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Alterations of the p53 Gene in Epstein-Barr Virus-Associated Immunodeficiency-Related Lymphomas

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Mutations of the p53 tumor suppressor gene are among the most common genetic alterations found in many different human malignancies, including those of the colon, lung, and breast. Alterations in wild-type p53 lead to loss of the suppressor function and thus contribute to tumorigenesis. The potential role of p53 mutations in a sampling of B-cell lymphomas, the majority of which were associated with Epstein-Barr virus (EBV), was investigated. Twenty-six biopsy specimens from immunocompromised patients, including allograft recipients and patients with AIDS, Wiscott-Aldrich syndrome, and human T-cell leukemia virus type 1 infection, in comparison with three Burkitt lymphomas and four Burkitt lymphoma cell lines were analyzed. Mutation in p53 was detected in all four Burkitt lymphoma cell lines as well as the three Burkitt lymphoma biopsy specimens. In patients with AIDS, 5 of 10 lymphomas were EBV positive, and 1 had a mutation in p53. Mutation in p53 was not detected in 14 EBV-positive lymphomas which arose in transplant recipients. These data indicate that with the exception of Burkitt lymphomas, p53 mutations are not involved in the majority EBV-positive B-cell lymphomas which develop in immunocompromised patients.

The pathogenesis of human cancers is thought to involve genetic abnormalities, including alterations in cellular oncogenes and tumor suppressor genes which have regulatory functions in cell proliferation and differentiation (14, 17). Wild-type p53 has been shown to negatively regulate cell growth, with loss of function appearing to be oncogenic. Inactivation of the p53 gene-encoded nuclear phosphoprotein can occur through virally encoded oncogenes such as the large T-antigen gene of simian virus 40 and the E6 gene of human papillomavirus or by genetic mechanisms such as deletions, rearrangements, or single-point mutations (17, 32, 34, 37, 43, 45). Mutations in the p53 gene on chromosome 17p are among the most common genetic alterations in many different human malignancies, including carcinomas of the colon, lung, brain, bladder, and breast (1, 37, 44). Transgenic mice expressing mutant p53 also have a high incidence of lymphoid tumors, suggesting that p53 may be involved in the development of lymphoid malignancies (33).

Epstein-Barr virus (EBV), a ubiquitous lymphotropic herpesvirus, is consistently associated with nasopharyngeal carcinoma (NPC), a clonal proliferation of epithelial cells, as well as endemic Burkitt lymphoma (BL) and lymphoproliferations arising in immunocompromised individuals (7, 9, 18, 35, 40, 41, 51). Mutations in p53 have not been detected in NPC but have been detected in EBV-positive BL tumors and cell lines (4, 11, 16, 19).

EBV is also associated with lymphoproliferative disease arising in immunosuppressed allograft recipients. These proliferations are thought to initially be polyclonal, with progression to monoclonal or oligoclonal outgrowths (24, 30). Therefore, to assess the contribution of p53 mutations to this clonal dominance, it is of interest to determine the frequency of p53 mutations in such malignancies. Mutations in p53 were analyzed in biopsy specimens from 29 B-cell lymphomas, including 26 arising in immunocompromised patients, including allograft recipients and patients with AIDS, Wiscott-Aldrich syndrome, and human T-cell leukemia virus type 1 infection, in comparison with three BL samples and four BL cell lines, using single-stranded conformation polymorphism (SSCP) analysis and PCR sequencing. Mutations in p53 were predominantly detected in the BL cell lines and BL biopsy specimens as opposed to lymphomas that develop in immunocompromised patients, suggesting that p53 mutations are not likely involved in the majority of EBV-associated B-cell lymphomas with the exception of BL.

