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Cleavage of the ALL1 gene in acute lymphoid leukemia before treatment disappears in relapse

EDUARDO ANGUITA,* ANA VILLEGAS,* ANNA SERRA,° FERNANDO ATAULFO GONZÁLEZ,* PALOMA ROPERO,* TRINIDAD CONTRA,# GIUSEPPE SAGLIO°

**Department of Hematology, Hospital Clínico San Carlos, Madrid, Spain; °Laboratorio di Medicina e Oncologia Molecolare, Ospedale San Luigi Gonzaga, Orbassano, Turin, Italy; #Department of Oncology, Hospital Niño Jesús, Madrid, Spain*

ABSTRACT

Background and Objective. **ALL1 gene rearrangements are frequently found in secondary acute leukemias (ALs). A site-specific cleavage of the ALL1 gene in a consensus sequence for topoisomerase II recognition has been considered to be the initial step leading to ALL1 rearrangement and subsequent therapy-related AL. The aim of the present study was to evaluate this cleavage in our patients, to analyze whether it is a laboratory-produced artefact and to check whether it persists or causes a real ALL1 gene rearrangement at relapse.**

Design and Methods. **We studied ALL1 rearrangement in 74 cases of AL before treatment by Southern blot avoiding room temperature exposure or delay in processing the samples which could produce ALL1 cleavage. DNA was available for two cases with ALL1 cleavage; it was analyzed by three different Southern blots in one and two in the other. One case with ALL1 cleavage was also studied in relapse.**

Results. **The presence of the cleavage of the ALL1 DNA was found in 3 of 74 (4%) patients. Two of these three patients had the ALL1 cleavage in three and two different analyses. One case was positive for ALL1 cleavage at diagnosis, but negative for both ALL1 cleavage and ALL1 rearrangement at relapse.**

Interpretation and Conclusions. **The fact that a constant pattern was obtained from the same patients in different DNA preparations, supports the notion that ALL1 cleavage is not a laboratory artefact. The absence of the cleavage in a sample from a relapsed patient suggests that the subclone with the ALL1 cleavage, in this case, did not play a clear role in the pathogenesis of disease recurrence.**

Key words: cleavage, ALL1, MLL, acute leukemia, Southern blot, RT-PCR

Correspondence: Professor Ana Villegas, Department of Hematology, Hospital Clínico San Carlos, C/ Profesor Martín Lagos S/N, 28040 Madrid, Spain.
Phone: international +34-91-3303321 – Fax: international +34-91-3303322 – E-mail: avillegasm@aehh.org

Abnormalities of chromosome 11 band q23 occur quite frequently in both myeloid and lymphoid acute leukemias (ALs). The vast majority of these abnormalities produce rearrangements of the ALL1 gene (also referred to as MLL, HRX, Hrtx 1) and lead to the formation of new fusion genes whose 5' portions retain the ALL1 sequences whereas the 3' ends derive from different partner genes.[1-3] More than 35 potential partners for the ALL1 gene have been identified so far[2,4] although the rearrangements most frequently found in ALs are ALL1/AF4, ALL1/ENL, ALL1/ELL, ALL1/AF6, ALL1/AF9 and ALL1 duplication.[2]

ALL1 rearrangements are also particularly frequent in therapy-related ALs associated with chemotherapy regimens incorporating topoisomerase II (topo II) inhibitors.[2] In these cases the breakpoints on chromosome 11 cluster within the telomeric region of the breakpoint cluster region of the ALL1 gene, where a high affinity scaffold attachment region, recognition sequences for topo II and a DNase I hypersensitive site have been identified.[5,6] A specific cleavage or pseudo-rearrangement of the ALL1 gene at this region has also been described to occur in a range of situations *in vivo* and *in vitro.*[7-10] This has been considered to represent an initial step leading to ALL1 rearrangement and to secondary leukemia.[6-10]

In this paper, we describe that ALL1 cleavage is quite frequently detected in the DNA taken at diagnosis from patients with myeloid and lymphoid ALs and that this phenomenon is not a laboratory-produced artefact. In addition, we report a case repeatedly positive for ALL1 cleavage at diagnosis that became negative at relapse.

