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MUTATIONAL ANALYSIS OF p53 IN 16 CASES OF ACUTE LYMPHOBLASTIC LEUKEMIA AND BURKITT'S LYMPHOMA

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†*In memoriam*

ABSTRACT

Background and Objective. Improvements in therapy for patients with B-cell acute lymphoblastic leukemia (ALL) and Burkitt´s lymphoma (BL) depend on the identification of subsets of patients who require more intensive therapy. Abnormalities of the p53 gene are the most common molecular lesions in human cancer, and may be of prognostic significance in hematologic malignancies. In this study, we examined the p53 gene status in a group of patients with ALL/BL to determine whether some types of mutants were more frequent in this selected group of patients

Methods. We selected a group of 16 patients with acute lymphoblastic leukemia (ALL) and Burkitt´s lymphoma (BL) in order to investigate the presence of p53 mutations. DNA obtained from affected organs (bone marrow, lymph node and a renal mass) was used for the molecular studies. Single-strand conformation polymorphism (SSCP) analysis of exons 5 to 9 of the gene was used to detect p53 mutants. After detecting an abnormal migration pattern on the SSCP, mutations were determined by direct sequencing.

Results. Point mutations were found in eight patients; a misense mutation in seven cases and a non-sense mutation in one case. The normal allele was also identified in 7 mutated samples. The same mutation at codon 282 was identified in three different patients, in whom an identical conformer was detected after SSCP analysis. Mutation at codon 282 was present in an extramedular relapse (renal) appearing after a BMT. No such alteration was present in the bone marrow analyzed at the same time.

Interpretation and Conclusions. Our findings suggest that p53 mutations are quite frequent in recognized clinical groups. The criteria chosen in this study allowed us to identify a high percentage of the samples with mutation. Different malignant phenotypes could be determined by functional heterogeneity of p53 mutants.

Key words: acute lymphoblastic leukemia, Burkitt lymphoma, p53, mutation, molecular genetics

Improvements in therapy for patients with B-cell acute lymphoblastic leukemia (ALL) and Burkitt´s lymphoma (BL) depend on the identification of subsets of patients who require more intensive therapy.[1,2]

Chromosomal translocations are detected in a large number of cases and many structural abnormalities appear to confer a poor prognosis. For example, chromosomal translocations leading to the bcr-abl or ALL-1/MLL rearrangements have been associated with a poor outcome.[1] In contrast, a *c-myc* rearrangement may have a favorable prognosis with standard therapy even in cases with p53 mutations.[4] In BL, risk stratification is assigned exclusively on the basis of clinical information. Abnormalities of the p53 gene are the most common molecular lesions in human cancer and may be of prognostic significance in hematologic malignancies.[5] Chronic lymphocytic leukemia,[6] BL,[6,7] relapsed T-ALL,[8] and transformed low-grade malignancies[9-12] seem to be the clinical settings in which p53 gene inactivation has been most frequently reported. However, the use of p53 as a potential prognostic marker in ALL appears to be limited as the incidence of p53 at diagnosis is low.[13,14] Despite this, p53 is frequently mutated in leukemias with ALL-1/MLL rearrangement[15] and identifies a subgroup of leukemias with E2A-PBX1 rearrangement and poor evolution.[16]

In this study, we examined the p53 gene status in a group of patients with ALL/BL. Our goal was to determine whether some types of mutants were

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more frequent in this selected group of patients. We describe one case of relapse after BMT in the renal parenchyma in which a p53 mutation at codon 282 was identified.

Materials and Methods

Patients

The patients were selected from cases referred to our Hospital for diagnosis and/or therapy. Diagnoses were made in all cases according to standard clinical, cytologic and immunological criteria.[17,18] The fraction of malignant cells was at least 60% according to cytofluorometric analysis of cell surface markers.

Patients in whom there was a possible p53 inactivation were selected based exclusively on clinical criteria and grouped as follows:

- group 1: BL/ALL-3 (10 cases);
- group 2: aggressive ALL defined by relapsed, induction failure or diagnosed in patients over the age of 60 (6 cases) (see Table 1).