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MATERIALS AND METHODS

Tissue and cell lines. The cell lines used, which were maintained in RPMI 1640 with 5 to 10% fetal calf serum, have been described previously (25, 31, 39, 48). The lymphoma tissue obtained at biopsy and preserved at −70°C has also been previously described (23) (Table 1). Only those tissues that contained predominantly neoplastic cells were used for the molecular studies. The frozen tissues were pulverized and dissolved in 4 M guanidine thiocyanate, and the DNA fractions were extracted twice with phenol and chloroform after dialysis and proteinase K treatment. All specimens were screened for EBV DNA and were compared with dilutions of the Raji cell line to determine the EBV genome copies. The EBV-positive biopsy specimens contained between 1 and >50 copies of EBV, and all were clonal for EBV with the exception of specimens from two allograft recipients, which were biclonal (23, 40). These results are included in Table 1.

SSCP analysis. Primers P1 and P2 (Table 2) were used in a standard PCR to amplify the 2.9-kb fragment of the genomic DNA which contains exons 5 to 8 of the p53 gene (36). The amplified fragments were purified from a 1% agarose gel after electrophoresis (Geneclean kit; Bio 101, Inc., La Jolla, Calif.). For the SSCP analyses, PCR was performed as previously described (11). Distinct primer pairs (Table 2) were used to amplify exons 5 to 8 of the p53 gene in separate PCRs for 30

TABLE 1. Clinical characteristics of lymphoma specimens

Lymphoma or cell line	Immune status[a]	Lymphoma type[b]	EBV status[c]
Lymphomas			
Hen[d]	TP-H	DLC	+
M9143	TP-BM	Polymorphic	+
CS707	TP-BM	DLC, immbl	+
CS336	TP-H	DLC	+
M9142	TP-BM		+
M9070	TP-BM	DLC, immbl	+
M9101	TP-BM	DLC, immbl	+
CS804[d]	TP-BM	DLC, immbl	+
CS803	TP-BM	DLC, immbl	+
CS788	TP-BM		+
CS305	TP	DLC	+
Fa	TP-H	DLC	+
T580	TP-H	Polymorphic	+
T594	TP-H	DLC, immbl	+
Ber	AIDS	DLC, immbl	+
Hou	AIDS	DLC, immbl	+
Jar	AIDS	DLC, immbl	+
Cas	AIDS	DLC, immbl	+
Wal	AIDS		+
Mal	AIDS	DLC	−
Mar	AIDS	DLC	−
Com	AIDS	DLC	−
Del	AIDS	DLC	−
Dav	AIDS	Burkitt	−
Blou	Normal	Burkitt	+
Coo	Normal	Burkitt	+
Burkitt	Normal	Burkitt	−
Hat	WAS	DLC, immbl	+
Boi	HTLV-1		
Cell lines			
Raji		Burkitt	+
Louckes		Burkitt	−
Namalwa		Burkitt	+
P3HR1		Burkitt	+

[a] TP-H, heart transplant; TP-BM, bone marrow transplant; WAS, Wiscott-Aldrich syndrome.
[b] DLC, diffuse large-cell lymphoma; immbl, immunoblastic subtype.
[c] +, EBV DNA detectable by Southern blot; −, EBV DNA not detectable.
[d] Biclonal for EBV.

TABLE 2. Sequences of oligonucleotides used as PCR and sequencing primers

Primer	Sequence[a]	Fragment length
P1	GACGGAATTCGTCCCAAGCAATGGATGAT	2.9 kb
P2	GTCAGTCGACCTTAGTACCTGAAGGGTGA	
P3	TTCCTCTTCCTGCAGTACT	209 bp
P4	AGCTGCTCACCATCGCTAT	
P5	GGCCTCTGATTCCTCACTGA	170 bp
P6	GCCACTGACAACCACCCTTA	
P7	TGTTGTCTCCTAGGTTGGCT	139 bp
P8	CAAGTGGCTCCTGACCTGGA	
P9	CCTATCCTGAGTAGTGGTAA	164 bp
P10	TCCTGCTTGCTTACCTCGCT	
P11	GACCTGGTCCTCTGACTGCT	320 bp
P12	ACGGCCAGGCATTGAAGTCT	

[a] Sequences for the p53 primers were taken from the published coding and intron p53 sequences (5).

cycles at 94, 55, and 72°C. One microliter of the PCR product was diluted 100-fold, and SSCP analysis was performed as previously described (11).

