Hyperdiploidy in Acute Myeloid Leukemia (FAB-M2)

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We present a case of acute myeloblastic leukemia (AML-M2) with a complex t(8;21) translocation and additional acquired chromosomes yielding a hyperdiploid karyotype. AML1/ETO transcript was observed by reverse transcription-polymerase chain reaction. Fluorescence in situ hybridization (FISH), spectral karyotyping (SKY), and comparative genomic hybridization (CGH) were performed to further identify the chromosomes observed by G banding. The patient was treated according to our current protocol for AML. He remains in complete remission for 11 months from diagnosis. Further follow-up of this patient and the analysis of a larger number of children are needed to define whether the gains of the specific extra chromosomes modify the good prognosis that t(8;21) confers to this subgroup of AML. Pediatr Blood Cancer 2008;50:651–654. © 2007 Wiley-Liss, Inc.

INTRODUCTION

The t(8;21)(q22;q22) translocation is one of the most common chromosomal aberrations found in acute myeloid leukemia (AML) [1]. It is closely associated with M2 subtype, according to the French–American–British (FAB) classification [2], and is considered an independent entity in the World Health Organization classification [3]. This translocation fuses the AML1 (CBFα2, RUNX1) gene located on chromosome 21 to the ETO (MTG8) gene located on chromosome 8, resulting in a chimeric protein product, the AML1-ETO. Patients with this translocation are known as having a high response rate to chemotherapy and good prognosis [4].

Since the t(8;21) is rarely found with numerous added acquired chromosomes, its prognostic significance, if any, has not been clearly established [1]. We report on a complex case of t(8;21) and hyperdiploidy [5].

MATERIALS AND METHODS

Case report

An 11-year-old male was referred due to purpuric syndrome, fever, and bone pain in lower extremities of 10 days of evolution. A complete blood count showed a WBC: 58.4 × 10^9/L with 87% blasts, hemoglobin level: 8.4 g/dl, and platelet count: 15 × 10^9/L. The bone marrow aspirate disclosed an infiltration of 78% by blasts, hemoglobin level: 8.4 g/dl, and platelet count: 15 × 10^9/L. The bone marrow aspirate disclosed an infiltration of 78% by blasts. The bone marrow aspirate disclosed an infiltration of 78% by blasts.

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myeloblasts with azurophilic granulation and Auer rods. The cytochemical reactions were positive for MPO (in 90% of blasts).

Immunophenotyping of bone marrow blasts by flow cytometry showed: cytoplasmic antigens: CD22: 2%, CD79: 3%, CD3: 3%, MPO: 91%; surface antigens: HLA-DR: 92%, CD34: 92%, CD45: 98%, CD13: 64%, CD15: 71%, CD33: 47%, CD2: 4%, CD3: 3%, CD7: 4%, CD19: 81%, CD20: 1%, CD56: 34%. These findings support the myeloid origin of the blasts with co-expression of CD19 as it was previously described in this group of AML [6]. The patient was registered in our current protocol for acute myeloblastic leukemia which is based on the AML-BFM-98 study. The schedule consists of a double induction phase. The first, AIE (cytarabine, idarubicin, etoposide) was followed by the second, HAM (high doses of cytarabine and mitoxantrone). The schedule was completed with a maintenance phase. The patient achieved complete remission after the second induction. He was administered consolidation and intensification phases with adequate tolerance. He remains in complete remission +11 months from diagnosis.

**CYTOGENETICS**

Bone marrow cells were cultured for 24 hr and chromosome preparations were G banded. Chromosome identification and karyotype designation were made according to the International System for Human Cytogenetic Nomenclature (ISCN) [7]. Two hundred nuclei and five metaphases were analyzed by fluorescence in situ hybridization (FISH) to detect the t(8;21)(q22;q22) translocation using a locus specific probe (Dual Color/Dual Fusion, Vysis, Downers Grove, IL). FISH using a MALT1 probe (Dual color/Break Apart, Vysis, Downers Grove, IL) was also performed to identify the 18q21 region. Spectral karyotyping (SKY) was performed using Spectral karyotyping Paint Kit, following the manufacturers’ instructions. Five metaphases were analyzed [8]. Comparative genomic hybridization (CGH) was performed as a standard procedure [9] with minimal changes. At least 20 metaphases were analyzed with Cytovysion software (Applied Imaging Systems).

**RT-PCR for AML1-ETO**

Total RNA from mononuclear bone marrow cells at the time of diagnosis was extracted using Trizol®. Reverse transcription (RT) and polymerase chain reactions (PCR) were performed as described at Biomed-1 protocol [10].

