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Short communication

Molecular cytogenetic characterization of breakpoints in 19 patients with hematologic malignancies and 12p unbalanced translocations

María D. Odero[a,*], Katrin Carlson[b], Idoya Lahortiga[a], María J. Calasanz[a], Janet D. Rowley[b]

[a]*Department of Genetics, University of Navarra, C/ Irunlarrea s/n, 31008, Pamplona, Spain*
[b]*Department of Medicine, Section Hematology/Oncology, University of Chicago, Chicago, IL, USA*

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Abstract Structural rearrangements of the short arm of chromosome 12 are frequent cytogenetic findings in various hematologic malignancies. The *ETV6* gene is the most common target for rearrangements in 12p13. Fluorescence in situ hybridization (FISH) investigations have shown that translocations of 12p other than t(12;21) are frequently accompanied by small interstitial deletions that include *ETV6*. Unbalanced translocations involving *ETV6* have rarely been described, and breakpoints outside *ETV6* appear to be strongly associated with complex karyotypes. We studied bone marrow samples from 19 patients known to have 12p unbalanced translocations and complex karyotypes, using FISH and spectral karyotyping. FISH analysis confirmed the hemizygous deletion of the *ETV6* and *CDKN1B* genes in 74% of cases. We found four cases with interstitial deletions. In these four cases and in two others (6/19, 31.5%), the fusion with the partner chromosome was in the subtelomeric region of 12p13.3, confirming that there is a recurrent breakpoint in this region.

1. Introduction

The short arm of chromosome 12 is frequently rearranged in hematologic malignancies of both myelocytic and lymphoid origin. The abnormalities include deletions and balanced and unbalanced translocations [1]. The *ETV6* gene (formerly called *TEL*), a member of the *ETS* family of transcription factors, is the most common target for rearrangements in 12p13 [1,2]. Since its initial description, *ETV6* has been found rearranged with more than 40 chromosome bands [3]. To date, 17 partner genes have been identified and cloned; however, several other genes mapping to 12p13 could also be relevant in leukemogenesis, including *CDKN1B/KIP1*, an important negative regulator of the cell cycle.

The *ETV6-CBFA2* fusion gene, resulting from a subtle t(12;21), has been characterized as the most common genetic lesion in pediatric acute lymphoblastic leukemia (ALL); it is associated with a favorable outcome. Frequently, this translocation is accompanied by the loss of the other *ETV6* allele [4–6], although this is a secondary event in ALL [7]. However, Andreasson et al. [8], reported that expression of *ETV6-CBFA2* is not sufficient for induction of growth factor independence in hematopoietic cell lines or hematologic disease in transgenic mice, and they concluded that additional genetic events, such as the deletion of the second wild-type *ETV6* allele, are important steps required for the development of *ETV6-CBFA2* ALL. Furthermore, fluorescence in situ hybridization (FISH) analyses have shown that translocations of 12p other than t(12;21) are frequently accompanied by small interstitial deletions, which may include *ETV6* [9–12]. These deletions in some cases are different from the region reported in *ETV6-CBFA2* positive ALL [12]. Unbalanced translocations involving *ETV6* have rarely been described [13,14], and cases with breakpoints outside *ETV6* appear to be strongly associated with complex karyotypes [14,15].

To define the chromosomal breakpoints and the region deleted, we studied bone marrow samples from 19 patients known to have 12p unbalanced translocations using FISH.

2. Patients and methods

Nineteen patients with hematologic malignancies and 12p unbalanced rearrangements studied at the University of Chicago (USA) (14 patients) and at the University of Navarra (Spain) (5 patients) are included in the present report. All samples were obtained with informed consent. Three pa-

* Corresponding author. Tel.: +34-948-425600; fax: +34-948-425649.
E-mail address: modero@unav.es (M.D. Odero).

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tients had acute myelocytic leukemia (AML) de novo, four secondary AML, four myelodysplastic syndrome (MDS), one biphenotypic acute leukemia, six ALL, and one mycosis fungoides (a lymphoproliferative disorder: LPD).