PCR sequencing. A portion of the preamplified and purified 2.9-kb fragments of the p53 gene was used in a second asymmetric PCR with primer pairs P3-P4 (exon 5), P5-P6 (exon 6), P7-P8 (exon 7), and P9-P10 (exon 8) (Table 2) (5, 11). For exons 4 and 9, a standard PCR was performed as described above from the genomic DNA, using primer pairs P11-P12 and P9-P2, respectively (Table 2) (5, 11). The amplified fragments were purified (Majic PCR Prep; Promega Corp., Madison, Wis.). For sequence analysis, the preamplified fragments containing exons 4 and 9 were used in a second asymmetric PCR with primer pairs P11-P12 (exon 4) and P9-P2 (exon 9) (Table 2) (11). Both strands were sequenced for each exon with the alternate less-abundant primer used to prime the sequencing reactions.

The product of the asymmetric PCR was purified and resuspended in 15 μl of water, and 7.5 μl was then used for each sequencing reaction. Sequencing was performed with the Sequenase kit (United States Biochemical Corp., Cleveland, Ohio). After electrophoresis on a 8% Long Ranger gel (AT Biochem, Malvern, Pa.) containing 5 M urea and 30% formamide, the gel was dried and exposed to Kodak XAR film overnight.

Detection of allelic loss. Differential PCR was performed with the gamma interferon reference gene as previously described (36). Amplification of sequences in exons 5, 7, and 8 of the p53 gene was performed in conjunction with the amplification of an 84-bp sequence within the gamma interferon gene. After electrophoresis and ethidium bromide staining of the PCR products, the degree of amplification was determined by densitometric analysis of the negatives.

Chromosome 17p has a variable number of tandem repeats; therefore, oligonucleotide primers which span the repeated pYNZ22 region were used (28). Amplification and analysis were performed as previously described (12, 28).

The known polymorphism in exon 4 within an *Acc*II site was also used to determine the number of alleles within a tissue (5). The polymorphism was detected by direct sequencing and/or by restriction digestion with *Acc*II. A fraction of the purified exon 4 PCR product from the procedure described above was digested with *Acc*II and subjected to electrophoresis on an 12% polyacrylamide gel. A 259-bp fragment represented allele 1, and 160- and 99-bp fragments represented allele 2 (12).

RESULTS

PCR analysis of p53. The DNA samples were screened for p53 deletions by PCR. A 2.9-kb fragment of p53 (primers P1 and P2 [Table 2]) including the coding sequences for exons 5 to 8 was amplified along with fragments containing exons 4 and 9, with successful amplification being indicative of intact coding sequences. The 2.9-kb fragment was successfully amplified from all samples except the P3HR1 cell line. Differential PCR was used to analyze the P3HR1 cell line for further deletions of exons 5, 7, and 8. Amplification of exons 5, 7, and 8 along with the gamma interferon gene as a control was performed. All three exons were present, suggesting that the P3HR1 cell line contains a deletion or mutation of sequences around primers P1 and/or P2 as well as the previously characterized mutation in exon 5 (data not shown) (16).

Detection of p53 mutations by SSCP analysis and PCR. Point mutations can significantly alter p53 function, with more than 98% of all mutations in p53 detected in exons 5 to 8 (27, 37). Therefore, SSCP analysis was used to screen the samples for sequence changes in exons 5 to 8. With this technique, radiolabeled PCR products when denatured and allowed to renature will assume intramolecular secondary structures