Design and Methods

Patients and samples

Seventy-four AL patients consecutively diagnosed between January 1996 and June 1998 were studied: 29 childhood ALL, 5 childhood AML, 20 adult ALL and 20 adult AML. Patients with acute promyelocytic leukemia (APL, FAB-M3) were excluded from the study. At least 50% of blast cells were present in all

Table 1. PCR conditions and sequences of the oligonucleotides used in this study. Total volume 5 µL.

PCR	Sense primer (20 pmol)	Antisense primer (20 pmol)	Annealing Temperature	MgCl (mM)	DMSO (%)	Formamide (%)
ALL1/AF6	ALL1 ex 5 ext 5'–GAGGATCCTGCCCCAAAGAAAAG–3	AF6 int 5'–TACTTGGGAGAGGACAGCATTCG–3'	60	2.5	5	–
ALL1/AF9	ALL1 ex 5 ext 5'–GAGGATCCTGCCCCAAAGAAAAG–3	AF9 AS 3 5'–TCACGATCTGCTGCAGAATGTGTCT–3'	60	2.5	5	–
ALL1/ENL	ALL1 ex 5 ext/ ALL1 p 5'–GAGGATCCTGCCCCAAAGAAAAG–3/ 5'–TCCAAACAGGCCACCACTCCA–3'	ENL AS 2 5'–AGCGTACCCCGACTCCTCTACTTTG–3' ENL AS 3 5'–GACGAAGAGTCGTCCTCGTCGGACT–3'	62	2	–	6
ALL1/ELL	ALL1 ex 5 ext 5'–GAGGATCCTGCCCCAAAGAAAAG–3	ELL int 5'–GCCGATGTTGGAGAGGTAGA–3'	57	1.5	–	–
ALL1–duplication	ALL1 ex 5 ext 5'–GAGGATCCTGCCCCAAAGAAAAG–3	ALL1 ex 3B.2 5'–AGAATCTTTCTTCTTGATCTTA–3'	57	2	–	–
ALL1/AF4	ALL1–A 5'–CCGCCTCAGCCACCTAC–3' ALL1–E5' 5'–AAGCCCGTCGAGGAAAAG–3'	AF4–B 5'–TGTCACTGAGCTGAAGGTCG–3' AF4–D 5'–CGTTCCTTGCTGAGAATTTG–3'	65	2.5	–	–

the bone marrow samples and, before being preserved at –70°C until analysis, they were further enriched by Ficoll gradient centrifugation within 4 hours of BM aspiration. As a part of the procedure of molecular characterization, all the samples were initially searched for ALL1 rearrangement by Southern blot. All the cases with positive Southern blot scores were further analyzed by RT-PCR for the presence of the following hybrid transcripts: ALL1/AF4, ALL1/ENL, ALL1/ELL, ALL1/AF6, ALL1/AF9 and ALL1 self-duplication.

DNA isolation and Southern blot

The DNA was extracted from the bone marrow mononuclear cells using a salting-out method.[11] Ten micrograms of DNA were digested with both BamHI and Bgl II restriction enzymes, electrophoresed on 0.8% agarose gels and transferred to nylon membranes (Hybon-N+, Amersham, UK). These membranes were hybridized with the B859 probe, labeled with ^{32}P. The B859 probe is a cDNA insert encompassing ALL1 exons 5 to 11 in the original nomenclature and it explores the entire ALL1 breakpoint cluster region.[1]

RT-PCR

RNA was extracted using the method described by Chomczynsky and Sacchi.[12] cDNA was synthesized using a previously described method.[13] To assess the efficiency of the reverse transcription step the Abl cDNA was amplified in each case. We amplified in one round of PCR 3 µL of cDNA for the fusion transcripts ALL1/AF4, ALL1/ENL, ALL1/ELL, ALL1/AF6, ALL1 duplication and ALL1/AF9 in a 47 µL mix with the PCR conditions and the primers described in Table 1. Some chimeric RNA (ALL1/AF4 and ALL1/ENL) were analyzed with different primers to confirm the positive results (see Table 1).

