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LETTER TO THE EDITOR

Point mutations in *TP53* but not in *p15*^{Ink4b} and *p16*^{Ink4a} genes represent poor prognosis factors in acute adult T cell leukemia/lymphoma

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Adult T-cell leukemia/lymphoma (ATL) is an aggressive mature T-cell malignancy (mainly CD4+) associated with the human T cell lymphotropic virus type-1 (HTLV-1). Approximately 2–5% of the HTLV-1 carriers may develop ATL after a latent period of 40–60 years post-infection, which generally occurs through breastfeeding. Therefore, leukemogenesis in ATL seems to be a long and multi-step process and it is thought to involve not only HTLV-1 infection, but also the accumulation of genetic and epigenetic alterations during the transformation of the infected cells.

ATL is classified into four clinical types: acute, chronic, lymphoma and smoldering [1]. The acute and lymphoma types have poor prognosis with a median survival time (MST) around 4 and 9 months, respectively [2]. The chronic and smoldering types are less aggressive [1,2]. Alterations mainly in acute ATL patients in the *TP53*, *p15*^{Ink4b} and *p16*^{Ink4a} genes have been previously reported in Japan [3–10]. However, studies about point mutations in these genes are still very limited and do not include the smoldering patients.

In the current study, we evaluated the presence of mutations in *TP53*, *p15*^{Ink4b} and *p16*^{Ink4a} genes in various clinical types of ATL. A total of 31 ATL patients from the Complexo Hospitalar Universitario Professor Edgar Santos of the Federal University of Bahia (UFBA), Brazil was included. The diagnosis of ATL was based on pre-established criteria, positive serology for HTLV-1 (positive ELISA confirmed by Western blot or polymerase chain reaction – PCR) and a histological and/or cytological diagnosis of leukemia/lymphoma of peripheral T-cell origin [1,2]. Cases with prolonged survival (more than 5 years) were considered atypical and HTLV-1 proviral integration was performed using Southern blot or long-inverse PCR. The clinical classification of ATL was made according to previous criteria [1]. Sixteen patients had the acute, 10 the chronic and five the smoldering type. The male-to-female ratio was 18/13 and the mean age was 45 years, ranging from 19–89 years (Table I). The Welfare Ethics Committee of the Climério de Oliveira Hospital, UFBA approved this study. Peripheral blood was drawn from the patients after obtaining written informed consent and before treatment with chemotherapy or a combination of zidovudine and interferon-α (IFN-α). DNA from peripheral blood mononuclear cells (PBMCs) was extracted using blood and a cell culture DNA Mini Kit (Qiagen, Valencia, CA). Since patients with smoldering ATL do not have lymphocytosis, as observed in acute and chronic types (Table I), the PBMC samples of the smoldering ATL were enriched with CD4 + cells before DNA extraction. So, the PBMC was sorted by flow cytometry with mouse anti-human monoclonal antibody CD4-PE (BD Biosciences, San Jose, CA) using the FACSAria and CellQuest software (BD Biosciences). The percentages of CD4^{+} cells observed in sorted samples varied between 66–98% (Table I). In these samples, DNA was extracted using the QIAamp DNA investigator Kit (Qiagen). The exons 4–9 of the *TP53* gene, exons 1 and 2 of the *p15*^{Ink4b} gene and exons 1–3 of the *p16*^{Ink4a} gene were amplified by PCR and directly sequenced using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The primers used were: sense 5′-GACCTGCTCCTCTGACTG CT-3′ and antisense 5′-CATTGAAGTCTCATGGAAG-3′ (*TP53, exon* 4); sense 5’-TGCCGTGTTCCAGTTG CTTTATCT-3’ and antisense 5’-ACTGACAACCACCCTT AACCCCTCC-3′(*TP53*, exons 5–6,); sense 5′-GCCACAGGTCTCCCCAAGG-3’ and antisense 5’-AGGGGTCAGCGGCAAGCAG A-3′ (*TP53, exon* 7); sense 5′-TGCCTCTTGCTTCTCTTTTCC-3′ and antisense 5′-TTACCTCGCTTAGTGCTCC CT-3′ (*TP53, exon* 8); sense 5′-GGAGACCAAGGGT GCAGTTAT-3′ and antisense 5-′GTTAGTTAGCTACAACCAGGAGCC-3′ (*TP53, exon* 9); sense 5′-CCAGAAGCAATCCAGGCGCG-3′ and antisense 5′-AATGCACACCTCGCCAA CG-3′ (*p15*^{Ink4b}*, exon* 1); sense 5′-CCCGGCCGGCAT CTCCCATA-3′ and antisense 5′-CGTTGTG GGCGGCTGGGGAACCT-3′ (*p15*^{Ink4b}*, exon* 2); sense 5′-CGGAGAGGGGGAGA GCAG-3′ (sense) and

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Table I. Clinical characteristics and results of *TP53* mutation analysis in patients with ATL.