TABLE 3. p53 DNA structure in lymphomas

Lymphoma or cell line	EBV status[a]	Structure[b]									
		Exon 4	Exon 5		Exon 6		Exon 7		Exon 8		Exon 9
		S	SSCP	S	SSCP	S	SSCP	S	SSCP	S	S
Lymphomas											
Hen	+	N	N	N	N	N	N	N	N	N	N
M9143	+		Weak	N	N		N		N		
CS707	+	N	N		N		N		N		N
CS336	+	N	N		N		N		N		N
M9142	+		Weak		N		N		N		N
M9070	+	N	Weak	N	N		Weak	N	?	N	N
M9101	+	N	N		N		N	N	N		
CS804	+	N	N		N		N	N	N		N
CS803	+		Weak	N	N		N		N		N
CS788	+		N		N		N		?	N	N
CS305	+		N	N	N		N		N		
Fa	+	N	N	N	N	N	N	N	N	N	N
T580	+	N		N		N		N		N	N
T594[c]	+	N		N		P		N		N	
Ber	+	N	N		N		N	N	N		N
Hou	+		N	N	N	N	N	N	N	N	N
Jar	+	N	N		N		N	N	N		N
Cas[c]	+	N	N	N	N	N	N	N	A	M	N
Wal	+	N	N		N		N		N		N
Mal	−	N	N		N		N	N	N		
Mar	−	N	N		N		N	N	N	N	N
Com	−	N	Weak	N	N		N	N	?	N	N
Del	−	N	N		N		N	N	N		N
Dav	−	N	N		N		N	N	?	N	N
Blou[c]	+	N	N	N	N	N	A	M	N		
Coo[c]	+	N	N	N	N	N	A	M	N	N	N
Burkitt[c]	−	N	N	N	N		A	M	N		N
Hat	+	N	N	N	N	N	N		N	N	N
Boi		N	N		N		N		N		N
Cell lines											
Raji[c]	+	N	N	N	?	M	A	M	N	N	N
Louckes[c]	−	N	N	N	N	N	A	M	N	N	N
Namalwa[c]	+		N	N	N	N	A	M	N		
P3HR1[c]	+	No 2.9									

[a] +, EBV DNA detectable by Southern blot; −, EBV DNA not detectable.
[b] S, sequence; N, normal; A, abnormal; M, mutation; P, polymorphism; ?, inability to identify a prominent abnormal band.
[c] Mutation or polymorphism.

unique for that sequence. SSCP analysis has successfully detected point mutations in the p53 gene as well as the *ras* gene family and the neurofibromatosis type 1 gene (6, 11, 12, 13, 38).

For SSCP analysis, exons 5 to 8 were amplified from the 2.9-kb fragment and subsequently subjected to electrophoresis under nondenaturing conditions. To confirm the sensitivity of the SSCP, controls included known examples of both normal and single-point mutations for the exon in question. In samples with mutations in p53, multiple abnormal migration patterns and extra bands were consistently detected when compared with the normal control samples. Of the 33 samples, abnormal migration patterns were detected in seven samples (Table 3).

To confirm the SSCP results, the abnormal specimens as well as a sampling of normal specimens were sequenced. Abnormal migration was not detected by SSCP analysis in exon 5, and sequencing confirmed this finding (Table 3).

Sequence analysis of exon 6 detected a polymorphism on one allele in the posttransplant lymphoma (PTL), T594, with a transition from CGA to CGG in codon 213 (Fig. 1A). This previously characterized polymorphism did not result in an amino acid change (Table 4) (3). The Raji cell line contained a transition from CGA to CAA in the same codon on a single allele, confirming previous studies (Fig. 1B; Table 4) (16).

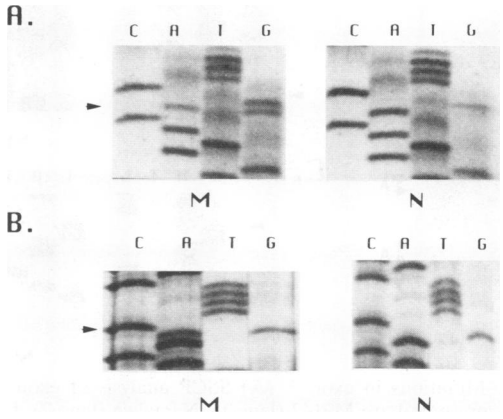

FIG. 1. Mutations in exon 6. (A) Reading downward, sequence of mutation in T594 (M) in codon 213 (CGA to CGG) on one allele compared with normal sequence (N). (B) Reading downward, sequence of mutation in Raji (M) in codon 213 (CGA to CAA) on one allele as compared with normal sequence (N).

