MLL tandem duplication in two cases of acute myelocytic leukemia with unbalanced translocations: der(16)(t(11;16)(q23;p13)) and der(18)(t(11;18)(q22;p11.2))

A. Aventín*a, R. La Starza,b, S. Casas,a J. Nomedédu,a M.P. Queipo de Llano,c G. Cimino,d F. Lo Coco,d J. Sierra,a C. Mecucci b

aDepartment of Hematology, University Hospital Sant Pau, UAB Barcelona, Spain
bHematology and Bone Marrow Transplantation Unit, University of Perugia, Perugia, Italy
cHospital Clínico, Málaga, Spain
dDepartment of Cellular Biotechnology and Hematology, University "La Sapienza," Rome, Italy

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Abstract

We describe two cases of acute myelocytic leukemia (AML), classified as M4 and M1 in the French-American-British classification, with unbalanced translocations der(16)(t(11;16)(q23;p13)) and der(18)(t(11;18)(q22;p11.2)), respectively. Molecular studies using Southern blot and reverse transcriptase-polymerase chain reaction showed an MLL rearrangement due to an internal duplication of the gene in both cases. Fluorescence in situ hybridization disclosed the presence of an extra copy of the MLL gene on 16p13 and 18p11.2, respectively, as a result of the partial trisomy of chromosome 11q. Our two cases clearly show that tandem duplication of the MLL gene may occur in AML with a partial 11q trisomy. Thus, systematic screening of this molecular defect should be performed in patients with unbalanced translocations involving 11q22–q23 region. © 2003 Elsevier Inc. All rights reserved.

1. Introduction

The MLL gene, located on 11q23, can be rearranged as a result of a reciprocal chromosomal translocation producing a fusion gene or by a duplication of an internal portion of the gene [1,2]. Partial tandem duplication of MLL has been described in patients with acute myelocytic leukemia (AML) and myelodysplastic syndrome with normal karyotype or trisomy 11 [2,3], and in a few cases of AML harboring other chromosomal abnormalities [4].

We describe two cases of AML with unbalanced translocations der(16)(t(11;16)(q23;p13)) and der(18)(t(11;18)(q22;p11.2)), respectively, in which Southern blot and reverse transcriptase polymerase chain reaction (RT-PCR) revealed an MLL rearrangement due to an internal duplication of the gene and fluorescence in situ hybridization (FISH) revealed the presence of an extra copy of the MLL gene on der(16) and on der(18), as a result of the partial trisomy of the long arm of chromosome 11.

2. Case reports

2.1. Patient 1

A 58-year-old man was admitted in February 2000 because of persistent fever and diffuse thoracic pain. Hepatomegaly was evidenced at clinical examination. Peripheral blood count laboratory values were as follows: hemoglobin 11.2 g/dL, white blood cells 135,000/mL (neutrophils 24%, lymphocytes 3%, monocytes 30%, eosinophils 2%, blast cells 4%), and platelets 42,000/mL. The lactate dehydrogenase level was 3909 U/L (normal: < 250 U/L). Bone marrow aspirate and biopsy were consistent with AML, M4 subtype. The immunophenotype of peripheral blast cells was as follows: CD13 (82%), CD34 (95%), CD14 (79%), CD33 (94%), CD34 (1%), CD16 (1%), sCD3 (3%), CD117 (1%), CD11b (66%), CD36 (93%), CD5 (19%), CD4 (95%), HLA-DR (1%), CD7 (3%), CD19 (4%), CD10 (5%), and CD20 (2%). After two therapeutic leukopheresis, the patient underwent an AML-99 GIMEMA protocol, which includes hydroxyurea, daunorubicin, cytosine-ara-C, and VP-16.
hematologic remission was obtained. A morphologic relapse occurred before the patient started consolidation therapy. He underwent treatment with IL2, and died in relapse 14 months after diagnosis.

2.2. Patient 2

A 42-year-old female was referred in June 2000 because of pancytopenia. Medical history was unremarkable. Physical examination on admission disclosed only conjunctival and cutaneous pallor. Hematologic data were as follows: hemoglobin 5.9 g/dL, white blood cells 39,000/μL (blast cells 90%), and platelets 29,000/μL. The lactic dehydrogenase level was 1018 U/L (normal: < 420 U/L). Bone marrow aspirate was consistent with AML. M1 subtype. Immunophenotyping of the blasts was: HLA-DR 23%, CD13 44%, CD33 44%, CD14 35%, CD117 48%, CD43 48%, CD15 27%, anti-MPO 60%, CD74 47%, CD10 3%, CD19 2%, CD20 2%. The patient was started on induction therapy according to the AML-99 CETLAM protocol; complete remission was achieved after one cycle of idarubicin, cytosine–arabinoside, and etoposide. She received a course of intensification treatment (mitoxantrone and cytosine–arabinoside) and an autologous peripheral progenitor transplantation was delayed because of abnormal liver function tests. She subsequently received one course of high-dose cytosine–arabinoside. In May 2001, a bone marrow aspirate revealed 12% blast cells. Despite treatment with salvage chemotherapy, she died from a cerebral hemorrhage 14 months after diagnosis with resistant disease.

Data were collected on an Olympus fluorescence microscope equipped with a cooled charge-coupled device (CCD) Sensys camera (Photometrics, Tucson, AZ, USA) running PathVysion software (Vysis, Stuttgart, Germany). 3.3. Southern blot analysis

High molecular weight DNA was extracted from mononuclear cells, digested to completion with BamHI and BglII restriction endonucleases, size-fractioned by electrophoresis in a 0.8% agarose gel, denatured, and transferred onto a nitrocellulose membrane. After prehybridization, the filter was hybridized overnight with the 32P random priming labeled probe B859, washed, and exposed for 48 hours at −70°C using intensifying screen. Prehybridization, hybridization, and washing conditions were as reported elsewhere [7]. The B859 probe is a cDNA insert, derived from ALL1 exons 5–11, that explores the MLL/ALL1 gene breakpoint cluster region [8].

