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p53 Mutation and Loss of Heterozygosity on Chromosomes 17 and 10 during Human Astrocytoma Progression[1]

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ABSTRACT

The human brain tumor, astrocytoma, typically progresses through three histopathologically defined stages with the passage of time: one premalignant stage, low-grade astrocytoma; and two malignant stages, anaplastic astrocytoma and glioblastoma multiforme. We correlated the results of a sequence analysis of the tumor suppressor gene, *p53*, and a restriction fragment length polymorphism analysis of chromosomes 17 and 10 in 45 patients with cerebral astrocytomas at different stages. To detect *p53* mutations in tumor DNA, we analyzed polymerase chain reaction products corresponding to every *p53*-coding exon for single-strand conformation polymorphisms and confirmed the mutations by sequencing. Loss of heterozygosity (LOH) was determined by Southern transfer analysis of somatic and tumor DNA from these same patients using polymorphic markers for various loci on chromosomes 10 and 17. *p53* mutations were found in 7 of 25 glioblastomas (28%), in 5 of 14 anaplastic astrocytomas (36%) but in 0 of 6 low-grade astrocytomas. *p53* mutations were found in 62% of patients with LOH on chromosome 17p. These results indicated that *p53* inactivation is a common genetic event in astrocytoma progression that may signal the transition from benign to malignant tumor stages. LOH on chromosome 10 was found in 61% of glioblastomas, in 23% of anaplastic astrocytomas, but in 0% of low-grade astrocytomas. LOH on chromosome 10 and *p53* mutation were found together only in patients with glioblastoma multiforme (22%), suggesting that these genetic changes may accumulate during astrocytoma progression.

INTRODUCTION

In cancer of the central nervous system, the affected cell is usually the astrocyte, and the resultant tumors (astrocytomas) typically progress through stages of increasing malignancy with the passage of time (1). One classification scheme based on histopathology recognizes three stages of astrocytomas: one benign stage, low-grade astrocytoma, and two malignant stages, anaplastic astrocytoma and glioblastoma multiforme (2). If untreated, astrocytomas progress to glioblastoma multiforme, an incurable, end stage of the disease.

Tumor progression is widely regarded as a multistep process that leads to increasingly deregulated cell growth. A major impediment to effective cancer treatment is incomplete knowledge of the molecular signals that underlie tumor progression. The tumor suppressor genes make up a class of genes whose products are believed to function normally to suppress cell proliferation. Mutations that inactivate tumor suppressor genes may define critical steps in tumor progression by removing the normal constraints to cell growth.

Studies using DNA markers that detect RFLPs[3] have shown that tumor suppressor genes important in the genesis or progression of human astrocytoma may be present on at least two different chromosomes. LOH for loci on chromosome 10 has been observed in many patients with glioblastoma multiforme (53–97%) but in very few patients with less malignant astrocytomas (0–15%) (3–5). On chromosome 17, LOH has been found in both glioblastoma and anaplastic astrocytoma patients with equal frequency (33–50%) (6–8). Taken together, these results suggested a model for tumor progression in the astrocyte lineage wherein tumor suppressor genes on chromosomes 17 and 10 may become inactivated in a preferred order.

Considerable experimental evidence supports the notion that the gene encoding the nuclear phosphoprotein, *p53*, is a tumor suppressor gene that is the target for the chromosome 17 deletions found so frequently in many human tumors, including malignant astrocytoma. The tumor suppressor gene hypothesis presupposes that both copies of the gene become inactivated in the cell before the malignant phenotype is expressed. Several laboratories have reported that, in tumors in which RFLP analysis showed loss of one copy of the *p53* gene by chromosomal deletion (LOH), sequence analysis showed that the remaining copy of p53 contained mutations that could have inactivated the gene product (9–12). The fact that similar results were obtained with several different tumor types indicated that *p53* inactivation is a key event in human oncogenesis and may define a common pathway in human tumor progression. By contrast, the loci on chromosome 10 that are lost in malignant astrocytomas have not been mapped with sufficient precision to identify a candidate region for a tumor suppressor gene (5).

