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Stem cell disorders

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

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ABSTRACT

Background and Objectives. **Rearrangements of the short arm of chromosome 12 have been described in different hematologic malignancies. 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).**

Design and Methods. **We have identified a chromosome 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.**

Results. **FISH studies demonstrated hemizygous loss of the *ETV6* and *CDKN1B* regions and two copies of the CCDN2 locus, as a result of the balanced translocation and an additional copy of the der(8).**

Interpretation and Conclusions. **Myeloid diseases with t(8;12)(q12;p13) have an interstitial deletion of 12p, including the *ETV6* and *CDKN1B* regions. A duplication of CCDN2 locus can also be found.**

Key words: cytogenetics, FISH, ETV6, t(8;12)(q12;p13), acute myeloblastic leukemia, myelodysplastic syndromes

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).[1-3] Some of these abnormalities showed a rearrangement of the *ETV6* gene. This gene was first described in patients with chronic myelomonocytic leukemia and t(5;12) in which *ETV6* was fused to PDGRFB on chromosome 5.[4] *ETV6* gene has also been found to be fused to *ABL* or *AML1* in ALL,[5,6] and to *MN1* or *EVI1* in myeloproliferative disorders.[7,8] Occasionally these translocations involving 12p show submicroscopic deletions of 12p with loss of *ETV6* and *CDKN1B* genes[9-11] (Figure 1). We describe a translocation t(8;12)(q12;p13) in two patients with myeloid disorders: one with refractory anemia (RA) and one with acute myelogenous leukemia, respectively; in the first case fluorescence *in situ* hybridization (FISH) studies showed a loss of the *ETV6* gene and duplication of the *CCND2* gene.

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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, CD13, 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-

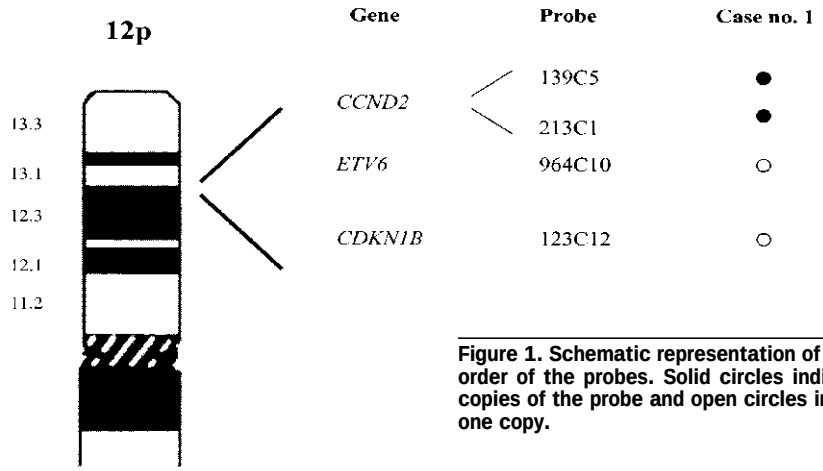

Figure 1. Schematic representation of 12p. The diagram gives the order of the probes. Solid circles indicate the presence of three copies of the probe and open circles indicate the presence of only one copy.

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, denaturated, pre-annealed with Cot-1 DNA and hybridized overnight on pretreated and denaturated 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 213 C1). The cosmid clone 123C12 for $p27^{Kip1}$ (*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