Patients	Sub-types	Sex	Age	Lymphocyte count ($\times 10^9$/L)	Sorting cell ($CD4^+$)	Mutation	Codon (Exon)	Domain function	Nucleotide change	Amino acid change	Predicted effect on protein function and structure*
1	Acute	M	41	27.0		−					
2	Acute	M	63	77.5		+	155 (5)	DNA biding	ACC → AAC	Thr → Asp	Non-functional
3	Acute	M	45	36.7		−					
4	Acute	F	45	5.4		−					
5	Acute	M	61	91.6		+	282 (8)	DNA biding	CGG → TGG	Arg → Trp	Non-functional
6	Acute	M	26	269.0		−					
7	Acute	M	44	27.6		+	248 (7)	DNA biding	CGG → TGG	Arg → Trp	Non-functional
8	Acute	F	22	61.3		−					
9	Acute	M	35	65.3		−					
10	Acute	M	66	128.6		−					
11	Acute	F	57	59.5		−					
12	Acute	F	73	16.3		+	181 (5)	DNA biding	CGC → CAC	Arg → His	Functional
13	Acute	F	64	51.7		−					
14	Acute	F	39	26.9		−					
15	Acute	F	19	204.0		−					
16	Acute	F	45	176.6		−					
17	Chronic	M	35	4.2		−					
18	Chronic	M	35	8.1		+	248 (7)	DNA biding	CGG → TGG	Arg → Trp	Non-functional
19	Chronic	M	20	30.7		−					
20	Chronic	M	89	130.0		−					
21	Chronic	M	40	4.7		−					
22	Chronic	F	48	5.0		−					
23	Chronic	M	49	2.1		−					
24	Chronic	M	32	9.5		−					
25	Chronic	F	51	11.3		−					
26	Chronic	M	42	8.4		−					
27	Smoldering	F	40	3.2	98%	−					
28	Smoldering	F	46	1.8	93%	−					
29	Smoldering	M	72	3.5	89%	−					
30	Smoldering	F	76	4.1	85%	−					
31	Smoldering	M	67	2.7	66%	−					

(+) point mutation; (−) no point mutation; * Kato *et al.* [11].

antisense 5′-TCCCCTTTTTCCGGAGAATCG-3′ (*p16^Ink4a^, exon* 1); sense 5′-GGCTCTGACCAT TCTGT-3′ and antisense 5′-AGCTTTGGAAGCT CT CAG-3′ (*p16^Ink4a^, exon* 2); sense 5′-TTTTCTTTCTGCCCTCTGCA-3′ and antisense 5′-TGAAGTCGACAGCTTCCG-3′ (*p16^Ink4a^, exon* 3). Survival of patients was assessed by the Kaplan-Meier method and compared using log-rank test; $p < 0.05$ was defined as statistically significant for all tests.

Mutations in *TP53* gene were found in five of the 31 (16%) patients (Table I). Among them, four presented the acute type (#2, #5, #7 and #12), corresponding to 25% of the acute patients, and one the chronic type (#18), corresponding to 10% of the chronic patients. None of the smoldering patients showed mutations in these exons, even after the enrichment of samples with CD4 + cells. The frequency of mutations in *TP53* is consistent with previous reports from Japan [3–5,7,9]. Moreover, we also observed a higher frequency of *TP53* mutations in the acute than in the chronic ATL, as previously demonstrated, suggesting that this mutation can be related to the development of the aggressive types of ATL [5,6].