We wanted to define further the roles of *p53* and the putative tumor suppressor gene on chromosome 10 in human astrocytoma progression. To accomplish this, we correlated the results of *p53* sequence analysis and RFLP analysis of loci on chromosomes 17 and 10 obtained from 45 patients with cerebral astrocytomas at various stages. To detect *p53* mutations in tumor DNA, we analyzed PCR products corresponding to every *p53*-coding exon for SSCPs and confirmed the mutations by cloning and sequencing those PCR products with altered electrophoretic mobilities. LOH was determined by Southern transfer analysis of somatic and tumor DNA from these same patients using polymorphic markers for various loci on chromosomes 10 and 17. *p53* mutations were found at both malignant astrocytoma stages (28% of glioblastomas and 36% of anaplastic astrocytomas) but not at the benign stage, low-grade astrocytoma. Among those patients with LOH on chromosome 17p, *p53* mutations were present in 62%. These results indicate that *p53* inactivation is a common genetic event in astrocytoma progression that may signal the transition from benign to malignant tumor stages. LOH on chromosome 10 and *p53* mutation were found together only in patients with end-stage glioblastoma multiforme (22%), suggesting that these genetic changes may accumulate during astrocytoma progression.

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[3] The abbreviations used are: RFLP, restriction fragment length polymorphism; LOH, loss of heterozygosity; PCR, polymerase chain reaction; SSCPs, single-strand conformation polymorphisms.

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MATERIALS AND METHODS

Human Tumor Samples. All tumor samples were removed surgically from the cerebral hemisphere prior to radiation or chemotherapy except in seven cases of tumors that recurred following treatment (A1, A3, U7, U16, U18, U24, U31). Histopathological grading of astrocytomas was based on the classification scheme of Burger *et al.* (2). All malignant astrocytoma samples were judged to contain >75% neoplastic cells, and low-grade astrocytoma samples were judged to contain >25%.

DNA Extraction and Southern Transfer Analysis. Extraction of genomic DNA from human tumor samples and from peripheral blood leukocytes, Southern transfer analysis, and radiolabeling of DNA markers was carried out as described previously (8). In some cases, DNA was prepared from cell lines derived from peripheral blood lymphocytes immortalized by Epstein-Barr virus. In cases in which tumor DNA showed LOH, quantitative densitometry scanning was carried out to measure any residual hybridization signal corresponding to the deleted allele due to nontumor cell DNA contamination. All cases of LOH described in "Results" showed >50% reduction in signal intensity in tumor DNA compared to leukocyte DNA.

DNA Markers. The following DNA markers for loci on the p arm of chromosome 17 were used in the RFLP analysis: YNZ22 (D17S30), YNH37.3 (D17S28), MCT35.1 (D17S31), BHP53 (P53), 10.5 (MYH2), and YNM67 (D17S29). The following DNA markers for loci on chromosome 10 were used: TB14.16 (D10S33), MHZ15 (D10S17), TBQ7 (D10S28), TB14.34 (D10S34), MEN203 (MEN2A), TB-IRBP-9 (RBP3), MCK2 (D10S15), TBQ16 (D10S30), TB10.163 (D10S22), TB10.171 (D10S19), CMM17.1 (D10S16), THH54 (D10S14), OS-2 (D10S20), EFD70.2 (D10S26), MCT122.1 (D10S36), EFD75 (D10S25). Original references for all markers used in this study were cited previously (5, 13).

Oligonucleotide Primers. Nine pairs of oligonucleotide primers (29-mers) were designed to span the 10 coding exons of the human *p53* gene (exons 2–11). Twenty bases in each primer were complementary to sequences flanking the intron/exon junctions according to intron sequence data published previously (14, 15). One primer pair (L1 and R1) spanned exons 2 and 3 as well as the intervening sequence (intron 2). The other primer pairs spanned only a single exon each. Each primer contained an artificially introduced restriction site at its 5′ end to facilitate cloning of the resultant PCR products into M13 for sequence analysis (*Hin*dIII on the left-hand primers and *Eco*RI on the right-hand primers). The primers were purified by electrophoresis through 12% polyacrylamide/7 M urea gels. The names and sequences of the primers were as follows:

L1, 5′-TATAAG-CTTCTGCCTTCCGGGTCACTGCC-3′;
R1, 5′-ACTGAATTCAACCCTTGTCCTTACCAGAA-3′;
L2, 5′-TATAAGCTTTTTTTCACCCATCTACAGTCC-3′;
R2, 5′-ACTGAATTCCTCAGGGCAACTGACCGTGC-3′;
L3, 5′-TATAAGCTTTTCCTCTTCCTGCAGTACTC-3′;
R3, 5′-ACTGAATTCGCCCCAGCTGCTCACCATCG-3′;
L4, 5′-TATAAGCTTCACTGATTGCTCTTAGGTCT-3′;
R4, 5′-ACTGAATTCAGTTGCAAACCAGACCTCAG-3′;
L5, 5′-TATAAGCTTGTGTTGTCTCCTAGGTTGGC-3′;
R5, 5′-ACTGAATTCCAAGTGGCTCCTGACCTGGA-3′;
L6, 5′-TATAAGCTTCCTATCCTGAGTAGTGGTAA-3′;
R6, 5′-ACTGAATTCGTCCTGCTTGCTTACCTCGC-3′;
L7, 5′-TATAAGCTTTTGCCTCTTTCCTAGCACTG-3′;
R7, 5′-ACTGAATTCCCCAAGACTTAGTACCTGAA-3′;
L8, 5′-TATAAGCTTCTCTGTTGCTGCAGATCCGT-3′;
R8, 5′-ACTGAATTCGCTGAGGTCACTCACCTGGA-3′;
L9, 5′-TATAAGCTTGCTTCTGTCTCCTACAGCCA-3′;
R9, 5′-ACTGAATTCGGAACAAGAAGTGGAGAATG-3′.

PCR-SSCP Analysis. For each patient, the nine primer pairs described above were used in individual PCRs to amplify *p53*-coding sequences using genomic DNA from tumor specimens and from peripheral blood leukocytes as a template. Each PCR mixture (50 μl) contained 0.5 μg genomic template DNA, 1 μM concentrations of each primer, 200 μM concentrations of each of the four deoxynucleotide triphosphates, 0.5 μCi [α-^{32}P]dCTP (3000 Ci/mmol), and 1.0 unit of Taq polymerase in buffer containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-Cl (pH 8.3), and 0.1% (w/v) gelatin. Thirty cycles of amplification were carried out in an automatic DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, CT) at 94, 53, and 72°C for 1, 2, and 2 min, respectively. Reaction products were analyzed on ethidium-stained 3% NuSieve 3:1 agarose gels (FMC Bioproducts, Rockland, ME) to ascertain that single, appropriately sized PCR products had been obtained. To detect SSCPs, the PCR mixtures were diluted, denatured, and electrophoresed through polyacrylamide gels under nondenaturing conditions as described by Orita *et al.* (16). We used 6% polyacrylamide gels, containing 90 mM Tris-borate (pH 8.3), 4 mM EDTA, and 10% glycerol, and electrophoresed them at 10 W for 15–18 h or at 30 W for 7 h with a cooling fan directed at the gel plate surface. The gels were dried and exposed to X-ray film for 1–7 days to generate autoradiograms.

Cloning and Sequencing DNA Fragments Recovered from SSCP Gels. Single-stranded tumor DNA fragments with electrophoretic mobilities different from comigrating leukocyte DNA fragments were purified as described by Hata *et al.* (17). Individual bands were excised from the dried gel and incubated in 100 μl H_2O at 37°C for 1 h to elute the single-stranded DNA. An aliquot (10 μl) of the gel eluate was subjected to a second PCR (100 μl reaction volume), and the resultant secondary PCR products were purified by phenol/chloroform extraction and ethanol precipitation. These double-stranded DNA fragments were digested sequentially with restriction enzymes *Hin*dIII and *Eco*RI and spin-dialyzed through Centricon-100 columns (Amicon; Danvers, MA). The PCR products were cloned into complementary M13 vectors, mp18 and mp19, and multiple independent clones were isolated. Single-stranded DNA was prepared from the M13 clones, and the inserts were sequenced by the dideoxy chain termination method (18) using Sequenase (United States Biochemical, Cleveland, OH). To control for sequencing artifacts introduced during PCR or M13 cloning, mutations were scored as positive only when they were observed in at least five independent clones.