Cytogenetic studies were done on unstimulated short-term cultures. Giemsa-banded karyotypes were described according to International System for Human Cytogenetic Nomenclature (ISCN 1995) [16]. FISH analysis was performed using 12 cosmid and 3 phage probes located on 12p12.1 to 12p13.3, as previously described [3]. *ETV6/TEL* was analyzed by 5 cosmids that cover the gene, and *KIP1/CDKNIB* using two P1 phage clones [17,18]. The order of the probes is shown in Fig. 1. The spectral karyotyping (SKY) probe mixture and hybridization reagents were obtained from Applied Spectral Imaging (Carlsbad, CA, USA). Slides for SKY were hybridized with the probe cocktail as previously described [19].

3. Results and discussion

One allele of *ETV6* and *CDKN1B* was deleted in 14 of 19 cases (74%). In 10 cases (1, 3, 5, 6, 7, 8, 9, 10, 12, and 14), the breakpoint was centromeric to *ETV6* and *CDKN1B* and resulted in the deletion of both genes as well as all other genes on distal 12p (Fig. 1). In case 13, the breakpoint was centromeric to *CDKN1B*, between P1-2097 and cos1C3, but both FISH and SKY analyses showed the 12p13 region was in the der(11) (Fig. 2A). We detected four other cases (11, 16, 17, and 19) with unbalanced translocations that also had large interstitial deletions similar to cases with del(12)(p12p13), with breakpoints both centromeric and telomeric to *ETV6*, resulting in loss of *ETV6* and *CDKN1B*. Interstitial deletions of 12p are known to occur frequently in primary and secondary MDS and in other hematologic malignancies, especially of myelocytic origin [1], however, few cases have been described in 12p unbalanced translocations. In all four of our cases, the most telomeric probes we used were retained, including cosmid 9A4, specific for the locus D12S235, indicating that the junction with the other chromosome was near to the telomeric region. In four patients—two with ALL (cases 2 and 4), one mycosis fungoides (case 15), and one MDS (case 18)—the breakpoint was telomeric to *ETV6* (Fig. 1). In the two ALL patients, it was telomeric to D12S235 (Fig. 2B), similar to cases 11, 16, 17, and 19. These data confirm the results of a recent report regarding a recurrent

