Two cases of myeloid disorders and a t(8;12)(q12;p13)

JESÚS MARÍA HERNÁNDEZ,* MARÍA BELÉN GONZÁLEZ,* JUAN LUIS GARCÍA,* MARÍA TERESA FERRO,°
NORMA CARMEN GUTIÉRREZ,† PETER MARYNEN,# JESÚS F. SAN MIGUEL*

*Servicio de Hematología, Hospital Universitario de Salamanca and Centro de Investigación del Cáncer, Universidad de Salamanca-CSIC, Spain; °Servicio de Genética, Hospital Ramón y Cajal, Madrid, Spain; #Flanders Institute of Biotechnology, University of Leuven, Belgium

ABSTRACT

Rearrangements of the short arm of chromosome 12 have been described in different hematologic malignancies such as acute lymphoblastic leukemias (ALL), acute myeloblastic leukemias (AML), and myelodysplastic syndromes (MDS). Some of these abnormalities showed a rearrangement of the ETV6 gene. We studied the 12p region in one case with a t(8;12)(q12;p13) by fluorescence in situ hybridization (FISH).

Results. FISH studies demonstrated hemizygous loss of the ETV6 and CDKN1B regions and two copies of the CCND2 locus, as a result of the balanced translocation and an additional copy of the der(8).

Design and Methods. We have identified a chromosomal translocation, t(8;12)(q12;p13) in two patients with myeloid disorders; one with acute myelogenous leukemia (AML) and one with refractory anemia (RA). FISH studies with specific probes (cosmids and YACs) for the 12p region were used to investigate one case.

Design and Methods

Case report

Case #1. A 73-year old man was referred to hospital because of fatigue and weight loss. He gave no history of previous exposure to toxic or mutagenic agents. Clinical examination showed moderate splenomegaly. The hemoglobin concentration was 82 g/L, the white blood cell count was 32·10^9/L, and the platelet count was 859·10^9/L. Bone marrow (BM) aspirate showed hypercellularity, 3% of myeloblasts, and marked dysplasia of the red cells and granulocytes. Immunophenotypic study revealed 3% of immature myeloid cells (positive for CD34/CD33/HLA-DR). A diagnosis of refractory anemia was made. Some improvement was achieved with hydroxyurea (500 mg/day). Four months after diagnosis hemoglobin levels decreased and red cell transfusions were needed. His platelet count dropped and he began to have symptoms of bleeding. His general condition deteriorated and the patient died 16 months after diagnosis.

Case #2. A 69-year old man with a history of two acute myocardial infarctions presented with fever, malaise, weight loss, and confusion. Clinical examination revealed disorientation and impairment of cortical functions. A computed tomography scan showed a hypodense area in the right frontal lobe. Routine laboratory examination revealed: hemoglobin of 34 g/L; WBC of 1·10^9/L (42% of blast cells), and a platelet count of 40·10^9/L. Bone marrow aspiration showed trilineage myelodysplasia and 38% of blasts cells. A diagnosis of AML, FAB subtype M2, was made. Blast cells were positive for CD34, CD33, CD11, CD15 and HLA-DR. The patient died 4 weeks after diagnosis.

Cytogenetic and FISH studies

Cytogenetic studies were done according to standard methods and karyotype recorded as recom
mended by the ISCN (1995).12
FISH analysis and probes. FISH studies were carried out in case #1 as previously described.13 In short, the probes were labeled either with biotin-11-dUTP or with digoxigenin-11-d-UTP, denatured, pre-annealed with Cot-1 DNA and hybridized overnight on pretreated and denatured chromosome spreads. After washing, the biotinylated probes were detected with two layers of avidin-FITC and the digoxigenin-labeled probes with two layers of TRITC-conjugated antibodies. The following probes were used: YAC 964c10, covering ETV6 at 12p13; cosmid clones 139C5 and 213C1, were obtained by screening the Lawrence Livermore chromosome 12 library LL12NCO1 with a probe for exon 2 of CCND2 (addresses: 139C5 and 213C1). The cosmid clone 123C12 for p27Kip1 (CDKN1B) was obtained from the same library as previously described.14 In addition, a centromere specific probe for chromosome 12 was used (CEP12 Spectrum Green, Vysis, Stuttgart, Germany). Dual color FISH using whole chromosome painting probes for chromosomes 8 and 12 (Coatasome 8 digoxigenin-labeled and coatasome 12 biotin-labeled, Oncor, Gaithersburg, MD, USA), was done according to the manufacturer’s recommendations.

Results

Cytogenetics
Cytogenetic analysis in case #1 revealed the karyotype 47,XY,t(8;12)(q12;p13), +der(8)t(8;12) in 18 out of the 20 cells analyzed. Case #2 showed 46,XY,del(5)(q13q31), t(8;12)(q12;p13) in all the 20 cells investigated.

FISH
The analysis with the painting probes (whole libraries of chromosomes 8 and 12) performed in case #1 confirmed the cytogenetic results (Figure 2A). FISH studies performed with YAC clone 964c10 (containing the entire ETV6 gene) and cosmid clone 123C12 (CDKN1B) showed a single copy of both probes placed on the normal chromosome 12 in 14 out of the 16 mitoses analyzed for the YAC clone 964c10 (Figure 2B) and in 12 out of the 14 mitoses studied for the cosmid 123C12 (Figure 2C).

Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Probe</th>
<th>Case no. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCND2</td>
<td>139C5</td>
<td>●</td>
</tr>
<tr>
<td>ETV6</td>
<td>213C1</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>964C10</td>
<td>○</td>
</tr>
<tr>
<td>CDKN1B</td>
<td>123C12</td>
<td>○</td>
</tr>
</tbody>
</table>

Figure 1. Schematic representation of 12p. The diagram gives the order of the probes. Solid circles indicate the presence of three copies of the probe and open circles indicate the presence of only one copy.

Figure 2. Results of FISH analysis on bone marrow metaphases from case #1. A) Double color hybridization with painting libraries for chromosome 8 (green) and 12 (red) showing one normal copy of chromosome 8 and 12 as well as one der(12) (yellow arrow) and two copies of der(8) (white arrow). B) FISH hybridization with a digoxigenin-labeled YAC 964c10 (green) showing only one copy of the probe in the normal chromosome 12. C) Hybridization with a biotin-labeled cosmid 139C5 (green) and a digoxigenin-labeled 213C1 (red) showing three copies of both probes, on the normal chromosome 12 (yellow arrow) and on both der(8) (white arrow).
12p proximal to CDKN1B and CCND2. In our case, studied by FISH, both ETV6 and CDKN1B were deleted and CCND2 was duplicated. Molecular mapping of 12p located sequences. Deletions of both genes have been reported independently or concurrently.3,9,11,17,19 The breakpoints in balanced rearrangements of 12p may be found not only in acute lymphoblastic leukemia of childhood, but also in myeloid disorders. **Potential implications for clinical practice**

- This case report suggests that the rearrangements of chromosome 12p may be found not only in acute lymphoblastic leukemia of childhood, but also in myeloid disorders.

**References**


**Disclosures**

Conflict of interest: none. Redundant publications: no substantial overlapping with previous papers.

**Manuscript processing**