RESULTS

***p53* Mutation Is a Frequent Genetic Change in Human Malignant Astrocytoma.** We utilized SSCP analysis to search for *p53*-coding sequence mutations in 45 patients with cerebral astrocytomas at the three different histopathological stages (25 glioblastomas, 14 anaplastic astrocytomas, and 6 low-grade astrocytomas). Significant electrophoretic mobility shifts were detected in 7 of the 25 glioblastomas (28%), in 5 of the 14 anaplastic astrocytomas (36%), but in none of the 6 low-grade astrocytomas. Fig. 1 shows examples of SSCP analysis of exons 7 and 8. Faint bands were often seen in the upper portions of the gels. These bands have been described before and represent different conformers of the same sequence (16).

Sequence analysis confirmed that mutations were present in tumor DNA from all 12 patients who showed significant SSCP shifts (Table 1). All of the mutations were located within four of the 10 *p53*-coding exons (exons 5, 6, 7, and 8). Most of the mutations were single-base changes that encoded single amino acid substitutions in the *p53* protein or premature translation terminations. In one case, a 14-base insertion was present that could have led to a frameshift during translation. Examples of the sequence analysis are shown in Fig. 2, *A* and *C*. In some cases, we isolated and sequenced tumor DNA from gel bands that were not shifted compared to leukocyte DNA. No mutation was found in any tumor DNA fragment with normal electrophoretic mobility. Thus, *p53* mutations were prevalent in malignant astrocytomas (31%), but no SSCP shifts were observed in the 6 patients with premalignant low-grade astrocytomas. Because of the small sample size, however, the observed differ-

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ences in *p53* mutation frequency between benign and malignant astrocytomas did not reach statistical significance when analyzed by the χ^2 contingency test ($P = 0.10$).

Loss of Heterozygosity on Chromosome 17p and *p53* Mutation Occur Concomitantly in Many Patients with Malignant Astrocytoma. In previous RFLP studies that we reported, 40% of malignant astrocytoma patients showed LOH for loci on the p arm of chromosome 17 (p11.2-pter), a region containing the *p53* gene (8). To determine whether *p53* was the target for these 17p deletions, we correlated the *p53* sequence data with the RFLP data (Table 2). In a group of 13 patients with malignant astrocytomas (8 glioblastomas and 5 anaplastic astrocytomas) in whom RFLP analysis showed LOH for loci on 17p that included the *p53* locus itself (band 13.1), 8 patients (62%) showed *p53* gene mutations (U4, U8, U15, U16, U21, A4, A14, A15). In five of the patients with LOH on chromosome 17p (U7, U14, U33, A1, A3), we did not detect a *p53* mutation by PCR-SSCP analysis. Fig. 2 shows examples of *p53* mutations and LOH on chromosome 17p. These findings indicated that p53 function is frequently lost in malignant astrocytomas since, in the majority of cases in which RFLP analysis showed that one copy of the p53 gene had been lost from the tumor cell by chromosomal deletion (LOH), the sequence analysis showed that the remaining copy of *p53* contained an inactivating mutation.

Among the patients in whom RFLP studies showed that both copies of chromosome 17p were present (no LOH found with informative markers for loci on 17p), the SSCP and sequence analysis showed a *p53* mutation in 3 cases (U23, A11, A17). In these 3 patients, both shifted and nonshifted bands were excised from the SSCP gels, and the DNA fragments were sequenced. The sequence analysis revealed that mutant and wild-type *p53* alleles were present in the same tumor samples, indicating that the tumor cells were most likely heterozygous.

Table 1 *p53 gene mutations in human astrocytomas*

Patient	Tumor type[a]	Exon	Codon	Sequence change	Amino acid substitution
U4	GBM	8	282	CGG → TGG	Arg → Trp
U8	GBM	7	234	TAC → TAA	Tyr → chain termination
U15	GBM	6	195	ATC → ACC	Ile → Thr
U16	GBM	5	131	AAC → deleted	Asn → deleted
U21	GBM	6	195	ATC → ACC	Ile → Thr
U23	GBM	8	282	CGG → TGG	Arg → Trp
U32	GBM	5	163	TAC → TGC	Tyr → Cys
A4	AA	7	248	CGG → TGG	Arg → Trp
A14	AA	6	213	CGA → TGA	Arg → chain termination
A15	AA	8	273	CGT → TGT	Arg → Cys
A17	AA	5	181	CGC → TGC	Arg → Cys
A11	AA	5		14 base insertion between Cys-141 and Pro-142 5′-CCTGTGCAGCTGTC-3′	

[a] AA, anaplastic astrocytoma; GBM, glioblastoma multiforme.