Results

ALL1 gene rearrangement was detected in 8 of the 74 patients with AL analyzed (10.8%). These 8 patients were analyzed before any treatment. In 5 cases (6.8%) the intensity of the rearranged bands was consistent with the rearrangement of one ALL1 allele, but in 3 cases (4%), the intensity was lower (Figure 1). These three cases presented *Bam*HI and Bgl II bands identical to those described by Aplan *et al.*[7] and Macintyre *et al.*[8] (6.7 and 1.5 Kb in the *Bam*HI digested DNA; 4.6 and 1.4 kb in the *Bgl* II digestion) in the cases carrying the site-specific ALL1 cleavage.

ALL1 chimeric transcripts were detected by RT-PCR in all the 5 patients with a strong rearrangement band in the Southern blot: two children with AML were positive for the presence of ALL1/AF6 transcripts, 2 children with T-ALL for ALL1/ENL and 1 child with precursor B-ALL for ALL1/AF4. In contrast, all three patients with low intensity rearrangements were negative for all the fusion transcripts analyzed. One of these three patients was a 16 year-old male with a T-ALL with more than 90% of blast cells in the bone marrow and a WBC count of 340×10^9/L at diagnosis. His karyotype was normal: 46,XY. In this case, the Southern blot showed a pattern compatible with the presence of ALL1 cleavage in three different and subsequent experiments that were performed starting from DNA extraction. The patient was treated with the LAL-93 AR protocol of the Spanish group PETHEMA for high risk ALL.[14] Because of the absence of a

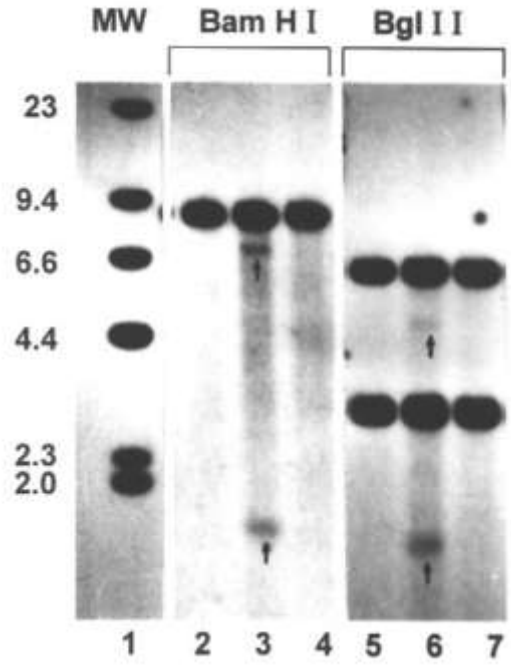

Figure 1. Southern blot analysis of BamHI and Bgl II digested DNA from AL patients. Lines 1 and 5, and 4 and 7 are negative controls. Lines 3 and 6 correspond to one of the patients described showing faint 6.7 and 1.5 Kb bands in the *Bam*HI digested DNA and 4.6 and 1.4 Kb bands in the *Bgl*II digestion (over the arrows) in addition to the germinal bands. Line 1 (MW): molecular weight marker (lambda phage / *Hind*III digest labeled with ^{32}P).

BM donor, after consolidation an unpurged autologous stem cell transplantation was performed. Thirteen months after the first diagnosis the patient relapsed, but, although the immunophenotype was the same, the ALL1 gene cleavage was no longer detectable in the DNA samples obtained in this phase. The patient was treated with rescue chemotherapy treatment and achieved a second complete remission which is presently persisting with a follow-up of 16 months.