Molecular studies

DNA obtained from the bone marrow or other pathologic samples (lymph node in one case and a renal mass in the other) was purified by digestion with proteinase K, extraction by the *salting out* method, and precipitation with ethanol.[19]

Immunoglobulin gene rearrangements were studied by means of a 2.5 kb JH gene probe.[20] The configuration of the c-myc locus was analyzed in three cases by hybridizing *Eco RI*- and *Hind III*-digested DNA to the MC413RC probe, representative of the third exon of the *c-myc* gene.[21]

Southern blot. Five µg of DNA were digested using *HindIII*, *BamH*I, *Bgl II* and *Eco RI* (New England Biolabs, Mass., USA) restriction enzymes. DNA was separated on a 0.7% agarose gel (Ecogen, Barcelona, Spain) and transferred to nylon membranes (Amersham Ltd, Amersham place, Buckinghamshire, UK) which were hybridized with probes labelled with ^{32}P-dCTP by the random primer extension method. Filters were washed in 0.2×SSC (NaCl/Na citrate)/0.5% sodium dodecyl sulphate (SDS) for 2 hours at 65°C and then autoradiographed using intensifying screens.

Polymerase chain reaction single strand conformation polymorphism (PCR-SSCP) of p53. SSCP analysis was modified from the method reported by Gaidano *et al*.[5] PCR was performed with 100 ng of genomic DNA, 30 pmol of each primer (exons 5, 6, 7, 8 and 9), 2.5 µM dNTP, 1 µCi of 32P-dCTP (Amersham Ltd, Amersham place, Buckinghamshire, U.K) specific activity, 3000 Ci/mmol), 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1 mM MgCl2, 7% DMSO, 0.5 U Ampli Taq polymerase (Perkin-Elmer, Norwalk, CT, USA) in a final volume of 50 µL. Thirty cycles of denaturation (94°C), annealing (annealing temperatures were optimized for each exon), and extension (72°C) were performed in a temperature controller (DNA Thermal Cycler, Perkin-Elmer). The final reaction was mixed with formamide. Samples were heated at 95°C for 5 min, chilled on ice, and immediately loaded (5 µL) onto a 6% acrylamide-TBE gel containing 10% glycerol. Gels were run at 8 W for 12-15 h at room temperature, air dried and analyzed by autoradiography using an intensifying screen for 24 h.

Table 1. Patient characteristics.

Patient	*Age*	*Sex*	*HIV*	*Sample*	*Diagnosis*	*Time*	*Therapy*	*Immunophenotype*
BVP	6y	M	–	BM	BL	D	No	22,Dr,TdT-CD10,19,20,
ACA	11y	M	–	BM	BL	R	Yes – ABMT	CD10,19,20,22,Dr,Igs+
FRR	10y	M	–	BM	BL	D	No	CD19,20,22,Dr,Igs+
DGP	8y	F	–	Lymph*	BL	D	No	CD10,19,20,Igs+
SMT	26y	M	–	BM	BL	D	No	CD19,20,Igs+
JMA	41y	M	+	BM	BL	D	No	–
ACM	46y	M	+	BM	BL	D	No	CD19,20,Dr+.
DGH	82y	M	–	BM	ALL-L3	D	No	CD10,19,20,22,Dr,Igs+
NGC	84y	F	–	BM	ALL-L3	D	No	CD10,19,20,22,Dr,Igs+
LCS	6y	F	–	BM	ALL-L3	R	Yes – CT	CD10,19,20,22,Dr,Igs-.
CVD	16y	F	–	BM	ALL	R	Yes – CT	CD10,19,20,Dr,Tdt+
RAC	89y	M	–	BM	ALL	D	No	CD10,19,Dr,Tdt+
VNA	68y	M	–	BM	ALL	D	No	CD10,19,20,Tdt-
ASR	70y	F	–	BM	ALL	D	No	CD10,19,20,22,34,Tdt-
JSN	21y	M	–	Renal°	ALL	R	Yes – BMT	CD19
JVA	3y	M	–	BM	ALL	D	No	CD10,13,19,22,Dr,Tdt-

**Abdominal lymph node obtained from a surgical resection. °Percutaneous punction from a nodular mass in the left kidney.*

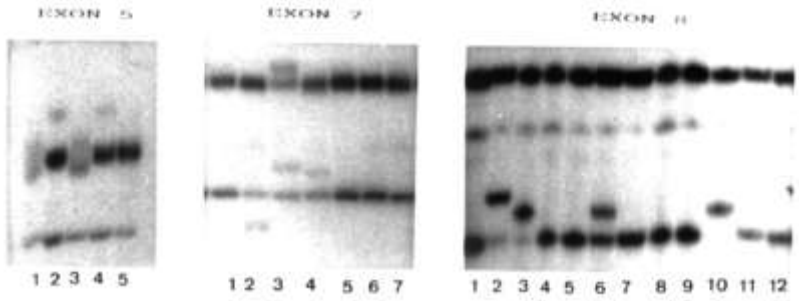

Figure 1. SSCP analysis of exons 5,7 and 8 showing abnormal migration patterns. In exon 5, lanes 1 and 3 correspond to patient BVP. Lanes 2 and 4 are a mutated control and lane 5 a non-mutated sample. In the second box the SSCP revealed three different conformers in lanes 2 (FRR), 3 (ACM9) and 4 (DGP). In these cases, the normal allele was also detected. SSCP on exon 8 disclosed two abnormal conformers: the first was present only in lane 2-NGC- and the second conformer was observed in three patients (lanes 3-RAC-, 6-JSN- and 10-ACA-). In lane 10 only the abnormal band was identified.

