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p53 gene mutations are uncommon but p53 is commonly expressed in anaplastic large-cell lymphoma

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Anaplastic large-cell lymphoma (ALCL), as defined in the World Health Organization, is a heterogeneous category in which a subset of cases is associated with the t(2;5)(p23;q35) or variant translocations resulting in overexpression of anaplastic lymphoma kinase (ALK). p53 has not been assessed in currently defined subsets of ALCL tumors. In this study, we assessed ALK+ and ALK− ALCL tumors for p53 gene alterations using PCR, single-strand conformation polymorphism and direct sequencing methods. We also immunohistochemically assessed ALCL tumors for p53 expression. Three of 36 (8%) ALCL tumors (1/14 ALK+, 2/22 ALK−) with adequate DNA showed p53 gene mutations. By contrast, p53 was overexpressed in 36 of 55 (65%) ALCL tumors (16 ALK+, 20 ALK−). p21, a target of p53, was expressed in 15 of 31 (48%) ALCL tumors including seven of 15 (47%) p53-positive tumors. p21 expression in a subset of ALCL suggests the presence of functional p53 protein. Apoptotic rate was significantly higher in p53-positive than p53-negative tumors (mean 2.78 vs 0.91%, P = 0.0003). We conclude that the p53 gene is rarely mutated in ALK+ and ALK− ALCL tumors. Nevertheless, wild-type p53 gene product is commonly overexpressed in ALCL and may be functional in a subset of these tumors.

Introduction

Anaplastic large-cell lymphoma (ALCL), as defined in the World Health Organization (WHO) classification system, is a high-grade lymphoma of T- or null-cell lineage with distinctive histologic features. The neoplastic cells also strongly and uniformly express CD30. In the WHO system, ALCL is heterogeneous, with a substantial subset of tumors associated with the t(2;5)(p23;q35) or variant translocations resulting in overexpression of anaplastic lymphoma kinase (ALK).[1–3] Accumulating evidence from in vitro studies in ALK+ ALCL has shown activation of well-established oncogenic pathways involved in cell proliferation and apoptosis.[4] Furthermore, deregulation of cell cycle-controlling proteins and a high proliferation rate have been reported previously in ALCL tumors regardless of ALK status.[5–10] Nevertheless, the mechanisms of ALCL oncogenesis are unclear.

The p53 tumor suppressor gene plays a crucial role in response to various cellular stress conditions by inducing the transcription of numerous genes controlling cell cycle arrest and apoptotic cell death.[11] p53 is inactivated in many human malignancies, and inactivating mutations of the p53 gene are the most common genetic alteration in human cancer.[12,13] p53 also can be inactivated by binding to oncogenic proteins.[14,15] In non-Hodgkin's lymphomas, previous studies have reported p53 overexpression in a subset of tumors, most frequently in high-grade tumors.[16] However, the mechanisms of p53 overexpression are incompletely understood and functional status has been rarely assessed in non-Hodgkin's lymphomas.[16] p53 also has not been systematically assessed in subsets of ALCL as they are currently defined in the WHO classification.

In this study, we assessed ALCL tumors for p53 gene mutations and expression. We also correlated the findings with ALK status, apoptotic rate (AR), proliferation index and expression of two proteins induced by wild-type p53 protein, $p21^{WAF1}$ (p21) and MDM2.[14,17] Our results demonstrate that p53 gene mutations are uncommon in both ALK+ and ALK− ALCL tumors. Nevertheless, variable levels of wild-type p53 protein are expressed in most ALCL tumors and p53 expression correlates with AR. In addition, the expression of p21 in a subset of ALCL tumors suggests that p53, at least in this subset, retains its function.

Materials and methods

ALCL tumors

The study group included 55 ALCL tumors from previously untreated patients accessioned at The University of Texas MD Anderson Cancer Center. The diagnosis of ALCL was based on morphologic and immunohistologic criteria as specified by the WHO classification.[1] All ALCL tumors were uniformly positive for CD30 and negative for CD15. None of the ALCLs expressed B-cell antigens, including CD20, CD79a or PAX5/BSAP. A total of 45 (82%) ALCL tumors expressed one or more T-cell or T-cell-associated markers including CD3, CD5 or CD43. ALK was assessed using the ALK-1 antibody (1:30; Dakocytomation, Carpinteria, CA, USA) and was positive in 24 (44%) cases. Of these ALK+ ALCL tumors, five (21%) showed only cytoplasmic ALK-1 immunoreactivity, suggesting a variant abnormality involving the alk locus (ie not NPM-ALK).