The second patient with ALL1 cleavage was a 33-year old woman with a B precursor-ALL with >90% of blast cells in the BM and 54.23×10^9/L WBC count, her karyotype was 52-53,XX/46,XX. The Southern blot analysis was repeated on this patient on two occasions with the same results. She was treated with allogeneic bone marrow transplantation and is in hematologic complete remission 9 months after diagnosis.

Finally, the third case was a 86-year old woman with a FAB-M2 AML with only 50% of blast cells in the BM, 14.5×10^9/L (42% of blast) WBC count in the peripheral blood and insufficient metaphases by cytogenetic analysis. This patient died 3 days after diagnosis.

Discussion

The ALL1 cleavage was first described in a case of T-ALL analyzed 16 hours after induction treatment with chemotherapy including doxorubicin.[7] A similar ALL1 cleavage pattern was induced treating *in vitro* cell lines and peripheral blood mononuclear cells (PBMC) from normal individuals with topoisomerase II inhibitors. On the basis of these observations, topo II inhibitors were suggested to be the causative agents of ALL1 cleavage. Nevertheless, Macintyre *et al.*[8] reported the occurrence of ALL1 cleavage in three cases of *de novo* AML before treatment (4.8% of their 62 AML cases) and recently Stanulla *et al.*[9,10] observed the same pattern when treating malignant cell lines and PBMC with stimuli able to induce apoptotic cell death including incubation for 24-60 hours at room temperature.

Our data confirm that ALL1 cleavage is relatively frequently detectable (4% of the cases) by Southern blot on DNA taken from AL patients at diagnosis before treatment even if the samples are processed shortly after BM aspiration. This event is not restricted to myeloid leukemias but can also occur in lymphoid ALs. The fact that a constant pattern was obtained from the same patient in three different DNA preparations further supports the notion that ALL1 cleavage is present *in vivo* and is not merely a laboratory artefact. As recently suggested by *in vitro* studies, a high degree of apoptotic cell death seems to be the most likely explanation.

Although ALL1 cleavage can be suspected on the basis of the low intensity and the size of the bands, its presence can complicate molecular characterization of ALs. The ALL1 gene rearrangement has been shown to confer a poor prognosis, at least in some in patients with lymphoid AL,[15] and patients with deletion of 11q23 or inversion (11)(p13q23) without ALL1 rearrangement have been shown to have favorable clinical features.[16] The prognosis significance of ALL1 gene rearrangement in acute myeloid leukemia is under discussion.[17-19] ALL1 rearrangement in patients with normal cytogenetics has been described[19,20] and some ALL1 fusion transcripts in normal human bone marrow and peripheral blood have been detected by nested PCR without evidence of ALL1 rearrangement by Southern blot.[21,22] Therefore, the correct classification of the ALL1 status by Southern blot is important because some patients with ALL1 rearrangement may benefit from more intensive therapy. The recognition of the ALL1 cleavage may avoid a misclassification of these cases.

ALL1 cleavage has been suggested to represent, particularly in secondary leukemias, the initial step leading to true ALL1 rearrangements and to leukemogenesis. Therefore, at least in some instances, the same phenomenon could also play a role in determining relapses. However, the case we reported was positive for ALL1 cleavage at diagnosis, but negative for both ALL1 cleavage and ALL1 rearrangement at

relapse. In this patient homozygous deletion of p16 gene and the same immunophenotype was detected at both diagnosis and relapse (data not shown) indicating this was a real relapse. This suggests that, at least in this case, the subclone with the ALL1 cleavage did not play a clear role in the pathogenesis of disease recurrence.

Contributions and Acknowledgments

EA did the molecular studies by means of RT-PCR and Southern blot and wrote the paper with AV and GS. AV designed the study with EA and GS. AS did the RT-PCR analyses with EA. FAG, PR and TC followed the patients clinically and revised the manuscript. GS took part in the conception of the study and gave final approval of the version to be published.

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Disclosures

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