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Cancer Genetics and Cytogenetics 142 (2003) 8–12

Cancer Genetics and Cytogenetics

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)

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Received 27 March 2002; received in revised form 9 August 2002; accepted 9 August 2002

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→qter.

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/μL (neutrophils 24%, lymphocytes 3%, monocytes 30%, basophils 2%, blast cells 41%), and platelets 42,000/μL. The lactic 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%), CD45 (95%), CD14 (79%), CD33 (94%), CD34 (1%), CD1a (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 leukophereses, the patient underwent an AML-99 GIMEMA protocol, which includes hydroxyurea, daunorubicin, cytosine–arabinoside, and VP-16. Complete

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0165-4608/03/$ – see front matter
doi:10.1016/S0165-4608(02)00736-7

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 blast cells was: HLA-DR 23%, CD13 44%, CD33 44%, CD34 33%, CD117 48%, CD45 48%, CD15 27%, anti-MPO 60%, CD7 4%, 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.

3. Materials and methods

3.1. Cytogenetics

Karyotypic analyses were performed on unstimulated bone marrow cells after short-term cultures. Chromosomes were G-banded and evaluated according to the Interantional System for Human Cytogenetic Nomenclature (ISCN 1995) [5].

3.2. FISH analysis

FISH methodology was as previously described [6]. Whole chromosome paint (wcp) was performed for chromosomes 11 and 16 (Oncor, Gaithersburg, MD, USA) in case 1 and for chromosomes 11 and 18 (Oncor) in case 2. The *MLL* gene was investigated with the LSI MLL Vysis dual-color probe (Vysis, Downers Grove, IL, USA). Additional FISH studies were performed with PAC 980J15 (kindly provided by M. Rocchi, University of Bari, Italy) spanning the *PLFZ* gene, PAC 891P24 (kindly provided by M. Rocchi) spanning the *ATM* gene, and PAC 770G7 (kindly provided by J. Flint, Oxford University, UK) for the 11q subtelomeric region. Centromeric probes for chromosomes 16 (Oncor) and 18 (L 1.84, kindly provided by M. Stul, University of Leuven, Belgium) were also used. At least five abnormal cells were analyzed in each experiment. 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 *Bam*HI and *Bgl*II 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 ^{32}P 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 guanidium thiocyanate–phenol chloroform method [9]. RT-PCR to detect the *MLL* gene duplication was performed using the oligoprimers 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 980J15, 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).