The median age of patients with ALK-positive tumors was 33 years compared with 54 years for patients with ALK-negative tumors (P = 0.0004 by Mann–Whitney U-test). All other clinicopathologic parameters between the two groups were comparable.

PCR-SSCP analysis

Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissues of 42 ALCL tumors using a QIAamp DNA mini kit (QIAGEN, Valencia, CA, USA) according to the

manufacturer's protocol. We chose to analyze six exons of the *p53* gene, exons 4–9, as these exons have had virtually all *p53* gene mutations reported in the literature.[18] The primers used for exons 4–9 of the *p53* gene and the size of PCR products are shown in Table 1. We also chose the *Pfu* Turbo Hotstart DNA Polymerase (Stratagene, La Jolla, CA, USA) for PCR because it is believed to be the least mutagenic polymerase. The PCR program included DNA denaturation at 95°C (5 min), followed by 35 cycles of 95°C (30 s), 56–58°C (30 s) and 72°C (1 min), and lastly extension at 72°C (5 min). The presence and quality of PCR products were tested using 1.5% agarose gels, UV light and the Alpha-Imager system (Alpha Innotech Corporation, San Leandro, CA, USA). The PCR products of 36 ALCL tumors (14 ALK+, 22 ALK−) that showed adequate amplification of all six *p53* exons were subsequently analyzed for possible *p53* gene mutations using a single-strand conformation polymorphism (SSCP) method.

SSCP analysis was performed on the GenePhor electrophoresis unit (Amersham Biosciences, Piscataway, NJ, USA) using appropriate gels, and PCR products were diluted in loading buffer containing formamide, 1% xylene cyanol and 1% bromophenol blue. Electrophoresis was performed using 80 V for 20 min followed by 320 V for 60 min at 12°C.

Subcloning and direct sequencing of PCR products

The PCR products of all ALCL tumors that showed mobility shifts by SSCP were subcloned into pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA) and sequenced. Sequencing was performed using the GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT, USA), fluorescently labeled M13 forward and reverse primers and AmpliTaq-FS DNA polymerase (Perkin-Elmer, Wellesley, MA, USA), according to the manufacturer's directions.

Tissue microarray and immunohistochemical methods

Tissue sections of ALCL tumors, 4–5 μm thick, were cut from a tissue microarray (43 tumors) or whole paraffin blocks (12 tumors). The tissue microarray included triplicate or quadruplet tumor cores from 43 tumors and two reactive lymph nodes and was constructed using a manual tissue arrayer (Beecher Instruments, Silver Springs, MD, USA) as described previously.[19]

The immunohistochemical methods used in this study were described previously.[8] For all antibodies, heat-induced epitope retrieval was performed. The panel of antibodies used in this study included p53 (DO-7), p21 (SX118) (both from Dakocyto-

mation), [Ser15]phosphorylated p53, [Ser20]phosphorylated p53 (Cell Signaling Technology, Beverly, MA, USA), MDM2 (N-20, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and MIB-1 (Ki-67, Immunotech, Westbrook, ME, USA). The DO-7 antibody is known to detect both wild-type and mutant p53. The slides were incubated with the p53, p21 and MDM2 antibodies at 4°C overnight, and with MIB-1 at room temperature for 1 h. Reactive small lymphocytes in all tissue sections served as internal positive controls for p21 immunoreactivity. Slides cut from formalin-fixed cell blocks of Karpas 299 cells served as positive controls for p53, MDM2 and MIB-1.