TABLE 4. p53 mutations in lymphomas

Lymphoma or cell line	Mutation
Lymphomas	
Cas	Codon 278, CCT to CTT (Pro to Leu)
Blou	Codon 241, TCC to ACC (Ser to Thr)
Coo	Codon 239, AAC to GAC (Asn to Asp)
T594	Codon 213, CGA to CGG (Arg to Arg)
Burkitt	Codon 248, CGG to CAG (Arg to Gln)
Raji	Codon 213, CGA to CAA (Arg to Gln)
Cell lines	Codon 234, TAC to CAC (Tyr to His)
Louckes	Codon 238, TGT to TAT (Cys to Tyr)
Namalwa	Codon 248, CGG to CAG (Arg to Gln)
P3HR1	Deleted for 2.9 kb

SSCP analysis of exon 7 revealed abnormal migration in six samples, three BL cell lines and three BL biopsies (Fig. 2A and 3A). Multiple additional bands were detected by SSCP in samples with a single mutation in p53 (Fig. 2). Sequence analysis confirmed the previously described base changes in codons 238 and 248 in the Louckes and Namalwa BL cell lines, respectively (Fig. 2B and 2C; Table 4) (16). An additional mutation in codon 234 was detected in the Raji cell line, with a transition from TAC to CAC resulting in an amino acid change of a tyrosine to histidine (Fig. 2B; Table 4) (10).

In the BL biopsy specimens, Burkitt had a transition from CGG to CAG in one allele of codon 248, resulting in a change of an arginine to glutamine (Fig. 3B; Table 4). The identical mutation was detected in the Namalwa cell line, and the two samples had identical abnormal migration patterns (Fig. 3A). Coo had a transition from AAC to GAC in one allele of codon 239, resulting in a change of an asparagine to an aspartic acid (Fig. 3C; Table 4). Blou had a transversion from TCC to ACC in codon 241 of one allele, with a subsequent amino acid change of serine to threonine (Fig. 3D; Table 4). All of the BL cell lines and samples had mutations within a specific region of exon 7, confirming the previous observation that p53 mutations are clustered (4).

SSCP analysis of exon 8 revealed abnormal banding in one of the AIDS-related lymphomas (ARL), Cas, compared with three normal samples in exon 8, Namalwa, Ber, and Com, and one known mutant control, CNE-1, which has a mutation in codon 280 (Fig. 4A). Sequencing detected a point mutation in one allele of codon 278 with a transition from CCT to CTT and a subsequent change in the amino acid from proline to leucine (Fig. 4B; Table 4).

In all cases, sequence analysis confirmed the normal and abnormal SSCP patterns. In reconstruction experiments, a mutation in p53 in 5% of the DNA produced an abnormal banding pattern by SSCP (11). Sequence analysis of mixtures of mutant and normal DNA in the same sample detected mutations with 20% mutant DNA (11). All samples with mutations were sequenced several times from different asymmetric amplifications to control for PCR-introduced mutation during amplification.

Although 98% of mutations in p53 have been detected within exons 5 to 8, mutations in exons 4 and 9 have also been

