CASE REPORT

Complex t(8;13;21)(q22;q14;q22)—A Novel Variant of t(8;21) in a Patient with Acute Myeloid Leukemia (AML—M2)

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Variants of the t(8;21)(q22;q22) involving chromosome 8, 21, and other chromosomes account for about 3% of all t(8;21)(q22;q22) in acute myeloid leukemia (AML) patients. We report a case of AML—M2 with t(8;13;21)(q22;q14;q22), not reported earlier. Using a dual-color fluorescence in situ hybridization (FISH) analysis with ETO and AML1 probes, we demonstrate an ETO/AML1 fusion signal on the derivative chromosome 8. Whole chromosome painting probes were used for chromosomes 8 and 13, to demonstrate the three-way translocation t(8;13;21)(q22;q14;q22). Involvement of chromosome region 13q14 has never been reported earlier, although region 13q12 as a variant in AML with t(8;21) has been reported earlier. The possible role of genes in this region in leuke-mogenesis, its response to the treatment and its clinical implications are dis-cussed.

Key Words: Variant translocation, Leukemia, Acute myeloid leukemia, Karyotype.

Introduction

Acute myeloid leukemia (AML) is well characterized by specific chromosomal aberrations that correspond to various subtypes based on French-American-British (FAB) classification of acute leukemia. Translocation (8;21)(q22;q22) is a frequent non-random cytogenetic anomaly in AML and is strongly associated with FAB subtype M2 (AML—M2). It occurs in ∼10—15% of AML patients, whereas >90% of t(8;21)-positive leukemias have FAB AML—M2 morpholgy (1) and is an independent entity according to the World Health Organization (WHO) classification (2).

At the molecular level, the two genes involved in t(8;21) are AML1 at 21q22 and ETO at 8q22 (3,4). AML1 gene fuses to the ETO gene in generating a chimeric AML1/ETO fusion gene on the der(8). AML1 and ETO are both involved in transcriptional regulation of genes in the hematopoietic precursor cells. In the overall majority of cases, the AML1/ETO fusion gene is the result of a balanced translocation between chromosomes 8 and 21 (5).

In approximately 3% of AML patients, t(8;21) occurs as a complex variant involving chromosomes 8 and 21 and a third or fourth chromosome (6). Patients with these variants show typical features of t(8;21) AML morphologically, and in a limited number of patients studied the AML1/ETO fusion transcripts detected were similar to those of t(8;21) (3,4). We report here a female patient with AML (FAB—M2) showing a complex t(8;13;21) involving a new break-point 13q14, which has previously not been reported in the literature.

Case Report

NSNR, a 33-year-old Omani female, was referred with a history of anorexia for 5 weeks and fever during 3 weeks. She was referred due to anemia, pancytopenia, and abnormal blood smear. There was no significant past medical history. On examination the patient looked pale, overweight, and with few ecchymotic skin lesions. There was no
palpable lymphadenopathy. Liver and spleen were not palpable. Investigations revealed Hb 6.6 g/dl, WBC 5.1 \times 10^9/L with myeloblast seen on blood smear, platelet count 39 \times 10^9/L. Bone marrow examination was reported as acute myeloid leukemia—FAB M2 (WHO) (Figure 1). Bone marrow biopsy showed a patchy and interstitial infiltrate of immature cells. These cells were large and had a large nucleus with fine chromatin pattern and one or two nucleoli, irregular nuclear membranes with clefting, and scanty cytoplasm consistent with acute myeloid leukemia. Flow cytometry with four-color analysis using monoclonal antibodies was performed on the marrow sample revealing CD13—63%, CD33—64%, CD34—50%, CD56—43%, CD64—48%, CD117—62%, HLA-DR—83%, and MPO—36% consistent with AML on 55% bone marrow cells. The patient’s liver functions were deranged with AST 59 IU (12—42), ALT 42 IU (12—42), S. bilirubin: 19 \mu mol/L (4—17), ALP 214 IU (32—92) albumin 28 g/L (35—50), total protein 61 g/L (64—83), LDH: 1533 U/L (90—180), CRP 59 mg/L (0—8). The patient was negative for HSV, hepatitis B and C, and HIV. She was started on IV hydration, allopurinol, alkalinization, and antibiotics with the placement of a central venous catheter in preparation for treatment. However, the patient discharged herself against medical advice and was lost to follow-up. She was readmitted following clinical deterioration and was immediately started on standard AML induction treatment consisting of ara-C (100 mg/m², continuous infusion, days 1—7) along with daunorubicin (45 mg/m², intravenous [IV] push, days 1—3). She responded to treatment with complete remission on bone marrow examination. Thereafter, she received consolidation chemotherapy in the form of one cycle of high dose ara-C (1 g/m², continuous infusion over 6—8 h, days 1—3) and second consolidation with ADE regimen (ara-C 200 mg/m², continuous infusion, days 1—5, daunorubicin 45 mg/m², IV push, days 1—3 and etoposide 100 mg/m², continuous infusion over 1 h, days 1—5). She tolerated the chemotherapy well and is now in complete clinical remission awaiting allogeneic bone marrow transplantation from her HLA-matched sibling.