Any nuclear staining of tumor cells was considered positive, irrespective of intensity. Expression levels for p53, p21 and MDM2 were determined by counting the percentage of positive tumor cells and, for the purpose of statistical analysis, a 10% cutoff was used to define positivity based on the distribution of data (histogram shown in Results) and previously published reports.[20]

TUNEL assay

AR was assessed using a modified terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) assay and designated as the percentage of TUNEL-positive tumor nuclei as described elsewhere.[8]

Statistical analysis

The χ^2 and Fisher's exact tests were used to compare expression of p53, p21 and MDM2 (positive *vs* negative) with various parameters. The Mann–Whitney *U*-test and Kruskal–Wallis tests were chosen for the nonparametric correlation of proliferation index and AR between various groups. The Spearman *R* correlation coefficient was used to assess correlations between continuous variables. All computations were carried out using the StatView statistical program (Abacus Concepts Inc., Berkeley, CA, USA).

Results

p53 gene mutations in ALK+ and ALK− ALCL tumors

Out of 42 ALCL tumors, 36 (14 ALK+ and 22 ALK−) had adequate quality DNA to allow for assessment of *p53* gene mutations using PCR-SSCP methods. In six tumors, all exons of

Table 1 Primers used for *p53* gene mutation analysis

p53 gene	Primer sequences	PCR product (bp)
Exon 4	Sense 5-TCC TCT GAC TGC TCT TTT CAC-3′ Antisense 5′-TGA AGT CTC ATG GAA GCC AG-3′	348
Exon 5	Sense 5′-CTT GTG CCC TGA CTT TCA ACT-3′ Antisense 5′-CAA CCA GCC CTG TCG TCT-3′	266
Exon 6	Sense 5′-TCT GAT CCT CAC TGA TTG CT C-3′ Antisense 5′-CCA CTG ACA ACC ACC CTT AAC-3′	187
Exon 7	Sense 5′-TCA TCT TGG GCC TGT GTT ATC-3′ Antisense 5′-AGT GTG CAG GGT GGC AAG-3′	169
Exon 8	Sense 5′-AGG ACC TGA TTT CCT TAC TGC C-3′ Antisense 5′-ATA ACT GCA CCC TTG GTC TCC-3′	237
Exon 9	Sense 5′-ACT TTT ATC ACC TTT CCT TGC C-3′ Antisense 5′-CAC TTG ATA AGA GGT CCC AAG AC-3′	134

the *p53* gene could not be amplified. Three of 36 (8%) ALCL tumors (one ALK+, two ALK−) tested showed mobility shifts indicating possible gene mutations and all three tumors over-expressed p53 protein (Figure 1a and b). Subcloning and direct sequencing of the PCR products revealed a frameshift mutation and two point mutations in the three tumors (Figure 1c and d). One of these tumors, an ALK-positive ALCL, showed a silent point mutation (CAA→CAC) that resulted in no change of the corresponding amino acid (Supplementary Table 1). A missense point mutation of exon 7 (TGG→CGG) leading to an amino-acid substitution (Thr→Ala) and a frameshift mutation were found in the two ALK-negative ALCL cases (Supplementary Table 1).

p53 expression in ALCL tumors

Using a 10% cutoff, p53 was overexpressed in 36 of 55 (65%) ALCL, including 16 of 24 (67%) ALK+ and 20 of 31 (65%) ALK− tumors (*P*>0.9, Fisher's exact test; Figure 2a–c and Table 2). In p53-positive tumors, the percentage of p53-positive tumor cells ranged from 12 to 99% with a median of 70% (Figure 3). In the three tumors with *p53* gene mutations, virtually all cells overexpressed p53 (Figure 2). However, p53 was also overexpressed in most ALCL tumors without evidence of *p53* gene mutation. In the unmutated cases, p53 was overexpressed by a variable percentage of tumor cells (Figure 2). In many tumors, the intensity of p53 staining was variable among the tumor cells, ranging from weak to strong (Figure 2).

Phosphorylation of p53 on serine 15 and serine 20 was assessed in a subset of 20 and 16 p53-positive ALCL tumors, respectively, using phospho-specific antibodies. Using a 10% cutoff, serine 15- and serine 20-phosphorylated p53 was expressed in 13/20 (65%) and 8/16 (50%) tumors, respectively. Staining intensity of phosphorylated p53 was generally weaker compared with that of total p53 (Figure 2d and e). All ALCL tumors positive for phosphorylated p53 were also positive for MDM2.