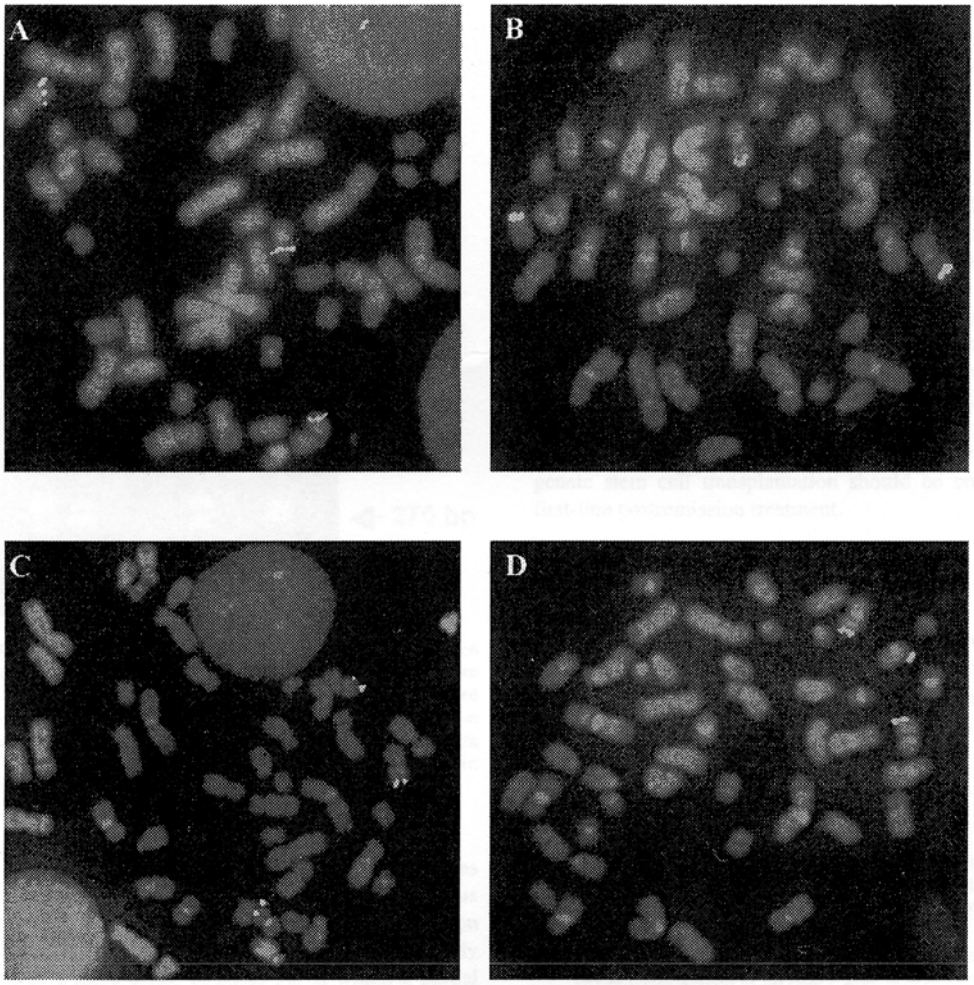

Fig. 1. (A, B) FISH) with the MLL dual probe (5′MLL and 3′MLL; Vysis) showing the presence of an entire additional copy gene in both cases. In patient 1, hybridization signals corresponding to the *MLL* gene are present on both normal 11 and on der(16) (A). In patient 2, hybridization signals are present 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/ALL1* 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/ALL1* duplication.

4.4. RT-PCR analysis

RT-PCR analysis using specific oligoprimers allowed us to amplify a 258-bp *MLL/ALL1* 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/ALL1* 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

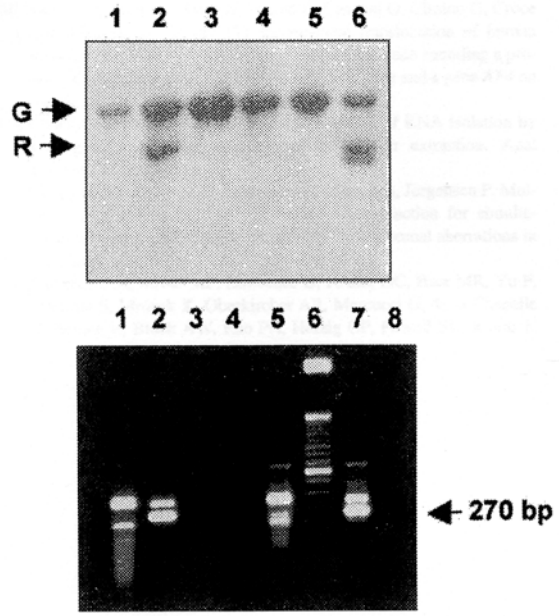

Fig. 2. Top: Southern blot analysis showed a rearranged band (R) on *Bam*HI digest in lane 2, which corresponded to the DNA obtained from patient 2. Lanes 1, 3, 4, and 5: nonrearranged samples. Lane 6: rearranged sample (positive control). Bottom: RT-PCR revealed an amplified exon 6/exon 2 transcript in patient 2 (lane 7). Lanes 1, 2, and 5: samples with *MLL* tandem duplication. Lane 8: negative control (H_2O). *Abbreviations*: G, germ line band; MW, molecular weight marker.

prognosis. A shorter duration of the complete remission was observed in AML patients with normal karyotype and this rearrangement, compared with those without the duplication of the *MLL* gene [11]. Adverse prognosis has been recently confirmed in a large series of cases [4] in which a partial tandem duplication of the *MLL* gene was also detected in cases with cytogenetic abnormalities other than trisomy 11. No recurrent structural or numerical changes, however, emerged from this series [4].

In the present study, we identified two patients with partial tandem duplication of the *MLL* gene in association with an unbalanced translocation involving a partial trisomy of chromosome 11q, from q22∼q23 to qter. By conventional cytogenetics, in our two cases, we could not identify the origin of the extra material on chromosomes 16p (case 1) and 18p (case 2). Genetic diagnosis was based on molecular studies used in our ongoing AML trials [12], such as Southern blot plus RT-PCR and FISH.

As far as we know, this is the first documentation of a partial duplication of the *MLL* gene in unbalanced translocations with partial trisomy of 11q in addition to partial monosomy of the terminal short arm of chromosomes 16, from 16p13, and 18, from 18p11.2. Michaux et al. [13], in a series of AML with *MLL* amplification, included an unbalanced t(11;11)(p15;q14), which could have been a self-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)(q3?4) 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.

Acknowledgments

This work was partially supported by FIS no. 00/0352. S.C. is a recipient of a fellowship from Autonomous Government of Catalonia. C.M. was supported by AIRC (Associazione Italiana per la Ricerca sul Cancro) and AIL (Associazione Italiana contro la Leucemia).

