p53 Mutations in Human Malignant Gliomas: Comparison of Loss of Heterozygosity with Mutation Frequency

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ABSTRACT

Mutations in the p53 gene were analyzed in 40 gliomas using the single strand conformation polymorphism assay together with restriction fragment length polymorphism analysis to assess loss of heterozygosity for 17p alleles in the same tumors. Mutations occurred in 40% of the gliomas and were found in exons 4–8 of the p53 gene. G:C to T:A transversions, which occur in high frequency in some lung (>50%), liver (>80%), breast (30%), and esophageal cancers (25%), were noted in >25% of the gliomas studied here. These transversions were clustered in exon 5 from codons 156 to 168, a region of the p53 gene not previously associated with a high frequency of mutation, and may represent a new hot spot for mutations in certain cancers. The majority of gliomas (27 of 38) analyzed here retained both 17p alleles. The frequency of p53 mutations was 37% in this group of tumors and increased to 64% in tumors with one 17p allele. Allelic loss for chromosome 17p occurred in 4 of 11 gliomas independently of mutations in the p53 gene. Absence of p53 mutations in 36% of the tumors with one 17p allele suggests that a tumor suppressor gene other than p53 may be located on chromosome 17p and involved in progression to malignancy of some gliomas.

INTRODUCTION

Cancers are thought to develop as a result of several sequential genetic events, occurring stepwise over time, which may involve activation of oncogenes or inactivation of tumor suppressor genes (1, 2). Tumor suppressor genes are now known to play an important role in the development of a wide variety of human cancers (3–5). The two most studied tumor suppressor genes are the Rb gene, located on chromosome 13 (6), and the p53 gene, located on chromosome 17 (7, 8).

Tumors of the central nervous system account for approximately 5% of all human cancers (9). Sixty % of all adult primary brain tumors are gliomas, of which astrocytomas are the most common (10). These tumors are progressive, tend to recur following treatment, and normally are fatal. The progression in histological grade of the astrocytic tumors from so-called “benign” astrocytomas through anaplastic astrocytoma to glioblastoma multiforme is reflected clinically by decreasing survival.

RFLP analysis of gliomas has shown nonrandom loss of heterozygosity for markers on chromosomes 9, 10, 13, 17, and 22 (11–17). Recent studies have reported the involvement of the p53 gene in some gliomas. One study by Nigro et al. (17) identified mutations in the p53 gene in 4 of 5 gliomas in which all had lost one chromosome 17p allele. Venter et al. (18) demonstrated loss of heterozygosity for the Rb locus in 4 of 9 gliomas in their study and found a partial deletion of the Rb gene in 1 of 4 tumors.

The aim of this study was to determine the genetic events in the development of malignant gliomas. To examine in greater detail the role that tumor suppressor genes play in the development of malignant gliomas, we analyzed a panel of 40 tumors using SSCP analysis to detect mutations in the p53 gene and RFLP analysis to assess loss of heterozygosity for chromosome 17p (p53) or structural alterations of the 13q (Rb) locus. In particular, we wanted to analyze the frequency of p53 mutations with respect to loss of alleles on chromosome 17. In colorectal cancers, there appears to be a mutation in one p53 allele accompanied by a near simultaneous loss of the remaining 17p allele (19). In addition, we examined the Rb gene for deletions to see whether inactivation of this gene contributed to the development of some gliomas. Here, we report that mutations of the p53 gene occurred in 40% of gliomas, whereas the Rb locus remained in the germline configuration in all of the tumors examined. Unlike colorectal tumors, p53 mutations occurred in a large number of gliomas that retained both 17p alleles. Moreover, the spectrum of mutations differed from previous reports for brain tumors in that >25% were G:C to T:A transversions. The implications of these findings for the development of malignant gliomas are discussed.

MATERIALS AND METHODS

Human Tumor Samples. Primary tumor samples from 27 patients and recurrent tumors from 13 patients (see Table 2 for clinical details) were obtained at surgery. Pieces of tumor tissue were snap frozen in liquid nitrogen and stored at −70°C until extracted for DNA. Histopathological grading of brain tumors was based on the classification scheme of Burger et al. (20).

DNA Extraction. Partially thawed tumor pieces were Dounce homogenized in DNA extraction buffer containing 10 mM Tris+Cl (pH 7.5), 150 mM NaCl, and 2 mM EDTA (pH 8.0). The cell suspension was digested with protease K (200 µg/ml) and SDS (0.5%) overnight at 37°C, and the genomic DNA was extracted by salting out with 6 M NaCl as described (21).