Expression of MDM2 and p21 in ALCL tumors

Expression of MDM2, a p53-regulatory protein, was assessed in 43 ALCL tumors (17 ALK+ and 26 ALK−). Of these tumors, 29 were p53-positive. MDM2 was expressed in 35 (81%) ALCL (Figure 2d), 16 (94%) ALK+ and 19 (73%) ALK− (*P*=0.1, Fisher's exact test). Among the MDM2-positive tumors, the percentage of MDM2-positive tumor cells ranged from 15 to 100% with a median of 80%. MDM2 and p53 were frequently coexpressed in ALCL (Table 2), as all but one p53-positive ALCL tumor were also positive for MDM2 (*P*=0.0007, Fisher's exact test; Table 2). When analyzed as continuous variables, the percentages of p53- and MDM2-positive tumor cells were
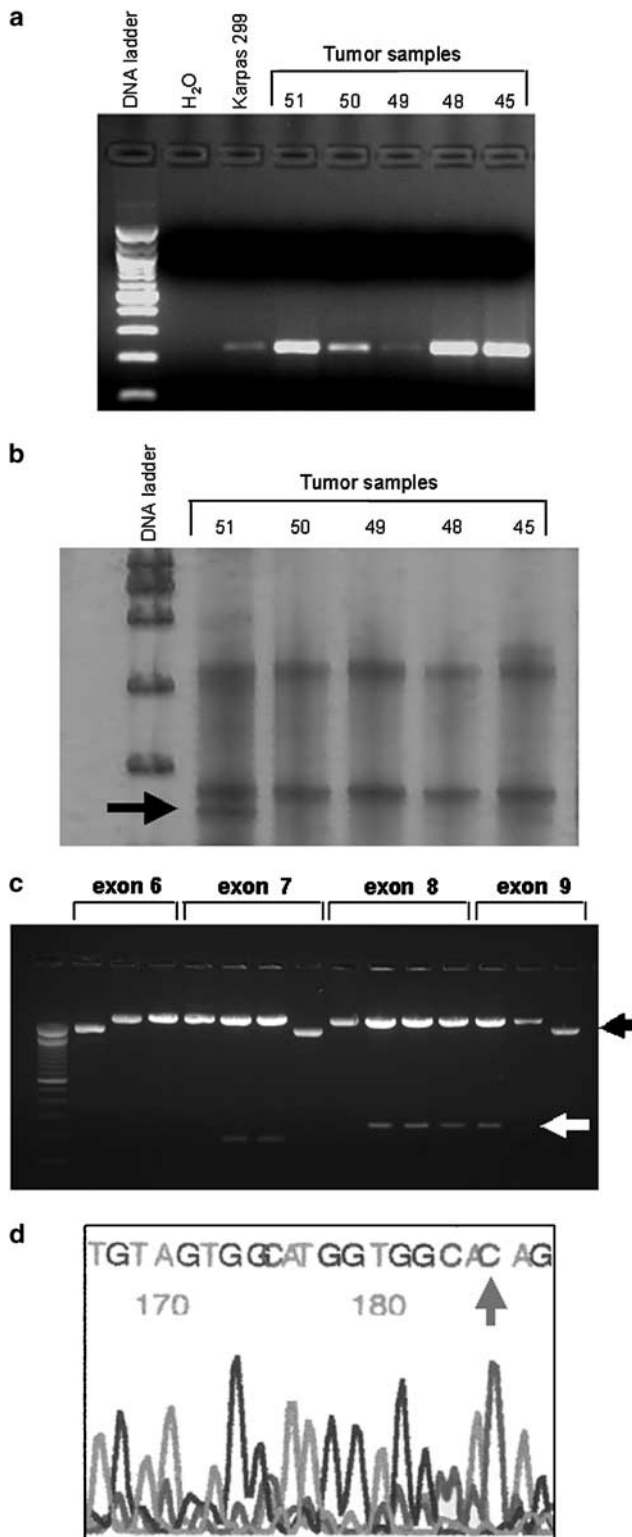
Figure 1 (a) Genomic DNA from ALCL tumors was amplified using primers specific for exons 4–9 of the *p53* gene. The quality of PCR products was tested using 1.5% agarose gels and ultraviolet light, which showed the presence of a single band corresponding to the known size of each product. The PCR products (169 bp) of exon 7 amplification are shown. (b) PCR products with adequate amplifica-tion of all six *p53* exons were subsequently analyzed for possible *p53* mutations using an SSCP method. Silver-stained gel from SSCP analysis shows a PCR product (case #51, exon 7, arrow) with a mobility shift indicating possible gene mutation. (c) PCR products with possible *p53* gene mutations were subcloned using the pCR2.1 TOPO vector that contains M13 sequences. Following transformation of *Escherichia coli*, the positive colonies (white arrow) were selected based on their resistance to ampicillin. The black arrow indicates the presence of the vector DNA. The subcloned products containing the M13 were subsequently sequenced. (d) Direct sequencing of the PCR products (forward and reverse) using the GeneAmp PCR System 9600 confirmed the presence of gene mutation. A nucleotide substitution (T→C) of case # 51 is shown with the arrow.
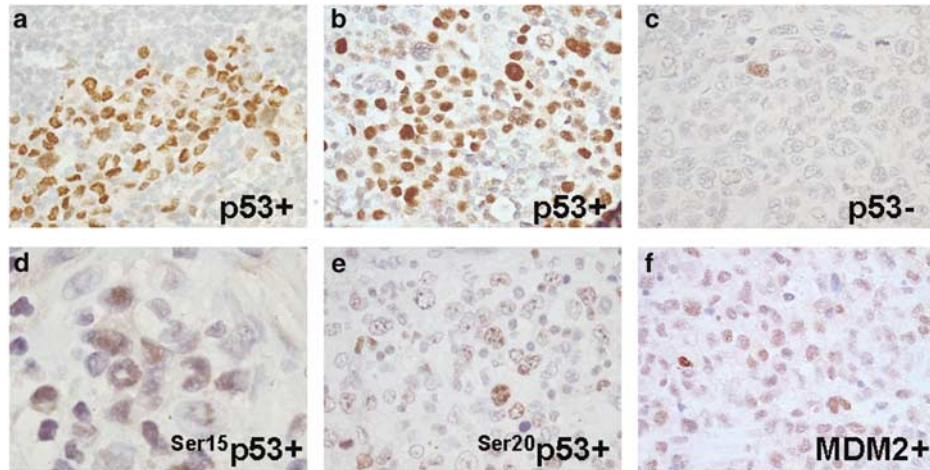
Figure 2 Expression of p53 and MDM2 in ALCL tumors. (a, b) p53 overexpression in cases of ALK + (a, case #14) and an ALK− (b, case #55) ALCL. *p53* gene was unmutated in both cases. (c) An ALK− ALCL case that is p53-negative. (d, e) Expression of serine 15- (d) and serine 20- (e) phosphorylated p53 in two cases of ALK + ALCL tumors. (f) Expression of MDM2 in an ALK + ALCL tumor.