Table 2. p53 mutations in aggressive lymphoid malignancies.

Patient	*Diagnosis*	*Exon*	*Mutated codon*	*Aminoacid*
BVP	Burkitt	5	158 CGC->CAC	Arg->His
ACM	Burkitt	7	238 TGT->TAT	Cys->Tyr
DGP	Burkitt	7	245 GGC->AGC	Gly->Ser
FRR	Burkitt	7	257 CTG->CAG	Leu->Gln
RAC	ALL	8	282 CGG->TGG	Arg->Trp
ACA	Burkitt	8	282 CGG->TGG	Arg->Trp
JSN	ALL	8	282 CGG->TGG	Arg->Trp
NGC	ALL-L3	8	306 CGA->TGA	Arg->Stop

Table 3. Survival analysis from the time of p53 status analysis.

Patient	*p53*	*Evolution*	*Cause of death*
BVP	mut	Dead (8 months)	Unresponsive disease
ACM	mut	Dead (1 month)	Untreated disease
DGP	mut	Alive (16 months)	—
FRR	mut	Dead (1 month)	Transplant related, unresponsive disease
RAC	mut	Dead (1 month)	Untreated disease
ACA	mut	Dead (1 month)	Unresponsive disease
JSN	mut	Dead (2 month)	Unresponsive disease
NGC	mut	Dead (1 month)	Unresponsive disease
CVD	wt	Dead (16 months)	Toxicity-related
JMA	wt	Dead (2 months)	Untreated disease
DGH	wt	Dead (3 months)	Untreated disease
VNA	wt	Dead (2 months)	Untreated disease
ASR	wt	Alive(36 months)	—
LCS	wt	Dead (4 months)	Disease-related
JVA	wt	Alive(8 months)	—
SMT	wt	Alive(12 months)	—

Direct sequencing of DNA PCR products. For the exons with an abnormal migration pattern on SSCP analysis, non-radioactive PCR was performed under the conditions described above. PCR products were purified using Microcon/Micropure devices (Amicon, Danvers, USA) following our rapid protocol.[22] Sequencing reactions were performed with reagents supplied with the Sequenase version 2.0 sequencing kit (USB, Cleveland, OH, USA). ^{33}P-dCTP was included in the sequencing mixture. Both strands were sequenced for each DNA fragment analyzed.

Results

Clonal rearrangement of the Ig locus was detected in all the patients. *c-myc* rearrangement was identified in two out of three cases investigated (DGP and AGP). SSCP analysis showed an abnormal electrophoretic mobility in 8 cases, 4 in exon 8, 3 in exon 7 and 1 in exon 5 (Figure 1). Sequence analysis of these polymorphic bands confirmed the SSCP results in all the cases and demonstrated that mutations in these tumors resulted from single base changes in the coding region of the respective exons (Table 2).

Mutations at the p53 locus were found in all the clinical groups: 6/10 in the BL/ALL-L3 group and 2/6 in aggressive ALL. Survival analysis from the time of p53 analysis detection revealed that p53 mutation was associated with a fatal evolution (7/8)(see Table 3).

Except for case NGC, all the mutations were included in the DNA-binding domain (aminoacids 102-292) of p53.[23,24] Three patients with the same conformer on the SSCP had the same mutation at codon 282 (Figure 1). In one case the mutation was identified exclusively in the DNA obtained from a renal mass. Simultaneous analysis performed in a sample obtained from bone marrow gave negative results. The wild type allele was also present in each of these tumors except in one case (see Figure 2),