Oligonucleotide Primers. The oligonucleotides used for PCR amplification were synthesized with an Applied Biosystems synthesizer. The sequences of the p53 primers used for exons 5–9 were previously described (22). In addition, the following primers were used for exon 4: P4-4, 5'-TTC-ACC-CAT-CTA-CAG-TCC-3' (nucleotides 602-619); P4-3, 5'-CTC-AGG-GCA-CTG-CTC-3' (nucleotides 892-909).

SSCP Analysis of p53 Mutations. Exons 4–9 of the p53 gene were amplified by PCR from 100 ng of genomic DNA. SSCP analysis was performed using the primers and amplification conditions described previously for exons 5–9 (22). Thirty cycles were used for amplification consisting of 30 s at 94°C, 30 s annealing (56°C for exon 4; 63°C for exons 5–7; 58°C for exons 8 and 9), and 1 min at 72°C for extension. The denaturation step was modified from that previously described, as follows: samples (2.5 of 10 µl PCR reaction were diluted in 9 µl sequencing stop solution, and then 1.5 µl of 0.08 n NaOH were added) were denatured 10 min at 95°C and then chilled to 4°C using the automated DNA thermal cycler (Perkin-Elmer Cetus) prior to loading (4 µl) onto 8% (6% for exon 4) acrylamide–TBE gels containing 10% glycerol. Gels were electrophoresed at room temperature for 18–20 h at 8–10 W. The gels were fixed in 10% acetic acid, dried under vacuum, and stained with ethidium bromide.
and exposed to X-ray film with intensifying screens at $-70^\circ C$ for 4–72 h. The level of sensitivity for detecting p53 mutations in artificial mixtures of genomic DNAs using the PCR-SSCP assay is 10% under our experimental conditions (data not shown).

Direct DNA Sequencing of p53 PCR Products. Genomic DNA (1 µg) was amplified by PCR, independent of the PCR amplification used for the SSCP assay, using 30 cycles, with each step consisting of 1 min. The primers and conditions used were as described above. DNA fragments from one-half of the PCR reaction mixture (50 µl) were isolated following electrophoresis from 2.1% low-melting-point agarose gels (Bethesda Research Laboratories). Sequencing reactions were performed using the Sequenase sequencing kit (United States Biochemical) according to the manufacturer's specifications. Both strands were sequenced for each DNA segment analyzed. Genomic DNA from control samples containing known wild-type and mutant p53 alleles was amplified by PCR, and the DNA fragments were sequenced in parallel when confirming mutations in samples that were positive in the SSCP assay.

Southern Blot Analysis of p53 and Rb Alleles. To assess loss of heterozygosity for p53 loci, genomic DNA (10 µg) was digested to completion with HindIII and separated by electrophoresis through 1.2% agarose gels. DNA fragments were transferred to nylon membranes (Biotrans; ICN Biomedicals, Inc.) and probed sequentially with two highly polymorphic markers for loci on chromosome 17, pYNZ22.1 (23) and p144D6 (24). To determine whether deletions of the Rb gene had occurred, genomic DNA (10 µg) was digested to completion with HindIII and separated on 0.7% agarose gels. Filters were probed sequentially with the 0.9- and 3.8-kilobase complementary DNA Rb probes derived from p4.7R, kindly provided by R. Weinberg (6). DNA probes (100 ng) were radiolabeled by random priming (Boehringer) and added to prehybridized filters in hybridization solution containing 50% formamide, 4x SSC, 5x Denhardt's solution, 0.1% SDS, 50 µM sodium phosphate (pH 7.2), and 250 µg/ml denatured salmon sperm DNA at 42°C (1x SSC = 150 mM NaCl-15 mM sodium citrate at pH 7.0). Filters were washed three times (200 rpm for 5 min) at room temperature in 2x SSC-0.1% SDS and twice at 60°C for 15 min in 0.1x SSC-0.1% SDS. Filters were exposed to X-ray film with intensifying screens at $-70^\circ C$ for autoradiography.