Table 2 Expression of p53, ALK, p21 and MDM2, and AR and proliferation index in ALCL tumors

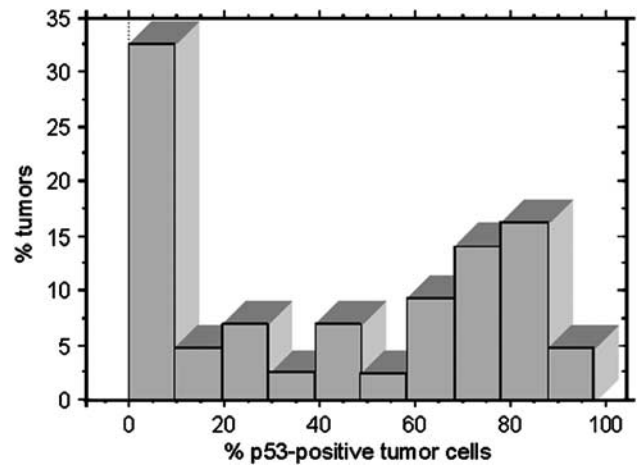|	p53 expression		P-value
	p53+	P53−	
ALK expression			>0.9
ALK+	16/24 (67%)	8/24 (33%)	
ALK−	20/31 (65%)	11/31 (35%)	
MDM2 expression			0.0007
MDM2+	28/35 (80%)	7/35 (20%)	
MDM2−	1/8 (13%)	7/8 (87%)	
p21 expression			>0.9
p21+	7/15 (47%)	8/15 (53%)	
p21−	9/16 (56%)	7/16 (44%)	
Apoptotic rate (%) (mean ± s.d.)	2.78 ± 2.13	0.91 ± 0.64	0.0003
Proliferation index (%) (mean ± s.d.)	73.4 ± 19.1	69.4 ± 18.9	0.38