All the nucleotide changes were missense mutations leading to alterations of the amino acid sequences. Mutations were located in codon 155 (Thr to Asp, patient #2) and codon 181 (Arg to His, patient #12) in exon 5, in codon 248 (Arg to Trp, patients #7 and #18) in exon 7 and in codon 282 (Arg to Trp, patient #5) in exon 8 (Table I). No mutations were observed in exons 4, 6 and 9. In accordance with previous reports [3,4,9], they are located in a high conserved region corresponding to the DNA binding domain of the p53 protein and could have an effect on protein function and structure [11]. Predominantly, they were G:C to A:T transition at CpG dinucleotides (patients #5, #7, #12 and #18, Table I).

Mutations in codons 248 and 282 in the *TP53* gene are referred to as hot spots in human cancers. The former has been previously reported in ATL [6], but the latter represents a new finding. Moreover, according to our knowledge, point mutations at codons 155 and 181 are also being reported in ATL for the first time. Interestingly, four of the five mutations observed here were G:C to A:T transitions at the CpG site, leading to amino acid change. Endogenous methylation of cytosines and transition of these methylated cytosines to thymidines by deamination were suggested as an important mechanism for *TP53* mutations [12]. We speculate that this mechanism may be involved in accumulation of *TP53* alterations during ATL development.

We then evaluated whether the presence of point mutation in *TP53* is related to survival time of ATL patients. Previous studies have shown that patients with acute ATL have shorter MST than the patients with the less aggressive types [2]. Therefore, we evaluated the MST regarding TP53 status in the acute type. The MST of the acute patients with mutations in *TP53* was shorter than the acute patients with the wild type *TP53* ($p = 0.047$; Figure 1). Our results suggest that aberrations in *TP53* may constitute a poor prognostic factor in acute

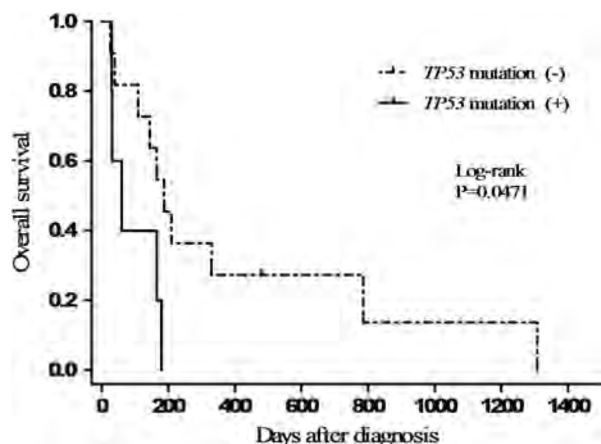

Figure 1. Survival curve of acute ATL patients with mutations in *TP53* gene (solid line) compared with ATL acute patients with wild type *TP53* (dotted line).

ATL. Japanese ATL patients with aberrant TP53 had a shorter survival than those without mutations, even considering the acute and chronic types together [4], forms that have different clinical features and outcomes. Nishimura *et al.* [10], showed a tendency for shorter MST in the presence of TP53 alterations (mutations and/or loss of heterozygosity) in patients with aggressive ATL (acute and lymphoma). It is noteworthy that the importance of *TP53* status in ATL patient's survival was shown in two different endemic areas for ATL. The survival time of the only chronic patient with mutation in TP53 was 1 month, while the MST of the chronic patients with the wild type *TP53* was 10 months. Studies with a large number of chronic and smoldering patients are required to further evaluate the prognostic significance of mutations in TP53 in the less aggressive types of ATL.

None of the 31 patients had point mutations in exons 1 or 2 of the $p15^{Ink4b}$ gene and exons 1, 2 or 3 of the $p16^{Ink4a}$ gene. The lack of alterations in $p15^{Ink4b}$ and $p16^{Ink4a}$ is consistent with previous studies [8]. Probably, other mechanisms such as deletion and hypermethylation can lead to inactivation of these genes in ATL [8,10,13]. In patient #10 a single nucleotide substitution at codon 140 in exon 2 of $p16^{Ink4a}$ was found. This alteration is a G:C to A:T transition at CpG dinucleotide, leading to alanine to histidine change, and was first reported as a point mutation, but now it is considered to represent a polymorphism [14]. Unfortunately, normal tissue was not available for comparative genotyping as this patient 5 five days after diagnosis.

The evaluation of *TP53* gene may be a useful tool for prognostic evaluation and appropriate therapeutic decisions in acute ATL, as the combination of zidovudine and IFN-α seems to produce therapeutic effects through the p53 pathway [15].

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal

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