**RESULTS**

The cytogenetic analysis revealed the following karyotype: 46,XY,t(8;21)(q22;q22)[3]/48,sl,þ4,þ6,[3]/52,sdl1,þ8,þ17,þ22,þmar[13]/46,XY[1] (Fig. 1A). FISH analysis showed a single fusion AML1-ETO in all nuclei analyzed (66% presented three copies of

![Fig. 1.](https://www.interscience.wiley.com)
ETO). Furthermore, the hybridization in metaphases showed that the fusion AML1/ETO appeared only in the der(8), and although a fusion was not observed in the der(21), a red signal and a green one were identified in the latter. We interpreted that the translocated material from chromosome 8 was inverted onto chromosome 21 (Fig. 1B, Supplementary Fig. 1A). Therefore, the karyotype was: 46,XY,t(8;21)(8pter→8q22::21q22→21qter;21pter→21q22::8q24.3→8q22::8q24.3→8qter). The application of SKY demonstrated that the extra marker belonged to chromosome 18 (Fig. 1C, Supplementary Fig. 1B).

The application of CGH confirmed gains of chromosomes 4, 6, 8, 17, and 22. It has also demonstrated a gain of 18q suggesting that the derivative 18 could be an isochromosome 18q (Fig. 2). To confirm these findings we performed FISH using MALT1 locus specific probe which demonstrated one signal in each arm (Supplementary Fig. 1C). Taking into account the FISH, SKY, and CGH studies, the following karyotype has been defined: 46,XY,t(8;21)(8pter→8q22::21q22→21qter;21pter→21q22::8q24.3→8q22::8q24.3→8qter) [3]/48,sl,+4,+6[3]/52,sdl1,+8,+17,i(18q),+22[13]/46,XY[1]. RT-PCR for AML1-ETO in mononuclear cells from a bone marrow sample and positive controls showed the expected size bands of 395bp and 338bp with AML1-A/ETO-B and AML1-E5/ETO-D primers, respectively.

DISCUSSION

A large number of recurring structural chromosomal aberrations have been described in acute leukemia and most of them have prognostic implications. Furthermore, cytogenetic abnormalities provide the strongest indicator of outcome in AML [11]. The t(8;21)(q22;q22) translocation is one of the most frequent cytogenetic aberrations in AML and it is associated with an overall favorable outcome, with high remission rates of 85–90% [12].

The presence of multiple additional chromosomes is a rare finding in AML and its prognostic significance is unknown. However, an unfavorable prognosis has been reported in a group of adult patients presenting this association [13]. In the largest study from the Medical Research Council (MRC) AML 10 trial, the authors found that additional cytogenetic abnormalities, irrespective of their nature or complexity, do not have a deleterious effect on the outcome of patients with t(8;21) [14].

The present case has a complex t(8;21) with gains of 4, 6, 8, 17, 18q and 22. Most likely, this karyotype is the result of a stepwise clonal evolution. A simple t(8;21) was the first event, with an inversion of the translocated material from chromosome 8, followed by gains of chromosomes. Three additional cases with t(8;21) and hyperdiploidy have been reported [15,16,17]. Each of the extra chromosomes identified in our case, except chromosome 17, was reported with t(8;21). Few cases of t(8;21) and trisomy 4 have been described. Although trisomy 8 is found in 10–15% of the AML, less than 5% are found with t(8;21). In each case, the trisomies do not seem to influence the relatively good prognosis of t(8;21). Gain of chromosome 18 is rarely found in AML [5]. The issue of the influence of other chromosomal findings on AML with the t(8;21) abnormality should be examined in current large cooperative groups trials.
Introduction

Aggressive natural killer cell leukemia (ANKL) is a rare disorder with fewer than 80 cases reported [1–6]. ANKL runs an aggressive course and is usually rapidly fatal, with a median survival of 61 days [1]. Only one survivor is reported in the literature [7].

Case Report

A 5-year-old male of Indonesian descent, born in Australia, presented with a 10-day history of persistent fevers, abdominal

References


Aggressive Natural Killer Cell Leukemia Presenting With Hemophagocytic Lymphohistiocytosis

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Aggressive natural killer cell leukemia (ANKL) is a very rare condition and when reported occurs almost exclusively in adults. We report a pediatric case of ANKL that presented with hemophagocytic syndrome, preceding the onset of leukemia by 12 weeks. Clinical and laboratory findings are discussed, along with morphology, immunophenotyping and cytogenetics, as well as the association with Epstein–Barr virus (EBV). This case is noteworthy for the expression of CD8 on the malignant cells, the cytogenetic findings that include abnormalities of chromosomes 6 and 7, as well as the age of the patient. Pediatr Blood Cancer 2008;50:654–657. © 2007 Wiley-Liss, Inc.

Key words: acute; Epstein–Barr virus; histiocytosis; leukemias; pediatric hematology/oncology

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