Figure 3 Histogram showing the distribution of ALCL tumors according to percentage of p53-positive tumor cells. Based on these data, a cutoff of 10% was chosen to define a tumor as p53-positive. x-axis, percentage of p53-positive tumor cells; y-axis, percentage of ALCL tumors.

significantly correlated (Spearman $R = 0.52$, $P = 0.0008$). Among MDM2-positive tumors, p53 was absent in six tumors. Absence of p53 and p21 expression was observed in three MDM2-positive tumors.

Expression of p21 was assessed in a subset of 31 ALCL (12 ALK + and 19 ALK−). p21 was expressed in 15 (48%) ALCL (Figure 4), eight (67%) ALK + and seven (37%) ALK− tumors. The association between p21 and ALK expression was not statistically significant ($P = 0.15$, Fisher's exact test). The association between p21 and p53 expression was also not statistically significant (Table 2). Of the 15 p21-positive ALCL tumors, seven (47%) were also positive for p53.

Association of p53, MDM2 and p21 expression with apoptotic rate and proliferation index

AR was available for 46 ALCL tumors.[8] In this group, the mean AR was 2.8% in p53-positive ALCL compared with 0.9% in the p53-negative ALCL ($P = 0.0003$, Mann–Whitney test; Table 2
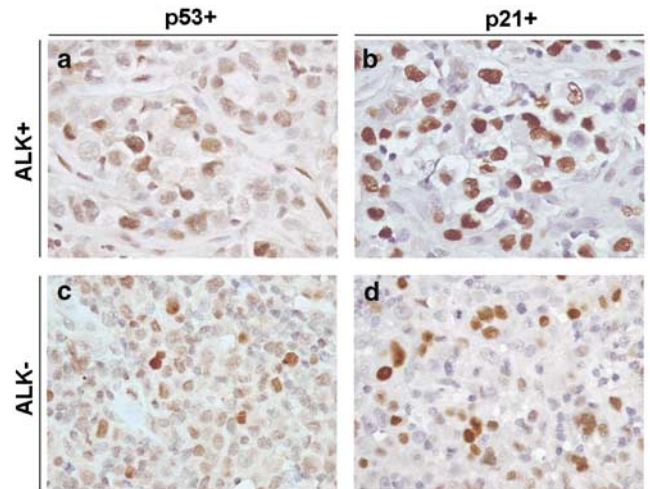

Figure 4 (a–d) p53 and p21 expression in ALK + (a, b) and ALK− (c, d) ALCL tumors. (a–d, immunohistochemistry).
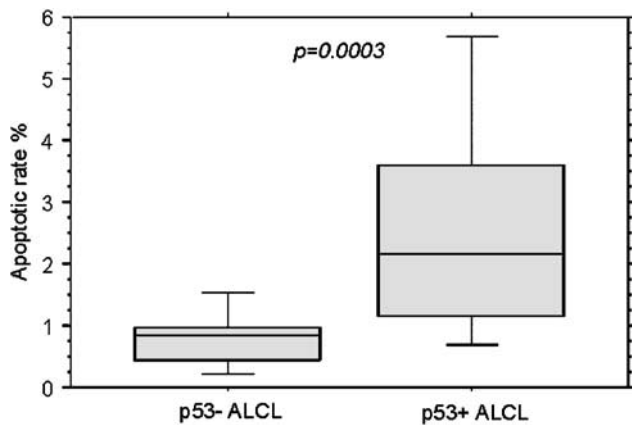
Figure 5 Box plots showing higher AR in p53-positive ALCL tumors than in p53-negative ALCL tumors ($P = 0.0003$).