3.4. RT-PCR analysis

Total RNA was extracted from mononuclear cells by the guanidinium thiocyanate–phenol chloroform method [9]. RT-PCR to detect the MLL gene duplication was performed using the oligonucleotides complementary to MLL/ALL1 exon 2 (antisense) exon 5 (sense) (case 1) and to MLL/ALL1 exon 3 (antisense) exon 5 (sense) (case 2); the experimental conditions reported elsewhere [10].

4. Results

4.1. Cytogenetics

4.1.1. Patient 1

All 20 metaphase cells analyzed from the unstimulated 24-hour culture showed the karyotype 46,XY,add(16)(p13). 4.1.2. Patient 2

Nine of 20 metaphase cells analyzed from the unstimulated 24-hour culture showed a 46,XX,add(18)(p11.2) karyotype. The 11 remaining cells were normal.

4.2. FISH analysis

4.2.1. Patient 1

FISH with wcp 11 and wcp 16 showed the presence of material from chromosome 11 on the short arm of der(16). FISH with PAC 98015, the LSI MLL probe, and PAC 770G7 all gave three fluorescence signals on both normal chromosomes 11 and on der(16) (Fig. 1A and C), whereas PAC 891P24 hybridized only with normal chromosomes 11. Thus, the partial 11q trisomy started downstream of PAC 891P24 (ATM gene).

4.2.2. Patient 2

FISH with wcp 11 and wcp 18 showed the presence of material from chromosome 11 on the short arm of der(18).
Fig. 1. (A, B) FISH with the MLL dual probe (5'MLL and 3'MLL; Vysis) showing the presence of an entire additional copy gene in both cases. In patient 1, hybridization signals corresponding to the MLL gene are present on both normal 11 and on der(16) (A). In patient 2, hybridization signals are present on both normal 11 and on der(18) (B, C, D) FISH with PAC 770G7 gives three signals of fluorescence in both cases. A signal is present on normal chromosomes 11 and on der(16) in (C), or on der(18) in (D).

FISH with PAC 891P24, PAC 980J15, the LSI MLL probe, and PAC 770G7 all gave three fluorescence signals on both normal chromosomes 11 and on der(18) (Fig. 1B and 1D). Thus, the partial 11q trisomy started upstream of PAC 891P24 (ATM gene).

4.3. Southern blot analysis

In both cases, Southern blot analysis of leukemic DNA with the B859 probe exploring the MLL/JALL1 locus revealed the presence of additional fragments of different size, compared with control placenta DNA. Abnormally migrating bands were confirmed in the two restriction endonuclease digestions. The detection of one single rearranged fragment in each digestion suggested the possible occurrence of MLL/JALL1 duplication.

4.4. RT-PCR analysis

RT-PCR analysis using specific oligoprimers allowed us to amplify a 258-bp MLL/JALL1 consistent with exon 6/exon 2 or exon 8/exon 2 fusion, depending on breakpoint location and occurrence of alternative splicing of exon 7 in case 1, and a 270-bp MLL/JALL1 consistent with exon 6/exon 2 in case 2 (Fig. 2).

5. Discussion

The partial tandem duplication of the MLL gene was first described in AML patients either with normal karyotype or with trisomy 11 as the sole chromosomal abnormality [2]. This molecular defect has been shown to confer a dismal
fusion of MLL. Unfortunately, molecular studies were not reported in detail. Unbalanced chromosomal anomalies are thought to be secondary cytogenetic changes related to disease progression rather than to tumor initiation [14]. In the two cases we describe, MLL duplication might instead be the primary genomic aberration, followed by a gross chromosomal unbalanced translocation. Similarly, Schnittger et al. [4] described two AML patients with MLL duplication who presented at relapse unbalanced chromosome aberrations (i.e., an add(7)(q37q4) and a del(17)(q23), respectively). Because the type of fusion transcripts, in both cases, were the same at diagnosis and at relapse, it was suggested that duplication was the primary event in the origin of leukemic transformation.

Regarding clinical outcome, both our patients died 14 months after diagnosis with a resistant disease, showing that AML with MLL internal duplication and partial 11q trisomy falls in the unfavorable prognosis subgroup, for which allogeneic stem cell transplantation should be considered as first-line postremission treatment.

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References


3.2.2 *Inversió del cromosoma 16 associada a trasnlocació (II)*


Es van analitzar per FISH amb la sonda LSI-CBFb 40 casos de LMA que presentaven al cariotip una inv(16)(p13q22) o t(16;16)(p13;q22) diagnosticada prèviament tant per ACC com per FISH utilitzant la sonda WCP-16p. D’entre els casos amb inv(16), en dos casos es va detectar la translocació de la part 3’ del gen CBFb a 10p13 en l’un, i a 1p36 en l’altre (Fig. 1A i C).

En el cas 1, els estudis addicionals de FISH dual amb les sondes TEL-10p i TEL-16q van permetre identificar una translocació reciproca de les dues regions subtelomèriques, essent per tant t(10;16)(p13;q22) (Fig. 1E). En el cas 2, on no es disposava de més material per realitzar estudis addicionals de FISH, es va estudiar retrospectivament i amb detall el cariotip, el qual es va reinterpretar com a una inv(16) associada a la translocació t(1;16)(p36;q22). L’estudi de RT-PCR va confirmar la fusió de CBFb/MYH11 en ambdós casos (resultats no presentats).