Loss of Heterozygosity on Chromosome 10 and *p53* Mutation Occur Together in Patients with Advanced Disease. We utilized RFLP analysis to determine the genotype with respect to chromosome 10 in the 45 patients in whom *p53* sequence analysis had been carried out (Table 2). We reported some of these RFLP data previously (5). Informative markers spanned both p and q arms of chromosome 10 in 23 glioblastoma patients and 13 anaplastic astrocytoma patients. LOH on chromosome 10 was found in 14 glioblastomas (61%) and in 3 anaplastic astrocytomas (23%). When analyzed by the χ^2 contingency test, these differences in LOH frequency were statistically significant ($P = 0.025$). We observed both LOH on chromosome 10 and *p53* mutations together in 5 of the glioblastomas (22%) but in none of the anaplastic astrocytomas. Neither *p53* mutation nor LOH on chromosome 10 was observed in any low-grade astrocytoma. The fact that *p53* mutation and LOH on chromosome 10 were found together only in glioblastoma multiforme suggested that the cumulative effects of both genetic changes may lead to end-stage disease in certain patients.

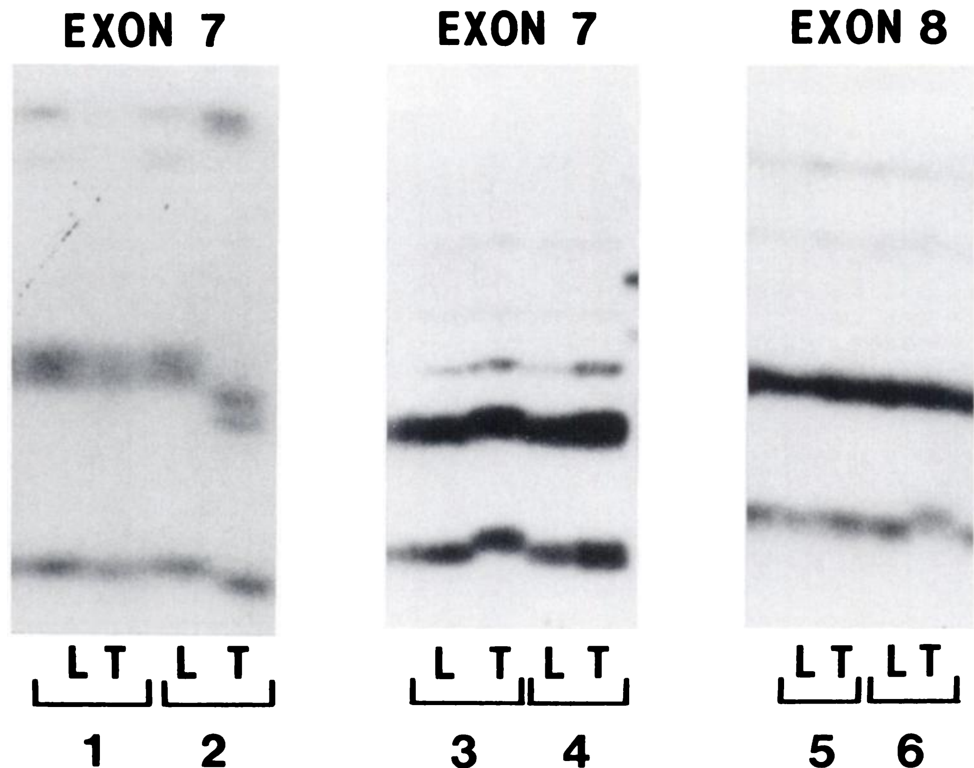

Fig. 1. Single-strand conformation polymorphism analysis of *p53*-coding sequences in 6 malignant astrocytoma patients. ^{32}P-labeled PCR products corresponding to *p53* exons 7 and 8 were synthesized using genomic DNA from peripheral blood leukocytes (L) and tumor (T) from each patient as a template. The PCR products were denatured, and the single-stranded DNA fragments were separated by electrophoresis through 6% polyacrylamide gels. The gels were dried and exposed to X-ray film for 6 days to generate the autoradiograms shown. Electrophoretic mobility shifts were present in tumor DNA fragments from patients 2 (A4), 3 (U8), and 6 (A15), but no shifts were present in patients 1 (A14), 4 (U7), or 5 (A8).