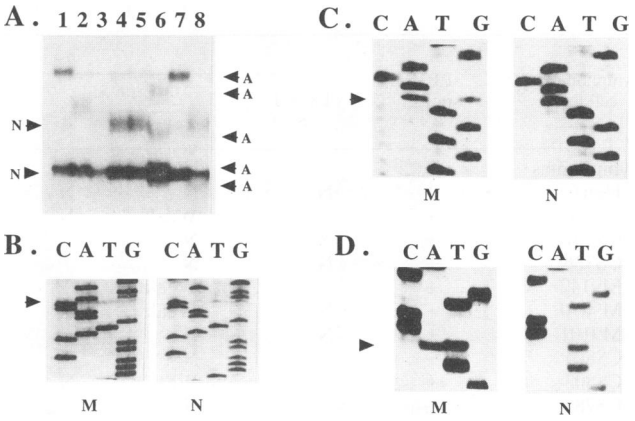

FIG. 3. Mutations in exon 7. (A) SSCP analysis of exon 7. The specimens presented are Namalwa (lane 1), Louckes (lane 2), Raji (lane 3), Mar (lane 4), Fa (lane 5), Blou (lane 6), Burkitt (lane 7), and Coo (lane 8). N, normal bands; A, abnormal bands. (B) Reading upward, sequence of mutation in Burkitt (M) in codon 248 (CGG to CAG) on one allele compared with normal sequence (N). (C) Reading upward, sequence of mutation in Coo (M) in codon 239 (AAC to GAC) on one allele compared with normal sequence (N). (D) Reading upward, sequence of mutation in Blou (M) in codon 241 (TCC to ACC) on one allele compared with normal sequence (N).

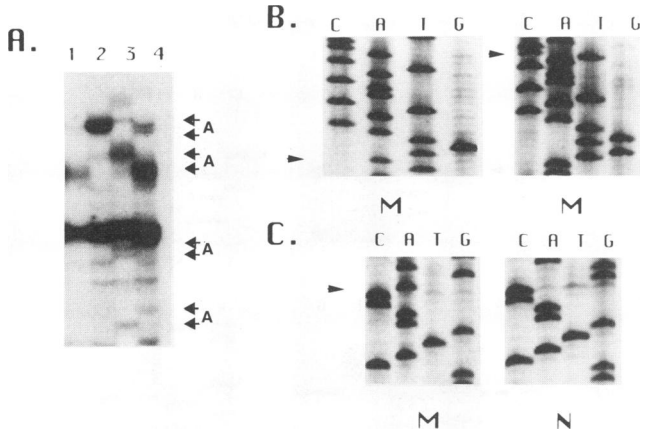

FIG. 2. Mutations in exon 7. (A) SSCP analysis of exon 7. The specimens presented are M9142 (lane 1), Namalwa (lane 2), Louckes (lane 3), and Raji (lane 4). Abnormal bands are marked by A. (B) Reading downward, sequence of mutation in Louckes (M) in codon 238 (TGT to TAT) and Raji (M) in codon 234 (TAC to CAC) on one allele. (C) Reading upward, sequence of mutation in Namalwa (M) in codon 248 (CGG to CAG) compared with normal sequence.

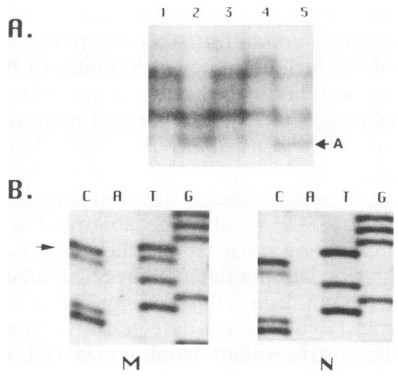

FIG. 4. Mutation in exon 8. (A) SSCP analysis of exon 8. The specimens presented are Namalwa (lane 1), CNE-1 (lane 2), Ber (lane 3), Cas (lane 4), and Com (lane 5). (B) Sequence of mutation in Cas (M) in codon 278 (CCT to CTT) on one allele compared with normal sequence (N).