Figure 1. May Grunwald-Giemsa staining of the bone marrow slide showing large agranular myeloblasts with Auer rods (arrow). Color version of this figure available online at www.arcmedres.com

Figure 2. G-banded karyotype of the bone marrow cells showing t(8;13;21)(q22;q14;q22). Arrows indicate the aberrant chromosomes. Color version of this figure available online at www.arcmedres.com
Chromosome Analysis

A bone marrow sample obtained from the patient was cultured using Marrow Max culture media (cat. #12260–022; Gibco, Grand Island, NY) for 24 and 48 h using standard protocols. Chromosomes were treated and stained with trypsin–Giemsa (GTG) banding (7). Chromosomal abnormalities were described per the International System for Human Cytogenetic Nomenclature (8). Chromosomal analysis of the bone marrow cells showed an abnormal karyotype—46,XX,t(8;13;21)(q22;q14;q22) in all the metaphases analyzed (Figure 2).

Fluorescence In Situ Hybridization (FISH)

FISH analysis was performed using whole chromosome painting probes of 8 and 13 (WCP 8 and WCP13; CytoCell, Ltd., Oxfordshire, UK) and probe LSI AML1/ETO dual-color, dual-fusion (Abbott, Vysis, Inc., Downers Grove, IL) were used. FISH using painting probes of chromosome 8 and 13 showed whole painting of normal chromosome 8 and 13, a part of der(8) was translocated on der(13), and a part of der(13) translocated on der(21). This result confirms t(8;13;21) with karyotype: 46,XX,t(8;13;21)(q22;q14;q22),ish t(8;13;21) (WCP8+, ETO+, AML1+; WCP13+, WCP+8, ETO+, AML1--; WCP8--, WCP13+, AML1+, ETO--) (Figure 3a).

FISH using probe LSI AML1/ETO dual-color, dual-fusion showed ETO-AML1 fusion in 98.5% of cells (300 nuclei cells analyzed). Metaphases showed one normal orange (ETO) and one normal green (AML1) signals on normal chromosomes 8 and 21. One orange green fusion signal (AML1/ETO) located on der(8), one small orange signal (ETO) on der (13), and one small green signal (AML1) on der (21). This result confirmed t(8;13;21) and AML1/ETO translocation (Figure 3b). (FISH was done commercially at Laboratory Marcel Mérieux, Paris, France).

Discussion

Translocation (8;21)(q22;q22) is a specific anomaly frequently observed cytogenetically in AML—M2. The AML1 rearrangement (4) or AML1/ETO fusion transcripts (9) detected by Southern blotting or RT-PCR analysis, respectively, in patients without cytogenetic evidence of t(8;21) indicated that AML1/ETO fusion gene could result from a variant or masked chromosomal rearrangement involving chromosomes 8 and 21.

Complex variants of t(8;21)(q22;q22) are occasionally observed in a small percentage of AMLs. Most are three-way translocations involving regions q822, 21q22 and a third chromosome. Chromosomes 1, 2, 3, 5, 6, 8, 10, 12, 13, 14, 15, 16, 17, 18, and 20 have been reported as the third chromosome involved in the variant three-way translocation (Table 1) (10–26). Very rarely, a four-way variant complex translocation is reported (27,28). In this study, we report an AML—M2 patient involving a new breakpoint 13q14 in a complex three-way t(8;13;21) (q22;q14;q22). FISH analysis with AML1/ETO-specific probes in a dual-color assay showed co-localization of AML1 and ETO signals on the rearranged chromosome 8 at q22. Involvement of chromosome band 13q14 has not been reported so far in the literature but was seen in our patient with the breakpoint at 13q14 along with 8q22 and 21q22. Band 13q14 is known to carry tumor suppressor genes for retinoblastoma, prostate cancer and chronic lymphocytic leukemia (29–31); however, it is unclear as to what role, if any, this breakpoint with complex three-way translocation has played in leukemogenesis.