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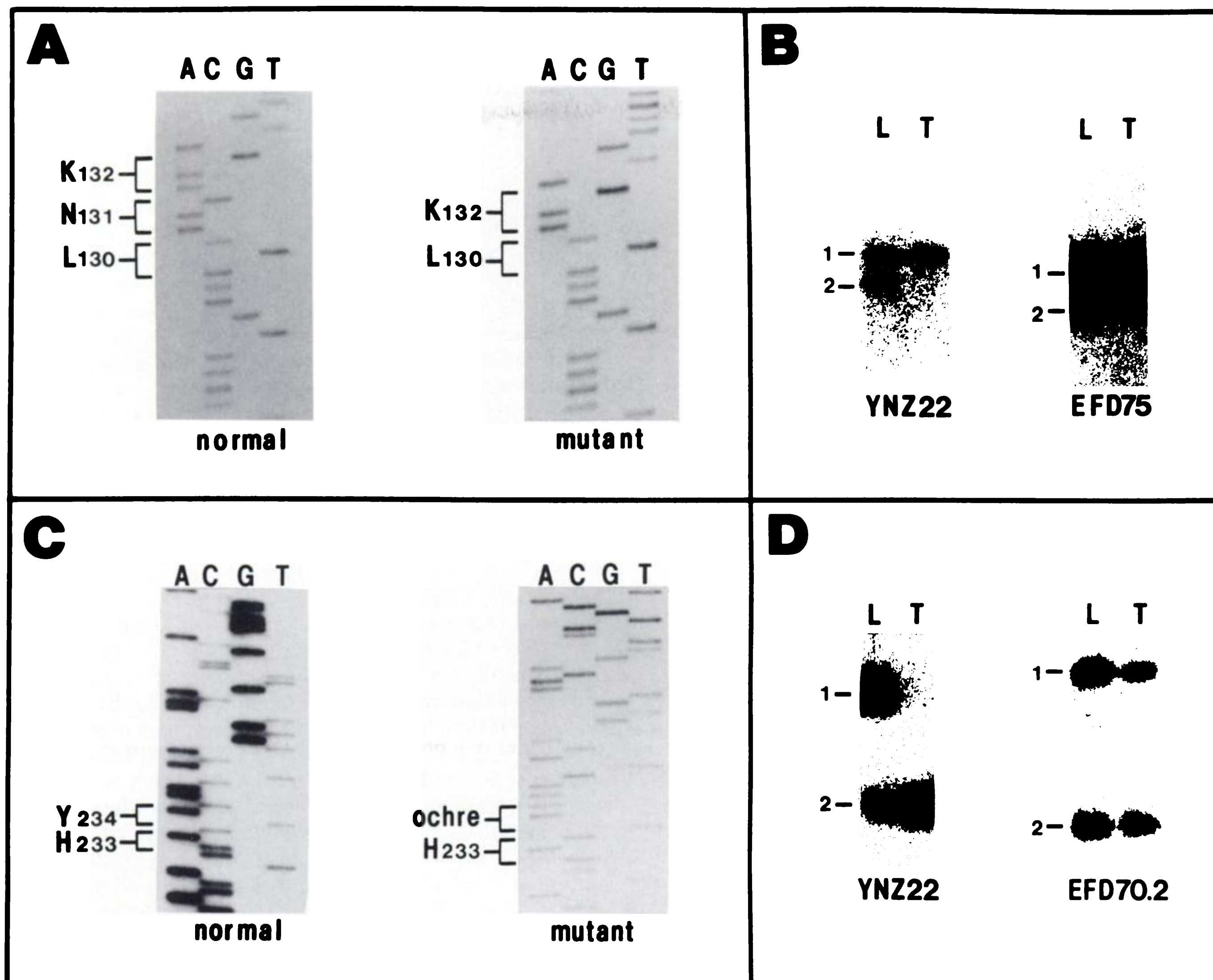