and Figure 5). As continuous variables, the percentage of p53-positive tumor cells and the AR were positively correlated (Spearman $R = 0.32$, $P = 0.03$). AR also correlated with MDM2 expression. The mean AR was 2.5% in MDM2-positive ALCL compared with 0.8% in MDM2-negative ALCL ($P = 0.0019$, Mann–Whitney test). In addition, the percentage of MDM2-positive tumor cells positively correlated with AR (Spearman $R = 0.34$, $P = 0.04$). No significant association between p21 expression and AR was observed.

The mean proliferation index was 73.4% in p53-positive ALCL compared with 69.4% in p53-negative ALCL ($P = 0.38$, Mann–Whitney test; Table 2). Proliferation index did not correlate with expression of MDM2 or p21.

Clinical outcome

Complete follow-up data were available for 43 of 51 patients with ALCL (18 ALK +, 25 ALK−) analyzed for p53 expression. The median follow-up period was 36 months (2–159 months). For the entire group, 5-year progression-free survival (PFS) was 57% for patients with p53-positive tumors compared with 80% for patients with p53-negative tumors ($P = 0.2$ by log rank). Survival analysis was also performed separately for the ALK-positive and ALK-negative groups. For 18 patients with ALK-positive ALCL, 5-year PFS was 64% for patients with p53-positive tumors compared with 100% for patients with p53-negative tumors ($P = 0.12$ by log rank). For 25 patients with ALK-negative ALCL, 5-year PFS was 66% for patients with p53-positive tumors compared with 71% for patients with p53-negative tumors ($P = 0.6$ by log rank). Similarly, overall survival at 5 years did not differ significantly between patients with p53-positive or p53-negative ALCL tumors.

Discussion

A large body of evidence accumulated over the past two decades suggests that alterations of the p53 pathway play a central role in tumorigenesis.[11] The *p53* tumor suppressor gene can be inactivated by a number of mechanisms including *p53* gene mutation, the most frequent genetic alteration in human cancer,[12] or defects in cell pathways that regulate p53 levels or inhibit p53 function.[11] Although *p53* gene alterations, most commonly point mutations, are detected in more than 50% of human cancers, their frequency is substantially lower in

lymphoid neoplasms compared with epithelial tumors.[16] The presence of *p53* gene alterations has been associated with clinically aggressive lymphomas, usually of high cytologic grade, or progression of low-grade non-Hodgkin's lymphomas to high-grade neoplasms.[16] However, the presence or absence of *p53* gene mutations has not been assessed in a group of ALCL tumors, incorporating ALK status and classified using the criteria of the current WHO classification system.

In this study, 8% of ALCL tumors (7% ALK +, 9% ALK−) carried a mutated *p53* gene and overexpressed p53 protein. In these tumors, virtually all cells overexpressed p53 (Figure 1). However, p53 was also overexpressed in most ALCL tumors without evidence of *p53* gene mutation. By contrast, in unmutated cases, p53 was overexpressed by a variable percentage of tumor cells (Figure 2). To our knowledge, only one earlier study by Cesarman *et al*[21] has assessed *p53* in ALCL. This study included 17 cases classified as ALCL using out-of-date classification criteria. In these 17 cases, ALK status was not available, and this study included B-cell cases; the latter are no longer considered ALCL.[1] Nevertheless, only one (6%) tumor in that study was found to carry a mutated *p53* gene.[21] Thus, our results support the conclusion by Cesarman *et al*[21] that *p53* gene mutation is uncommon in ALCL, and extend their work by showing that *p53* gene mutation is uncommon in both ALK + and ALK− ALCL.