TABLE 5. Allelic loss

Lymphoma or cell line	No. of alleles or fragments		Polymorphism in exon 4 by:		Mutation(s)[c] in exon:		
	Differential PCR[a]	pYNZ22[b]	Sequence	*Acc*II digestion	6	7	8
Lymphomas							
Hen	2	1	C+G	C+G			
M9143							
CS707	2	2	G	G			
CS336	2		C+G	C+G			
M9142							
M9070	2	2	G	G			
M9101	2	2	G	G			
CS804	2	1	G	G			
CS803							
CS788							
CS305							
Fa	2	2	C+G	C+G			
T580	2	1	C+G	C+G			
T594	2	2	G	G	A+G		
Ber	2	2	C+G	C+G			
Hou	2	1	G	G			
Jar	2	2	G	G			
Cas	2	2	G	G			C+T
Wal	2	2	C+G	C+G			
Mal	2	1	C+G	C+G			
Mar	2	1	C+G	C+G			
Com	2	1		G			
Del	2	1	G	G			
Dav	2	2	G	G			
Blou	2	2	G	G		T+A	
Coo	2	2	G	G		A+G	
Burkitt	2	2		G		G+A	
Hat	2	2	C+G	C+G			
Boi	2	1	C+G	C+G			
Cell lines							
Raji	2	2	C+G	C+G	G+A	T+C	
Louckes	2	1					
Namalwa	1	1					
P3HR1	1						

[a] Number of alleles detected with PCR by comparison with the reference gamma interferon gene.
[b] Number of fragments generated by PCR with the pYNZ22 primers.
[c] Mutations that support the presence of both alleles.

detected in a few sarcomas (47). Sequence analysis of exons 4 and 9 in 25 of the specimens analyzed in this study did not detect any mutations (Table 3).

Tables 3 and 4 summarize the p53 mutations detected in B-cell lymphomas and BL cell lines. Alterations in p53 were not detected in lymphomas arising under immunosuppression, while they occurred at high frequency in BL and BL cell lines. In all lymphoma samples, the changes were point mutations leading to amino acid changes, with retention of the wild-type allele.

Detection of allelic loss. In a recent study of bone and soft tissue sarcomas, allelic loss of p53 was detected with the retention of a wild-type p53 allele (47). This finding suggested that allelic loss at the p53 locus with a possible decrease in the p53 gene product could confer a growth advantage and contribute to oncogenesis. Therefore, to determine whether allelic loss had occurred in the lymphoma samples with wild-type p53, the specimens were analyzed by several methods, including differential PCR and sequence analysis of known polymorphic regions.

Differential PCR was performed with the gamma interferon gene as a reference gene in comparison with four BL cell lines previously reported to contain one or two p53 alleles. Comparison of the intensities of exons 5, 7, and 8 with that of the reference gene confirmed allelic loss in the BL cell lines, Namalwa and P3HR1, and the presence of two alleles in the Raji cell line (Table 5) (16). Differential PCR with the gamma interferon reference gene did not indicate a loss of p53 sequences in the PTL, ARL, or BL samples (Table 5).

To confirm this determination of the number of alleles, a polymorphism within exon 4 which encompasses an *Acc*II site was analyzed by sequencing and *Acc*II digestion. *Acc*II digestion identified two polymorphic alleles in 10 of 24 samples, while 14 of the samples were not polymorphic at this site (Table 5). However, 4 of the 14 nonpolymorphic samples (T594, Cas, Blou, and Coo) had mutations in the p53 coding sequence, with detection of both the mutant and normal sequences (Table 5). This result indicates the presence of both the wild-type and mutant alleles and confirmed the differential PCR analyses. The Raji cell line, which contains two alleles, was polymorphic at the *Acc*II site, and both mutant and normal sequences were detected at the site of mutation in exons 6 and 7 (Fig. 1 and 2; Table 5).

An additional approach analyzed a region on chromosome 17p, pYNZ22, which contains a variable number of sequence repeats (28). At least 10 alleles have been identified, and 86% of the population is polymorphic at this site. Amplification with the pYNZ22 oligonucleotide primers producing two fragments

indicates the presence of two alleles, while the amplification of a single fragment could indicate either a single allele or homozygosity (Table 5). Two alleles were detected in 14 samples, with detection of a single pYNZ22 allele in 9 samples (Table 5). In five of the nine samples, two p53 alleles were detected by *Acc*II digestion. As reference DNA was not available for these samples, this result could indicate a higher proportion of nonpolymorphic individuals in this sampling or could suggest a possibility of allelic loss at the pYNZ22 site with retention of two p53 alleles.