RESULTS

Frequency of p53 Mutations in Brain Tumors. A total of 40 primary and recurrent brain tumors were analyzed by SSCP to screen for point mutations in the p53 gene. The results of this survey are summarized in Table 1. Representative SSCP patterns for exons 4–8 of the p53 gene are shown in Fig. 1. The overall frequency of p53 mutations was 40% (16 of 40). The highest-grade tumor, glioblastoma multiforme, comprised the majority of tumors examined (33 of 40). The frequency of p53 mutations in 23 primary tumors (10 of 23) was similar to that observed in recurrent tumors (3 of 10) obtained from patients who had received either radiation treatment or chemotherapy or both. In two patients (cases 15 and 155), we obtained tumor samples before and after treatment. In both instances, the primary tumor already contained mutations in the p53 gene prior to treatment. These data, taken together, suggest that radiation therapy and/or chemotherapy does not appear to influence the mutation frequency of the p53 gene. In addition, 3 anaplastic astrocytomas and 4 gliosarcomas, representing different histopathological grades of gliomas, were analyzed for p53 mutations. Although the sample size was small, there was no difference in the frequency of p53 mutations associated with different grades of malignancy.

Identification and Location of p53 Mutations in Brain Tumors. To confirm the p53 mutations detected by SSCP, the relevant exons were amplified by PCR and directly sequenced. Table 2 summarizes the results, and representative DNA sequences are shown in Fig. 2. In 14 of 16 cases, a single nucleotide change resulted in missense mutations. In one case, an insertion of one nucleotide caused a frameshift mutation. One chain-terminating mutation was also observed.

Five regions of the p53 gene are highly conserved between species (25). The majority of p53 mutations identified occur in these regions (26). Historically, the classification scheme of Burger et al. (20) was used to categorize these mutations. In this study, p53 mutations were classified according to the classification scheme of Burger et al. (20).
### Table 2
Loss of 17p alleles and p53 mutations in patients with glioblastomas

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* NF, patient diagnosed with von Recklinghausen neurofibromatosis; R, recurrent tumor.

† GBM, glioblastoma multiforme; AA, anaplastic astrocytoma; GS, gliosarcoma.

* Patients received radiation therapy, chemotherapy, or a combination of both treatments.

Loss of heterozygosity was confirmed by DNA sequencing: * hemizygous patients had barely detectable or no wild-type sequence at the site of mutation; † heterozygous patients had wild-type and mutated sequences of equal intensity.

* ND, not determined.

* A frameshift mutation was caused by an insertion of an A: GTG to AGTG.

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**Fig. 2.** DNA sequences of p53 mutations detected in PCR-amplified fragments. Representative DNA sequences of p53 mutations are shown for codons 161, 248, and 275. Two cases were heterozygous (#) for the p53 gene (cases 150 and 81), with both wild-type and mutated sequences present, and one hemizygous case (*) having a barely detectable wild-type sequence (see legend to Table 2). Arrows, the first and last nucleotide of the DNA sequence displayed at the right. The nucleotide change from the wild-type sequence resulting in an amino acid substitution follows the slash bar.
within these regions in exons 4–9, spanning codons 110–307 of the *p53* gene (4, 5). The majority of mutations that we identified were localized in exon 5 (50%, 8 of 16) or exon 7 (24%, 4 of 16). Approximately half of these mutations (7 of 15) occurred at sites of CpG dinucleotides (codons 175, 213, and 248), which are recognized hot spots for mutations within the *p53* gene in several types of human cancer (4, 5). Two mutations occurred in exon 8, and one each was found in exon 4 and exon 6. No mutations were observed in exon 9, and no patient had more than one *p53* mutation. This is in contrast to our finding in B-cell chronic lymphocytic leukemia, in which 5 of 80 cases screened to date have contained mutations in two different exons of the *p53* gene.

The spectrum of mutations observed in the *p53* gene differs with respect to cancers of different tissue types (4, 5). In the 15 brain tumors that contained mutations of the *p53* gene, G:C to A:T transitions were the most frequent, occurring in 73% of the cases (11 of 15). More than half of these transitions occurred at CpG dinucleotides. These data are in agreement with the spectrum of mutations recently reported by Hollstein et al. (4) for brain tumors.

As noted above, a large fraction of the mutations of *p53* in this study of brain tumors occurred in exon 5. Four of 8 of these mutations were clustered within codons 156–168, an area of the *p53* gene corresponding to a region of unknown biological function which lies outside of domains II and III (25). One of the four mutations was a G:C to A:T transition. However, there were three exceptions where G:C to T:A transversions were observed. Transversions of this type have been noted in lung cancer (26), hepatocellular carcinomas (27, 28), and esophageal tumors (29), but they have not previously been noted in brain tumors.