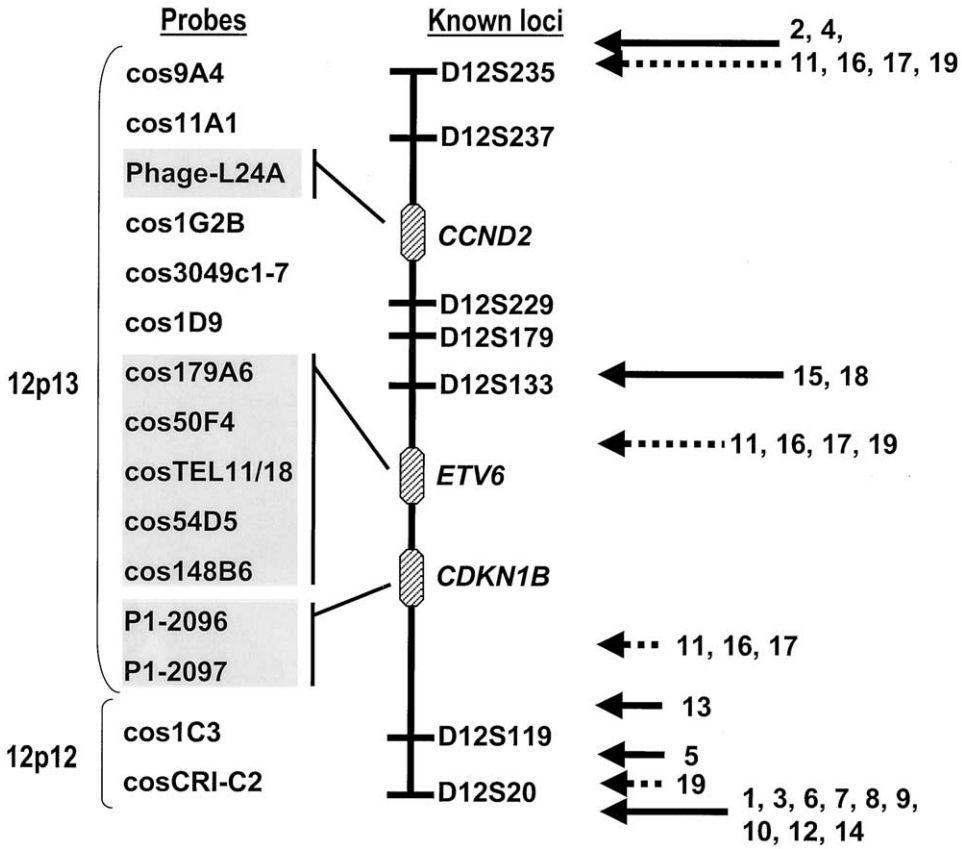

Fig. 1. Results of FISH analysis of 19 patients with 12p unbalanced translocations. Cases 11, 16, 17, and 19 (dashed arrows) had a normal chromosome 12 and a der(12) with three breakpoints on 12p; the junction with the partner chromosome was telomeric to the 9A4 probe. These four cases had an interstitial deletion with two breakpoints, centromeric and telomeric to *ETV6*, resulting in deletion of *ETV6* and *CDKN1B*. The probes used in the hybridization are shown on the left.

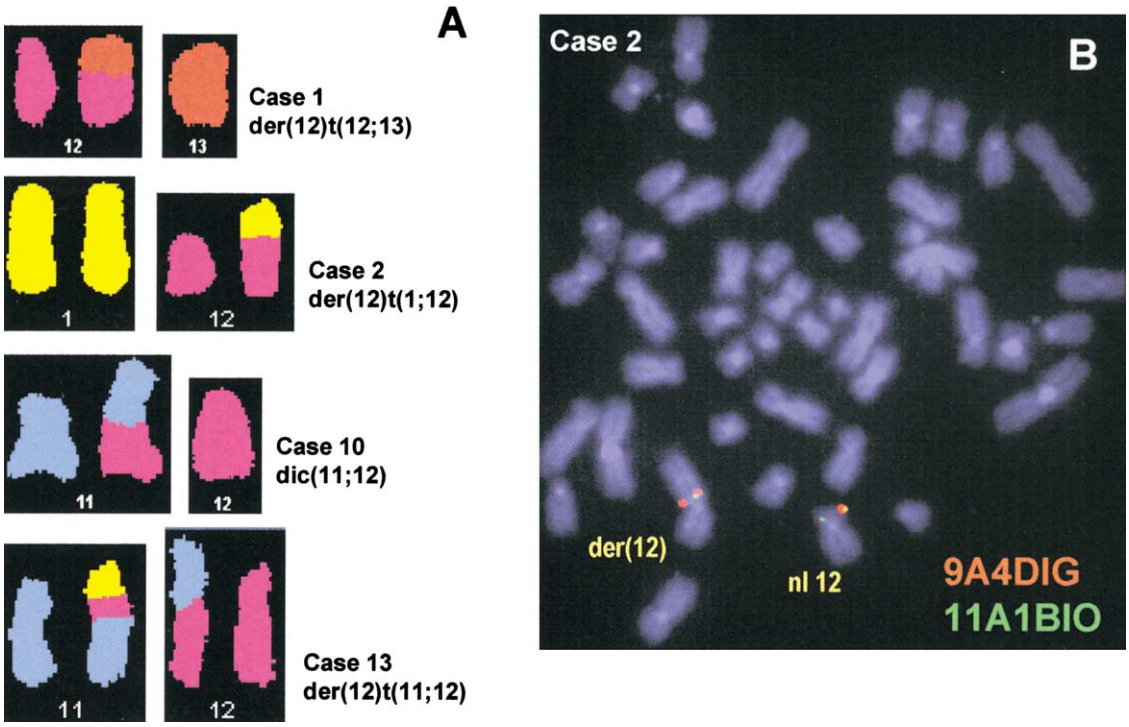

Fig. 2. Examples of SKY and FISH analysis. (A) Partial SKY karyotype of patients 1, 2, 10, and 13. (B) FISH analysis of patient 2 showed that the most telomeric probes used are retained. Cosmids 9A4 (red) and 11A1 probe (green) label both normal chromosome 12 and der(12)t(1;12)(q24;p13).

breakpoint in the subtelomeric region of 12p [14]. The localization of the most telomeric probe used by La Starza et al. [14] is between 12p13.32 and 12p13.33 (D12S158), the same region as our probe (D12S235).