Fig. 2. Correlation of sequence analysis of the *p53* gene and RFLP analysis of chromosomes 17 and 10 in two patients with glioblastoma multiforme. Patient U16 showed a deletion of codon 131 (asparagine) in exon 5 of the *p53* gene (*A*) and loss of heterozygosity for the loci defined by markers YNZ22 on chromosome 17p and EFD75 on chromosome 10q (*B*). In patient U8, a single-base transversion in codon 234 of exon 7 converted the normal sequence for tyrosine (TAC) to a chain termination signal (TAA, ochre mutation) (*C*). Patient U8 showed loss of heterozygosity with chromosome 17p marker, YNZ22, but no loss with chromosome 10q marker, EFD70.2 (*D*). In the sequence analysis (*A* and *C*), the mutant sequence from tumor DNA is shown in comparison to the normal sequence from another individual. In the RFLP analysis (*B* and *D*), the results of Southern transfer analysis of leukocyte DNA (L) and tumor DNA (T) are shown. *1* and *2*, alleles with the larger and smaller restriction fragment lengths, respectively. The restriction enzymes used were *Taq*I (markers YNZ22 and EFD75) and *Pvu*II (EFD70.2). *L*, leucine; *N*, asparagine; *K*, lysine; *H*, histidine; *Y*, tyrosine.

DISCUSSION

We have shown that mutations in the protein-coding region of the *p53* gene occur frequently in human malignant astrocytomas. No mutations were found in any patient with premalignant low-grade astrocytoma, suggesting that *p53* mutation may signal a transition from benign to malignant tumor stages during astrocytoma progression. Correlation of *p53* sequence data with chromosome 10 RFLP data showed that *p53* mutation and LOH on chromosome 10 occurred together in certain cases of advanced disease.

Work in other laboratories has shown that *p53* gene mutations occur commonly in many human cancers including astrocytoma (9–12). Our results support the hypothesis that *p53* is a tumor suppressor gene involved in astrocytoma tumorigenesis, particularly since *p53* mutations were found so frequently in patients in whom RFLP studies showed LOH on chromosome 17p. In these patients, one copy of *p53* had been removed from the tumor cell by chromosome deletion and the other copy inactivated by mutation. In 3 tumors, the presence of mutant and wild-type *p53* alleles indicated that the tumor cells were heterozygous with respect to *p53*. In these heterozygous cases, the normal activity of *p53* in the cell may be suboptimal through a mechanism whereby the normal and mutant gene products complex to form a dysfunctional heterodimer (19).

The location of mutations that we found in astrocytomas (exons 5, 6, 7, and 8) is consistent with *p53* mutations in other types of human tumors (reviewed in Ref. 20). These exons contain sequences that are highly conserved in evolution and probably encode functionally important regions of the *p53* protein.

The role of *p53* in astrocytoma progression may be similar to that in colorectal cancer progression wherein *p53* mutation is usually a late event signaling the transition from adenoma to carcinoma rather than an early event in tumor initiation or

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Table 2 *Correlation of p53 mutation with RFLP analysis in 45 astrocytoma patients*

Patient	p53 mutation	LOH CHR.17P	LOH CHR.10	No. of informative chromosome markers 17P	10P	10Q
Glioblastoma multiforme ($n = 25$)						
U4	Y[a]	Y	Y	4	5	6
U6	N	N	N	5	2	7
U7	N	Y	N	6	1	6
U8	Y	Y	N	4	2	7
U9	N	N	Y	3	1	1
U10	N	N	Y	2	4	4
U11	N	N	N	3	1	6
U13	N	N	Y	4	3	7
U14	N	Y	N	4	2	3
U15	Y	Y	Y	3	5	3
U16	Y	Y	Y	2	3	5
U18	N	N	Y	3	3	2
U20	N	N	Y	1	3	6
U21	Y	Y	Y	3	3	4
U22	N	N	Y	1	4	3
U23	Y	N	Y	1	2	4
U24	N	N	N	2	1	6
U27	N	N	Y	1	2	2
U28	N	N	N	1	1	5
U31	N	nd	Y	0	2	2
U32	Y	nd	N	0	3	2
U33	N	Y	Y	2	3	1
U35	N	nd	N	0	2	5
U25	N	N	N	1	1	0
U36	N	N	N	1	2	0
Anaplastic astrocytoma ($n = 14$)						
A1	N	Y	N	4	3	3
A2	N	N	N	5	1	5
A3	N	Y	N	4	2	4
A4	Y	Y	N	3	3	4
A6	N	N	N	3	1	4
A8	N	N	N	5	1	4
A10	N	N	Y	4	4	4
A11	Y	N	nd	4	0	0
A13	N	N	Y	4	1	3
A14	Y	Y	N	2	3	3
A15	Y	Y	N	3	2	2
A16	N	nd	N	0	2	2
A17	Y	N	N	5	3	4
A18	N	N	Y	1	3	2
Low-grade astrocytoma ($n = 6$)						
L1	N	N	N	1	2	2
L2	N	N	N	1	2	0
L3	N	nd	N	0	1	2
L4	N	N	N	1	2	2
L5	N	N	N	1	2	0
L6	N	nd	N	0	2	0