References

[1] Huret JL, Dessen P, Le Minor S, Bernheim A. The atlas of genetics and cytogenetics in oncology and hematology on the Internet and review on infant leukemias. Cancer Genet Cytogenet 2000;120:155–9.

[2] Caligiuri MA, Schichman SA, Strout MP, Mrózek K, Baer MR, Frankel SR, Barcos M, Herzig GP, Croce CM, Bloomfield CD. Molecular rearrangement of the *ALL-1* gene in acute myeloid leukemia without cytogenetic evidence of 11q23 chromosomal translocations. Cancer Res 1994;54:370–3.

[3] Caligiuri MA, Strout MP, Schichman SA, Mrózek K, Arthur DC, Herzig GP, Baer MR, Schiffer CA, Heinonen K, Knuutila S, Nousiainen T, Ruutu T, Block AMW, Schulman P, Pedersen-Bjergaard J, Croce CM, Bloomfield CD. Partial tandem duplication of *ALL1* as a recurrent molecular defect in acute myeloid leukemia with trisomy 11. Cancer Res 1996;56:1418–25.

[4] Schnittger S, Kinkelin U, Schoch C, Heinecke A, Haase D, Haferlach T, Büchner T, Wörmann B, Hiddemann W, Griesinger F. Screening for *MLL* tandem duplication in 387 unselected patients with AML identify a prognostically unfavorable subset of AML. Leukemia 2000;14:796–804.

[5] ISCN. An international system for human cytogenetic nomenclature. Mitelman F, editor. Basel: S. Karger. 1995.

[6] Dierlamm J, Wlodarska I, Michaux L, La Starza R, Zeller W, Mecucci C, Van den Berghe H. Successful use of the same slide for consecutive fluorescence in situ hybridization (FISH) experiments. Genes Chromosomes Cancer 1996;16:261–4.

[7] Cimino G, Rapanotti MC, Biondi A, Elia L, Lo Coco F, Price C, Rossi V, Rivolta A, Canaani E, Croce CM, Mandelli F, Greaves MF. Infant acute leukemias show the same biased distribution of *ALL1* gene breaks as topoisomerase II related secondary acute leukemias. Cancer Res 1997;57:2879–83.

[8] Gu Y, Nakamura T, Alder H, Prasad R, Canaani O, Cimino G, Croce CM, Canaani E. The t(4;11) chromosome translocation of human acute leukemias involves fusion between *ALL-1* gene encoding a protein with homologies to *Drosophila trithorax* gene and a gene *AF4* on chromosome 4. Cell 1992;71:701–8.

[9] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–9.

[10] Pallisgaard N, Hokland P, Riishoj DC, Pedersen B, Jorgensen P. Multiplex reverse transcription-polymerase chain reaction for simultaneous screening of 29 translocations and chromosomal aberrations in acute leukemia. Blood 1998;92:574–88.

[11] Caligiuri MA, Strout MP, Lawrence D, Arthur DC, Baer MR, Yu F, Knuutila S, Mrózek K, Oberkircher AR, Marcucci G, de la Chapelle A, Elonen E, Block AW, Rao PN, Herzig GP, Powell BL, Ruutu T, Schiffer CA, Bloomfield CD. Rearrangement of *ALL1*(*MLL*) in acute myeloid leukemia with normal cytogenetics. Cancer Res 1998;58:55–9.

[12] Aventín A, Casas S. Leucemia mieloide aguda: caracterización genética [Acute myeloid leukemia: genetic characterization]. Haematologica 2001;86(Suppl 1):219–26 [in Spanish].

[13] Michaux L, Wlodarska I, Stul M, Dierlamm J, Mugneret F, Herens C, Beverloo B, Verhest A, Verellen-Dumoulin C, Verhoef G, Selleslag D, Madoe V, Lecomte M, Deprijck B, Ferrant A, Delannoy A, Marichal S, Duhem C, Dicato M, Hagemeijer A. *MLL* amplification in myeloid leukemias: a study of 14 cases with multiple copies of 11q23. Genes Chromosomes Cancer 2000;29:40–7.

[14] Johansson B, Mertens F, Mitelman F. Primary vs. secondary neoplasia-associated chromosomal abnormalities: balanced rearrangements vs. genomic imbalances? Genes Chromosomes Cancer 1996;16:155–63.

3.2.2 Inversió del cromosoma 16 associada a trasnlocació (II)

Aventin A, Espadaler M, Casas S, Duarte J, Nomdedeu J, Sierra J. Chromosome 16 inversion-associated translocations in acute myeloid leukemia elucidated using a dual-color CBFB DNA probe. Cancer Genet Cytogenet 2002;134(2):142-4.

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 recíproca 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).