**Polymorphisms Detected by SSCP Analysis.** The SSCP method has proved to be a rapid and extremely sensitive method for detecting changes of one nucleotide within a DNA sequence (30). In our panel of 40 brain tumors, two nucleotide sequence polymorphisms were easily detected. One silent mutation in codon 213, exon 6, was identified, for a frequency of 2.5%. This polymorphism was noted earlier by us (22) and has recently been documented in the Italian population by Serra et al. (31). A second polymorphism was detected in exon 4, codon 72 (32).

The detection of this polymorphism by SSCP analysis was recently reported by Murakami et al. (33). In a screen of 28 cases, 6 were found to be positive, for a frequency of 21%.

**RFLP Analysis of Brain Tumors for Loss of 17p and 13q Alleles.** Loss of heterozygosity for the chromosome region 17p in astrocytomas and glioblastomas has been well documented by RFLP analysis (12, 14–17, 34). To determine the frequency of loss of 17p alleles in our panel of brain tumors, we used Southern blot analysis to assess the number of 17p loci using two highly informative polymorphic probes which map near to the *p53* locus (23, 24). Although paired samples of normal blood and tumor tissue were not available from these patients, we could assess constitutive loss within 95% confidence limits when both of the informative DNA probes showed a loss of one 17p allele, as previously reported (17). The results of this analysis are presented in Table 2. Fig. 3 shows the pattern of 17p alleles from representative tumor samples with the two DNA probes, pYNZ22 and p144D6. Of 38 tumors assessed for allelic loss of 17p, 27 cases were scored for retaining both alleles, whereas 11 cases showed a loss of one allele, for an overall frequency of 29%. DNA sequencing confirmed heterozygosity or hemizygosity of all patients with *p53* mutations (Table 2; Fig. 2). The frequency of loss of 17p loci was somewhat lower than that reported in the study by Fults et al. (14), in which 40% of both anaplastic astrocytomas and glioblastoma multiforme cases showed loss of 17p alleles. Similarly, in our panel of brain tumors, loss of 17p alleles occurred with the same frequency in tumors of different grades of malignancy.

**RFLP analysis has shown a loss of chromosome 13 in 15% of malignant gliomas (11, 15). Chromosome 13 is the site of the retinoblastoma gene (*Rb*) locus (6). A recent report noted a partial deletion of the *Rb* gene in 25% of gliomas.
for the 13q14 allele (18). To determine the frequency of deletion or structural alterations of the Rb gene in our panel of gliomas, we used two complementary DNA probes which recognize the 3' and 5' ends of the more than 200 kilobases of the genomic locus of the Rb gene to probe Southern blots. Of 40 brain tumor samples assessed for the integrity of the Rb gene, none showed deletions or rearrangements of the Rb locus.

Comparison of Allelic Loss of 17p with Frequency of p53 Mutation. Although several groups have reported loss of 17p alleles (11, 12, 14-16) or the presence of mutations in the p53 gene in malignant gliomas (4), only one report to date has assessed loss of heterozygosity for chromosome 17 and determined the frequency of mutations in p53 in five tumors (17). To determine the frequency of loss of 17p alleles with acquisition of mutations in the p53 gene, we compared the frequency of p53 mutations in tumors with either one or both chromosome 17p loci. Table 3 summarizes the results of this analysis. The overall frequency of p53 mutations in all tumors with two alleles was 33% (9 of 27) and increased to 64% (7 of 11) in tumors with one allele. In tumors that had lost one 17p allele, the frequency of p53 mutations was significantly increased at \( P < 0.01 \). However, it is important to note that 36% (4 of 11) of the cases which had lost one 17p allele did not have mutations in p53.

DISCUSSION

The genetic events which predispose to the development of brain tumors are largely unknown. In this report, we have analyzed malignant gliomas, frequently fatal tumors of adults, for specific genetic changes that may contribute to their progression. Cytogenetic evidence for loss of chromosome 17, the site of the p53 gene, has been documented in a variety of human tumors and is frequently associated with the presence of point mutations in the remaining p53 allele (3-5). In this study, we evaluated the frequency of mutations of the p53 gene together with 17p allele loss to determine (a) whether these genetic events were tightly linked in the development of gliomas, as has been shown for some other cancers, (b) whether loss of 17p alleles and mutation frequency of the p53 gene were related to grade of malignancy, and (c) whether the spectrum of p53 mutations observed in our panel of gliomas was similar to that reported for other human cancers. Finally, we determined whether the Rb tumor suppressor gene was involved in the progression of gliomas.

Comparison of p53 Gene Mutations with Deletions of 17p Alleles in Gliomas. In our study, we found that 64% of gliomas with one 17p allele had mutations in the p53 gene, which agrees with the finding of others who have reported frequencies of 60-86% in brain, breast, colon, lung, hepatocellular, and gastric cancers (17, 19, 26, 28, 35).