[a] Y, yes; N, no; nd, not determined.

promotion (21). Two of our observations supported this idea: (*a*) no *p53* mutations were found in patients with low-grade astrocytoma, a premalignant stage of the disease, although the number of benign tumors that we studied was small; (*b*) *p53* mutations were found in both malignant stages of astrocytomas with similar frequencies (36% of anaplastic astrocytomas and 28% of glioblastomas).

We did not find a *p53* mutation in every malignant astrocytoma examined. In fact, five patients with LOH for loci on chromosome 17p that included the *p53* locus itself showed no SSCP gel shifts in tumor DNA. It is possible that *p53* mutations were present in these tumors but remained undetected with the techniques used in this study. Specifically, certain coding sequence mutations may not have imparted sufficient conformational change to the single-stranded DNA molecules to permit detection by SSCP electrophoresis. Point mutations at intron-exon boundaries that were masked by the normal primer sequence could have gone undetected as well. Furthermore, mutations outside the coding exons could have inactivated the *p53* gene product by altering gene expression or splicing. An alternative explanation for the absence of *p53* mutations in those patients with LOH on chromosome 17 is the possibility of a second tumor suppressor gene on chromosome 17 distinct from p53.

The existence of a tumor suppressor gene on chromosome 10 that is important in astrocytoma tumorigenesis is based on cytogenetic and molecular genetic studies showing that sequences from chromosome 10 are lost frequently in glioblastoma multiforme (53–97%) (3, 4, 22). In fact, patients with LOH for loci on chromosome 10 have typically lost one entire copy of the chromosome in tumor cells (8). The absence of a small chromosome 10 deletion has impeded progress toward identifying the putative tumor suppressor gene. Lacking a candidate gene, we treated LOH on chromosome 10 in this study as a specific genetic event occurring during astrocytoma progression and correlated that event with mutation in the known tumor suppressor gene, *p53*. This correlation revealed that *p53* mutation and LOH on chromosome 10 occurred together only in glioblastomas, suggesting that sequential inactivation of *p53* and the putative tumor suppressor gene on chromosome 10 may cause tumors to progress to end stage. However, if *p53* mutation and LOH on chromosome 10 define a preferred order of genetic events in astrocytoma progression, then one would expect to find both genetic changes together in a large proportion of malignant astrocytomas, especially in the end-stage glioblastomas. In fact, only 22% of glioblastomas showed both changes. This small percentage may reflect inherent limitations of PCR-SSCP analysis in detecting *p53* mutations and of RFLP analysis in detecting small chromosome 10 deletions. Alternatively, astrocytoma progression may take place along several different pathways in which *p53* mutation and LOH on chromosome 10 occur as independent genetic events rather than sequential steps. Ultimate proof that *p53* mutation and LOH on chromosome 10 occur in a preferred order during astrocytoma progression will require analysis of sequential tumor samples from individual patients with progressive disease.

Tumors that share a common histopathological identity often have different molecular genetic profiles. In the group of malignant astrocytomas that we studied, for example, tumors with various combinations of LOH on chromosome 10 in association with wild-type and mutant *p53* sequence were identified. Although classification of tumors into three histopathological stages (low-grade astrocytoma, anaplastic astrocytoma, and glioblastoma multiforme) does correlate with clinical prognosis, patients with tumors within each stage differ somewhat with respect to survival and response to treatment (2). For this reason, it will be important to correlate molecular genetic changes such as *p53* mutation and LOH on chromosomes 10 and 17 with various clinical features in order to delineate subgroups of patients within current histopathological stages. Classification of astrocytomas based on genetic tumor markers may enable oncologists to predict better a patient's response to treatment and perhaps to design improved treatment strategies in the future.

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