Subtelomeric regions are interesting from a genomic perspective. Although the telomeric regions of human chromosomes are believed to have the highest concentration of genes [20], it has been shown that many nonfunctional

Table 1
Partial Giemsa-banded and revised karyotype modified by SKY and FISH of 19 patients with 12p unbalanced translocations

Patient no.	Diagnosis	G-banded Karyotype	Revised Karyotype
1	AL	der(12)t(7;12)(p11;p12)	der(12)t(12;13)(p12;q11)
2	ALL	der(12)t(1;12)(q24;p13)	der(12)t(1;12)(q24;p13.33)
3	ALL	dic(9;12)(p13;p11)	dic(9;12)(p13;p11)
4	ALL	der(12)t(12;15)(p13;q22)	der(12)t(12;15)(p13.33;q22)
5	ALL	der(12)t(12;17)(p12;q12)	der(12)t(12;17)(p12.3;q12)
6	ALL	t(4;12)(q21;p13)	der(12)t(4;12)(q21;p12)
7	ALL	der(12)t(12;17)(p12;q11)	der(12)t(12;17)(p12.3;q11)
8	sAML	t(6;12)(p21;p12)	der(12)t(6;12)(p21;p12)
9	sAML	add(12)(p12)	der(12)t(3;12)(q?21;p12)
10	AML	dic(11;12)(p13;p13)+del(12)(p12p13)	dic(11;12)(p13;p12)+del(12)(p12p13)
11	sAML	der(12)t(6;12)(p22;q24)	der(12)del(12)(p12p13.2)t(10;12)(p12;p13.33)
12	AML	der(12)t(1;12)(q21;p12)	der(12)t(1;12)(q21;p12)
13	AML	der(12)t(11;12)(q11;p11)	der(12)t(11;12)(q11;p11)
14	sAML	der(12)t(12;14)(p13;q11)	der(12)t(12;14)(p12;q11)
15	LPD	add(12)(p12)	der(12)t(12;21)(p13;q?)
16	MDS	der(12)t(3;12)(q13;p13)	der(12)del(12)(p12p13.2)t(3;12)(q13;p13.33)
17	MDS	der(12)t(12;17)(p13;q21)	der(12)del(12)(p12p13.2)t(12;17)(p13.33;q21)
18	MDS	der(12)t(2;12)(q21;p12)	der(12)t(2;12)(q21;p13)
19	MDS	add(12)(p13)	der(12)del(12)(p12p13.2)t(12;13)(p13.33;q?)

Abbreviations: AL, acute leukemia; ALL, acute lymphoid leukemia; AML, acute myelocytic leukemia; LPD, lymphoproliferative disorder (specifically, mycosis fungoides); MDS, myelodysplastic syndrome; sAML, secondary acute myelocytic leukemia.

[a]Patient 7 is patient 8 in [3]. Patients 1, 9, 10, 11, 12, 13, 14, 16, 18 and 19 are patients 1, 4, 5, 9, 10, 12, 2, 13, 14 and 15, respectively, in [23].

Table 2
Summary of clinical features and karyotype modified by FISH of patients not previously reported