In summary, in the 24 clinical samples, 4 samples were not polymorphic at the *Acc*II site or the pYNZ22 locus; however, differential PCR with the gamma interferon gene did not indicate a loss of p53 sequences. Surprisingly, 3 of 8 samples of PTL and 5 of 10 ARLs which were polymorphic at the *Acc*II site or had not lost p53 sequences were homozygous at the pYNZ22 site.

DISCUSSION

In the human tumors studied, 98% of the p53 mutations have fallen in the highly conserved regions of the gene (exons 5 to 8) or in the binding site for simian virus 40 large T antigen, with single-point mutations conferring transforming properties to the p53 gene (27, 37). Several previous studies have detected p53 mutations in examples of BL and ARL, with detection of p53 mutations in both EBV-positive and EBV-negative samples (2, 4, 16, 19). The data presented here confirm that the p53 mutations in BL occur within a specific region in exon 7, regardless of the presence of EBV or geographic location (4, 19). The progression to neoplasia in BL seems to involve not only the c-*myc* translocation but also p53 inactivation (8). In the AIDS lymphomas analyzed here, a single EBV-positive specimen contained an altered p53 gene.

In many tumors previously studied, p53 mutations were detected in the absence of a normal allele (37, 45). However, in all of the samples analyzed in this study, a wild-type allele was retained. Similarly, in a series of EBV-negative non-Hodgkin's B-cell lymphomas, mutations were detected in approximately 20% of the samples, with retention of the wild-type allele in all but one (29). These results suggest that in lymphomas, the transdominant inactivation of wild-type p53 by mutant forms of p53 can contribute to the emergence of a malignant clone in vivo.

The detection of p53 mutations in all four BL cell lines raises the possibility that p53 mutations give a growth advantage in cell culture or arise during passage. Notably, the mutation detected in P3HR1 p53 is different from the mutation in the parent cell line Jijoye, and the P3HR1 and Raji cell lines cultured in our laboratory contained mutations in addition to those previously described (16). In this study, a deletion and/or mutation in the P1 and/or P2 region of P3HR1 and an additional mutation in codon 234 of exon 7 of Raji as well as the mutation in codon 213 were detected (10). These additional alterations further support the idea that mutations may develop during passage in cell culture. Previous analyses suggested that mutations in p53 predispose malignant cells to growth in nude mice or may develop during passage in nude mice, since 77% of NPC tumor xenografts contain p53 mutations, compared with 3% of primary NPC tumors; similar results have been found for prostate carcinoma cell lines (11, 12).

In contrast to the development of p53 mutations in BL or ARL, although clonal or oligoclonal PTL develops from an initial polyclonal proliferation, mutations in p53 were not detected in any of the PTL samples analyzed here. This suggests that p53 mutations do not contribute to the development of these tumors and the emergence of a dominant clone, similar to the absence of p53 mutation in NPC (11, 40). The fact that BL has p53 alterations while NPC and PTL do not, even though EBV is associated with all three diseases, suggests that EBV viral gene expression may influence the selection for p53 mutations. EBV gene expression is known to be different in BL compared with NPC and PTL. In BL, only the EBV nuclear antigen EBNA1 is thought to be expressed, while in NPC, EBNA1, the latent membrane proteins LMP1 and LMP2, and a gene encoded by the *Bam*HI A fragment are consistently expressed (15, 20, 26, 42, 49). Recent data also indicate that EBNA2 and LMP1 are expressed in lymphoproliferative disorders which develop during immunosuppression (21, 46, 50). As LMP1 expression has been shown to suppress apoptosis or programmed cell death in human B cells, it is possible that LMP1 or another EBV gene product expressed in the PTLs and NPC may indirectly suppress p53 function, thereby altering the negative growth-regulatory properties of p53 (22).

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