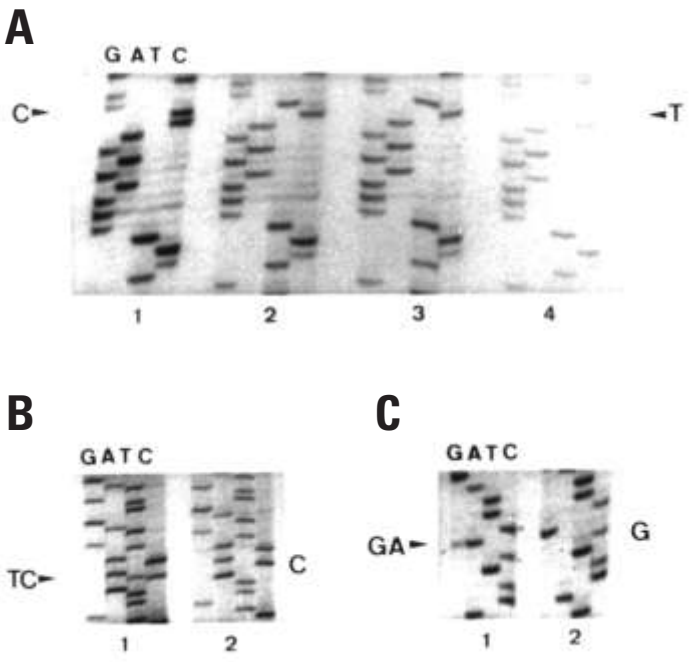

Figure 2. Characterization of p53 gene mutations by direct sequencing.

A) 1. Control. 2, 3 and 4: mutation at codon 282 (CGC/TGG).
B) 1. Point mutation (arrowhead) at codon 238 (TGT/TAT) detected using the reverse primer; 2. Control.
C) 1. Mutation at codon 306 (CGA/TGA). 2. Control non-mutated. Sequencing reaction performed using the reverse primer.

which could represent an admixture of normal cells infiltrating the pathologic sample. Alternatively, the heterozygosity may be due to a mutant with a dominant effect[27,28] or to a previous stage in leukemogenesis progression to homozygosity and full abnormal p53 function.[29]

Discussion

In human cancers analyzed for p53 mutation, there appears to be a consistent association between inactivation of normal p53 gene function and poor prognosis.[5,30] Mutations usually cluster in four highly conserved regions of the protein (aminoacid residues 132-145, 171-179, 239-248 and 272-286). In cases of BL from South America, more than 50% of mutations are reported to concentrate in a small stretch of 33 aminoacids (codons 213 to 248).[7] Other studies performed in Europe do not show such a defined pattern.[4] This discrepancy may be due to environmental factors that modulate molecular evolution in BL.[31]

Whether p53 acts as a true tumor suppressor gene or as a dominant oncogene[28] depends on the type of mutant. The central region of p53 ranges from aminoacid residues 100 to 300.[32] This core region which binds to DNA is flanked by a carboxy-terminal region mediating oligomerization[33] and an amino-terminal region containing a strong transcription activation signal. p53 function thus involves the sequence specific transactivation of target genes. The majority of mutants found in tumors are defective in sequence-specific transactivation. However, other functions of p53 such as the induction of apoptosis are not dependent on transactivation activity.[34] In a clinical sample it is important to determine the mutated codon since structural and functional studies may predict the degree of aggressivity of the tumor. In experimental models, defects in apoptosis caused by inactivation of p53, can produce treatment-resistant tumors.[35]

Although p53 mutation is not frequent at diagnosis of ALL, detection of some mutants early in disease could become a useful prognostic indicator.[14]

The detection of the same mutation at codon 282 in three different cases is noteworthy. This lesion has frequently been associated with solid neoplasms.[26] In one case reported in our study, the mutation was present exclusively in the DNA obtained from a renal mass appearing after BMT. This finding suggests that patterns of relapse can be influenced by the type of p53 mutation. Recently, Greiner *et al.* reported p53 mutation clustering at codon 248 in patients with blastic mantle-cell lymphoma.[12] Mutations at codon 282 have previously been associated with relapsed T-ALL[8] and histologic transformation of follicular lymphoma.[9] Furthermore, this mutation has been found in pedigrees of the Li-Fraumeni syndrome.[25] Mutations at codon 306 have not been associated with lymphoid malignancies. This molecular lesion has been reported in cases of brain and breast tumors.[26]

Despite the small number of cases analyzed, we successfully selected p53 mutants exclusively on the basis of clinical data. In this selected group of patients and in our geographical area, mutations at codon 282 are particularly frequent. Identification of these mutations may have clinical relevance.

Overall, from a clinical perspective, lymphoma genetic lesions serve three purposes:[36] a) they assist and complement histologic diagnosis; b) they provide a molecular marker with prognostic relevance; c) they allow evaluation of minimal residual disease through highly specific and highly sensitive technologies.

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