Other studies assessing *p53* gene and p53 expression in T-cell lymphomas of various types have been performed, although p53 function has not been investigated. Matsushima *et al*[22] detected *p53* gene mutations in 9% of tumors although p53 was overexpressed in 50%. Similarly, Quintanilla-Martinez *et al*[20] reported *p53* gene mutations in 15% of a series of extranodal T/NK lymphomas of nasal type whereas p53 was expressed in 60%. Others have not identified *p53* gene mutations in peripheral T-cell lymphomas.[23] The high frequency of p53 expression in high-grade T-cell lymphomas including ALCL, and the low frequency of *p53* gene mutations suggest that mechanisms other than *p53* gene alterations stabilize the wild-type p53 gene product. The presence of wild-type p53 in most ALCL tumors is further supported by our results showing that a *p53*-induced gene, *p21*,[24] is expressed in a subset of ALCL tumors (Table 2 and Figure 4), suggesting that p53 protein is capable of inducing expression of target genes. As *p53* gene mutations are uncommon in ALCL, the mechanism of p53 overexpression in these tumors is uncertain.

It is known that p53 functions as a transcriptional factor through binding of its activation domain to DNA specific sites inducing expression of multiple genes.[11] Thus, p53 can be inactivated by binding to oncogenic proteins, such as MDM2, that conceal the activation domain of p53.[14,15] In this study, p53 and MDM-2 expression levels were significantly correlated in ALCL tumors (Table 2). It is likely that, at least in a subset of these tumors, increased levels of MDM2 can inhibit p53 transcriptional activity resulting in cell cycle deregulation in ALCL. MDM2 inhibition of p53 transcriptional activity is further indicated by the absence of p21 in a subset of ALCL cases that we assessed. Nevertheless, the detection of p21 in a subset of tumors suggests that p53 is functional in some cases.

Apart from its p53 inhibitory activity, MDM2 is also an E3-ubiquitin ligase that targets p53 for degradation through the ubiquitin–proteasome system. Therefore, one would expect that high MDM2 levels would lead to increased degradation of p53.[14] There are several possible explanations for the inability of MDM2 to degrade p53 in these tumors. One possibility is that phosphorylation of p53 by ATM on serine 15,[25,26] or by Chk2 on serine 20,[27,28] can impair the ability of MDM2 to bind p53

resulting in p53 stabilization and increased p53-dependent transactivation. In this study, we found that phosphorylated p53 on serine 15 and 20 is expressed in 65 and 50%, respectively, of the ALCL tumors assessed; all of these phosphorylated p53-positive tumors expressed MDM2 (Figure 2). Therefore, phosphorylation of p53 ALCL may explain, at least in part, p53 stabilization in the presence of MDM2 in ALCL. Other possibilities include the presence of splice variants of MDM2 transcripts that may not contain the p53 binding site,[29] functional inactivation of MDM2 through phosphorylation by ATM kinase on serine 395 or sequestration of MDM2 in the nucleolus by p14[ARF] or promyelocytic leukemia (PML) proteins.[30–32]

AR was significantly higher in p53-positive than p53-negative ALCL tumors in this study (Table 2). Apart from its cell cycle regulatory function, p53 normally promotes apoptosis by inducing expression of numerous apoptotic genes, including genes involved in both the intrinsic and extrinsic apoptotic pathways.[11] This is additional evidence suggesting that p53 in ALCL is, at least in part, functional. In 35 ALCL tumors with available data from a previous study,[8] p53 expression correlated with BAX, a p53-induced proapoptotic protein (data not shown), suggesting that wild-type p53 may induce expression of BAX, and probably other apoptotic genes, resulting in a higher AR in ALCL tumors.

In conclusion, p53 gene mutations are uncommon (<10%) in ALK+ and ALK− ALCL. Nevertheless, p53 is frequently expressed in ALCL tumors at a variable level and appears to be capable of inducing expression of target genes, such as p21, in a subset of cases. p53 is serine phosphorylated in a subset of ALCL tumors and, in part, phosphorylation may protect p53 from binding to MDM2 and subsequent degradation. Whether or not high expression levels of MDM2 found in most cases of ALCL might inhibit p53 transcriptional activity resulting in cell cycle deregulation merits further investigation. The regulatory p53–MDM2 system is being currently used as a target for investigational therapy.[33] These approaches may result in release of fully functional p53 capable of inducing cell cycle arrest and apoptosis in tumor cells.

Acknowledgements

Supplementary Information

Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu).

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