The relevance of the der(8) chromosome, which contains the AML1/ETO fusion gene in the pathogenesis of AML—M2,
Table 1. Variant complex translocations reported in AML

<table>
<thead>
<tr>
<th>No.</th>
<th>Year</th>
<th>Translocation</th>
<th>FAB type</th>
<th>Authors</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1985</td>
<td>t(8;21):15</td>
<td>M2</td>
<td>Ayraud et al.</td>
<td>10</td>
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<td>2</td>
<td>1986</td>
<td>t(1;8;21)</td>
<td>M2</td>
<td>Tagushi et al.</td>
<td>11</td>
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<tr>
<td>3</td>
<td>1988</td>
<td>t(8;18;21)</td>
<td>NA</td>
<td>Uchida et al.</td>
<td>12</td>
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<tr>
<td>4</td>
<td>1992</td>
<td>t(8;15;21)</td>
<td>M2</td>
<td>Sundareshan et al.</td>
<td>13</td>
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<tr>
<td>5</td>
<td>1994</td>
<td>t(8;13;21)</td>
<td>M2</td>
<td>Gallego et al.</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>1995</td>
<td>t(8;21;16;21)</td>
<td>NA</td>
<td>de Greet et al.</td>
<td>28</td>
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<tr>
<td>7</td>
<td>1997</td>
<td>t(8;12;16;21)</td>
<td>M2</td>
<td>Saito et al.</td>
<td>14</td>
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<td>8</td>
<td>1998</td>
<td>t(3;21;8)</td>
<td>NA</td>
<td>Giles et al.</td>
<td>15</td>
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<tr>
<td>9</td>
<td>1998</td>
<td>t(8;20;21)</td>
<td>M2</td>
<td>Wong et al.</td>
<td>16</td>
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<tr>
<td>10</td>
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<td>t(5;8;21)</td>
<td>M2</td>
<td>Kikuchi et al.</td>
<td>17</td>
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<td>11</td>
<td>1999</td>
<td>t(6;21;8)</td>
<td>M2</td>
<td>Shinagawa et al.</td>
<td>18</td>
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<td>12</td>
<td>2001</td>
<td>t(8;17;15;21)</td>
<td>M2</td>
<td>Vieira et al.</td>
<td>27</td>
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<td>13</td>
<td>2001</td>
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<td>M2</td>
<td>Watanabe et al.</td>
<td>19</td>
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<td>14</td>
<td>2001</td>
<td>t(8;21;8)</td>
<td>M2</td>
<td>Xue et al.</td>
<td>20</td>
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<td>15</td>
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<td>t(8;21;14)</td>
<td>M2</td>
<td>Ishida et al.</td>
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<td>16</td>
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<td>t(8;14;21)</td>
<td>M1</td>
<td>Takahashi et al.</td>
<td>22</td>
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<td>17</td>
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<td>M2</td>
<td>Barra et al.</td>
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<td>18</td>
<td>2005</td>
<td>t(8;10;21)</td>
<td>M2</td>
<td>Lee et al.</td>
<td>24</td>
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<td>2005</td>
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<td>M1</td>
<td>Lau et al.</td>
<td>25</td>
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<td>2007</td>
<td>t(8;13;21)</td>
<td>M2</td>
<td>Udayakumar et al.</td>
<td>Present report</td>
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AML, acute myeloid leukemia; NA, not available.

is reinforced by these complex variant translocations. These variants stress the need for co-localization of ETO/AML1 genes by applying FISH and/or for detection of AML1/ETO fusion transcripts by RT-PCR in AML patients showing variant rearrangements of t(8;21), which allows them to be assigned to the correct risk group for the purpose of treatment (22,27).

The distal long arm of chromosome 21 at q22 was translocated to the long arm of chromosome 8, whereas the end of chromosome 8 was translocated to a third chromosome. The remainder of the third chromosome was translocated to chromosome 21 (32,33). Exactly the same mechanism was observed in our patient, but the third chromosome was 13 involving band q14. These events clearly indicate that the AML1/ETO fusion from t(8;21) is one of the main causes of leukemogenesis in the variant translocations associated with t(8;21). Clinopathological features of AML carrying variant t(8;21) are less well characterized (34). Although t(8;21) is associated with a good prognosis, the clinical relevance and implications of this new variant t(8;13;21) is yet to be determined. In our patient, there have been no complications. She has tolerated the chemotherapy well and has achieved complete clinical remission as is often seen in uncomplicated AML patients with t(8;21). However, being unsure as to whether the genes in the region 13q14 have any new role to play in leukemogenesis, it was decided to recommend allogeneic marrow transplantation.

Acknowledgments
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