Patient no.	Sex/ age	Diagnosis	Status	Sample	G-banding
2	M/19	ALL	RL	Bone marrow	46,XY,del(6)(q15q23)[5]/46,idem,der(12)t(1;12)(q24;p13)[4] NCA1:46,idem,der(6)t(6;8)(q24;?),der(12)t(1;12)(q24;p13) NCA2:46,idem,der(6)t(6;20)(q24;?),der(12)t(1;12)(q24;p13)
3	M/15	ALL	Dx	Bone marrow	46,XY,+8,dic(9;12)(p13;p11)[17]/46,XY[4]
4	F/11	ALL	RL	Bone marrow	46,X,−X,der(12)t(12;15)(p13;q22),der(15)t(12;15)(p13.33;q22),del(15)(q15q22)t(15;21)(q15;q11),+16,der(21)t(15;21)(q15;q11)[21]/46,XX[2]
5	M/18	ALL	Dx	Bone marrow	45,XY,der(6)t(2;6)(q?;p23),del(9)(p21),der(12)t(12;17)(p13.3;q12),−17[13]/46,XY[17]
6	F/36	ALL	Dx	Bone marrow	45,XX,−4,−5,del(6)(q1?5q2?5),t(9;22)(q34;q11.2),add(11)(q21 or q22),der(12)t(4;12)(q21;p12),−13,add(14)(q32),+22,+mar,inc[4]/46,XX[22]
8	M/64	sAML	Dx	Peripheral blood	47,XY,+8,inv(9)(p11q13)c[13]/47,idem,del(9)(q11q22 or q22q34)[2]/46,XY,inv(9)(p11q13)c[5],der(12)t(6;12)(p21;p12)
15	F/68	LPD	Dx	Lymph node	42∼44,X,del(X)(q13q27),der(1)t(1;10)(p36;?),der(1)t(1;12)(q32;q?),−2,der(3)t(3;8)(q26;p11),der(4)t(4;11),t(6;12)(p23;q?)x2,+6,del(7)(q11),−8,der(8)t(3;8)(q26;p11),der(9)t(7;9)(q?;q12),−10,−10,der(11)t(10;11)(q11;p11),der(11)t(1;10)t(10;11),−12,der(12)t(12;21)(p13;q?),der(13)t(12;13),der(15)t(X;15),der(16)t(5;16),der(17)t(8;10)t(10;17),−18,der(18)t(1;8)t(8;18),der(19)t(15;19)(q2?;p13),−21[cp8]
17	M/38	MDS	CR	Bone marrow	46,XY,−5,−7,−8,der(12)del(12)(p12p13.2)t(12;17)(p13.33;q21),−17,+4mar[6]/46,XY[29]

Karyotypes of patients 2 and 15 have been modified after SKY analysis.

Abbreviations: ALL, acute lymphoid leukemia; Dx, new diagnosis; F, female; LPD, lymphoproliferative disorder (mycosis fungoides); M, male; MDS, myelodysplastic syndrome; RL, relapse; sAML, secondary acute myelocytic leukemia.

pseudogenes map to the subtelomeric regions [21,22]. The pseudogenes and repeat sequences share homology between nonhomologous chromosomes, providing the opportunity for nonhomologous telomere pairing, which could lead to exchange events and gene–dosage imbalance.

Ninety five percent of our patients (18/19) presented with a complex karyotype. Cases with unbalanced 12p translocations appear to be closely associated with complex karyotypes, and in most cases the breakpoints have been reported to be outside *ETV6,* as was true for all our patients [14,15]. SKY is an important tool to characterize the aberrations in these cases [23]. Samples with complex aberrations, deletions, and unbalanced translocations are particularly prone to misinterpretation based on G-banding alone, especially when chromosomal regions that have a similar G-banding pattern are involved. In our study, SKY allowed for the more complete characterization of the karyotype of the leukemia samples (Table 1 and Fig. 2). Our SKY analyses of 11 of the 19 cases have been reported [3,23]. The clinical features and the karyotype of the remaining 8 cases are given in Table 2.

Deletions of the short arm of chromosome 12 are frequent cytogenetic findings in hematologic malignancies of both myelocytic and lymphoid origin [10,11,17,24]. The smallest region deleted has been delineated by FISH analyses and loss of heterozygosity studies in 12p aberrant hematologic malignancies; this deleted region includes *ETV6* and *CDKN1B* [6,11,24]. In a few cases, the deleted region include *CDKN1B* but not *ETV6*, or *ETV6,* but not *CDKN1B* [2]. Interestingly, most deletions are interstitial [6,17]. These findings suggest a minimally deleted region on 12p13 located between *ETV6* and *CDKN1B*. This genomic region was recently thoroughly mapped by Baens et al. [25], who narrowed the commonly deleted region to a 600-kb segment between *ETV6* and D12S358, excluding *CDKN1B*. In our cases with 12p unbalanced translocations, FISH analysis confirmed the hemizygous deletion of the *ETV6* and *CDKN1B* genes in 14 (74%) of cases, including 4 with interstitial deletions. In the four cases with myelocytic disorders, and in two patients with ALL (6/19, 31.5%), the fusion with the partner chromosome was in the subtelomeric region of 12p, confirming a recurrent breakpoint in this region. These data confirm our analyses of other complex karyotypes, which showed that deletion of critical genes often occurs as a consequence of unbalanced translocations [23].

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