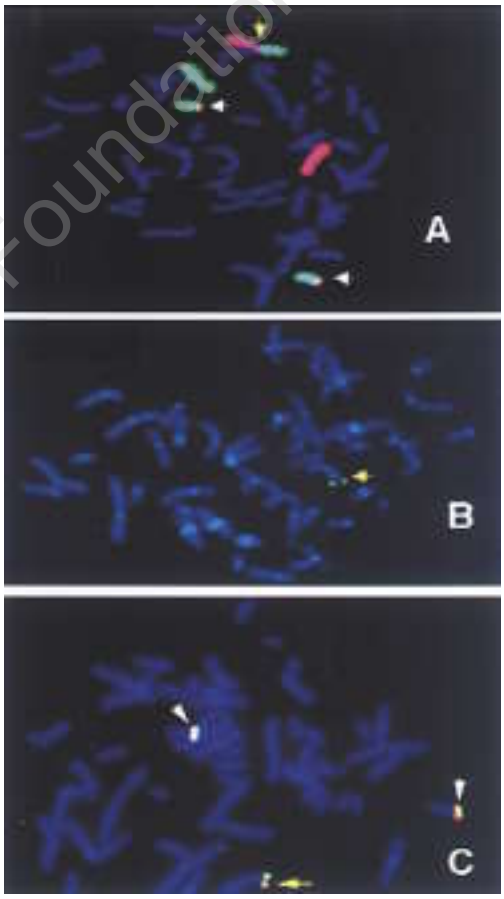

Figure 2. Results of FISH analysis on bone marrow metaphases from case #1. A) Double color hybridization with painting libraries for chromosome 8 (green) and 12 (red) showing one normal copy of chromosome 8 and 12 as well as one der(12) (yellow arrow) and two copies of der(8) (white arrow). B) FISH hybridization 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).

123C12. No residual material either from the YAC or from the cosmid, seen as a reduced signal, was detected on the aberrant chromosomes indicating that both probes were deleted in the translocation. FISH studies performed with the 139C5 and the 213C1 probes (*CCND2*) labeled with two colors showed three signals each, one on the normal chromosome 12 and the two other signals on both derivative chromosomes 8, in 23 out of the 25 mitoses analyzed (Figure 2C). These results indicated a breakpoint on chromosome 12 proximal to *CCND2* and a duplication due to the presence of two der(8) (Figure 1).

Discussion

Rearrangements of chromosome 12p are commonly found in acute lymphoblastic leukemias of childhood, but they are infrequent in myeloid disorders. We describe a translocation t(8;12)(q12;p13) in two patients with myeloid disorders, acute myeloblastic leukemia (AML) and myelodysplastic syndrome (MDS). Case #2, with a diagnosis of AML, presented the t(8;12) in association with a del(5q) and trilineage dysplasia. The other patient had a diagnosis of refractory anemia. This suggests that the t(8;12) could be associated with MDS. Translocations of 12p have been observed with different chromosomes in AML, ALL and MDS.[1-3,9,15-18] Wlodarska *et al*. in 1996 described an ALL case with a t(8;12) but with different breakpoints.[19] Recently Streubel *et al.*[3] reported two cases of AML with t(8;12); one of these cases had been diagnosed as AML-M3 and showed a t(8;12) with a breakpoint at 8q22 and associated with t(15;17); the other patient, with a diagnosis of AML-M2, showed an unbalanced translocation: der(12)t(8;12)(q11;p11.2) in a complex karyotype. Sato *et al*. described a t(8;12) with a possible breakpoint on 8q11 in two patients, one with a diagnosis of MDS and the other of chronic myeloid leukemia (CML).[2] However no cases with two copies of the der(8), as we found in our case #1, have been previously reported.

In our case, studied by FISH, both *ETV6* and *CDKN1B* were deleted and *CCND2* was duplicated. Molecular mapping of 12p located *CCND2* telomeric to *ETV6* (Figure 1). Our FISH results indicate a 12p breakpoint proximal to *CCND2* and a submicroscopic interstitial deletion of both *ETV6* and *CDKN1B* sequences. Deletions of both genes have been reported in different hematologic malignancies such as ALL, AML, MDS and non-Hodgkin's lymphomas, either independently or concurrently.[3,9,11,17,19] The breakpoint of *ETV6* appears to be rather heterogeneous in myeloid disorders with t(8;12): *ETV6* was lost in the case reported by Streubel *et al.* with a der(12) and stayed on the der(12) of the CML case #2; and moved to the der(8) in the MDS case #2. In our case, *CCND2* is duplicated and probably so are all the genes located distally on 12p. This is the direct consequence of the second copy of the der(8). The biological significance of this observation is unclear.

Contributions and Acknowledgments

JMH: general design and writing of the paper. MBG: FISH studies. JLG: cytogenetic and FISH studies of case #1. MTF: clinical and cytogenetic data of case #2. NCG: clinical data of case #1 and FISH studies. PM: design of FISH experiments and provided the probes. JFSM: final design and corrections of the paper. The authors thank Prof. Anne Hagemeijer for her critical review of the manuscript and C. Hilliker, R. González-Sarmiento, M. A. Sánchez and P. Fernández for their technical assistance.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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Potential implications for clinical practice

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

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