Only two reports have assessed the frequency of p53 mutations and 17p allele loss in a large number of tumors. Baker et al. (19) found that 17% (5 of 30) of all colorectal tumors with both 17p alleles had mutations in p53; the frequency of p53 mutations increased significantly to 86% (24 of 28) in tumors with only one allele. Chiba et al. (26) studied 39 cases of nonsmall cell lung cancer. No tumors with both 17p alleles had p53 mutations, but in 20% (8 of 39) of the cases with one chromosome 17p, there was a 75% (6 of 8) incidence of p53 mutations. In both of these studies, the frequency of p53 mutations was low (0-17%) in tumors with two 17p alleles, but it increased significantly (75-86%) in tumors with one 17p allele. These findings indicate that mutations of the p53 gene and deletions of the normal 17p allele occur close in time in cancers of the colon and the lung. The results of our study showed no such correlation. In our study, the majority of gliomas (27 of 38) retained two 17p alleles, and 33% (9 of 27) of these tumors had mutations of the p53 gene, a frequency twice that observed for colon cancers with two 17p alleles (19). Our findings suggest that p53 mutations occur prior to and independent of deletions of the 17p locus in a large proportion of gliomas. We also observed loss of one 17p allele in one-third (4 of 11) of the tumors without an accompanying mutation in the p53 gene. Several groups have noted nonrandom loss of heterozygosity for loci on chromosome 17p in astrocytic brain tumors, a pattern of loss which suggests that a tumor suppressor gene may be located there (14-16, 34). Whether the p53 gene is the only target for loss on chromosome 17p remains to be determined in future studies. Our data, which show loss of 17p alleles in the absence of p53 mutations in 36% of gliomas, may indicate that some other tumor suppressor gene on 17p is the target for loss in the development of some gliomas. This notion is strongly supported by the recent findings of Saylor et al. (36) in medulloblastomas. In that study, 8 of 8 medulloblastomas that showed loss of heterozygosity for one 17p allele lacked mutations in the p53 gene.

Loss of Heterozygosity for 17p and Grade of Malignancy in Gliomas. Glioblastomas and gliosarcomas are considered high-grade (grade IV of IV) malignancies (20). We analyzed 34 grade IV gliomas (Table 2), of which >70% (25 of 34) retained both 17p alleles. This finding differs markedly from those in cancers of the colon and bladder, where loss of chromosome 17p alleles has been associated with the transition from the benign to the malignant state (19, 37). The low frequency of loss of heterozygosity for chromosome 17p, observed in gliomas of the highest grade analyzed here, again suggests that progression of low-grade astrocytomas to high-grade gliomas occurs independently of 17p allele loss. Studies of low-grade astrocytomas for loss of 17p alleles and/or the presence of p53 mutations are currently in progress to explore this issue further. However, similar findings have been reported for astrocytomas, non-small cell lung cancers, and gastric cancers (12, 16, 26, 38), in which loss of 17p alleles occurs at all grades of malignancy and has not been associated with tumor progression. These findings again point to other genes, oncogenes as well as tumor suppressor genes, on other chromosomes that play a role in progression to malignancy in these different tumors. Putative tumor suppressor genes have been mapped to chromosomes 9 and 10 using RFLP analysis of gliomas (13, 15). Loss of heterozygosity for these loci is restricted to higher-grade astrocytomas, indicating that genes on these chromosomes are more closely linked with malignant progression. Studies are currently in progress to ascertain loss of heterozygosity for loci on chromosomes 9 and 10 in the panel of high-grade gliomas studied here.

Spectrum of p53 Mutations in Gliomas. The incidence of p53 mutations in gliomas with one 17p allele was 64% in our study compared to 80% reported by Nigro et al. (17). The distribution of mutations that we observed was in the highly conserved regions of the p53 gene (exons 5-8), as reviewed elsewhere (4, 5).
p53 MUTATIONS IN HUMAN MALIGNANT GLIOMAS

Only a few studies to date have examined mutations in exon 4 (codons 43-125) of the p53 gene (26, 33, 35). In our survey of p53 mutations in gliomas, we found one tumor that contained a mutation in exon 4, a C to A transversion in codon 87. It is interesting to note that this glioma was from a patient diagnosed with NF. Patients with von Recklinghausen neurofibromatosis are at higher risk for developing tumors of the nervous system (39). Menon et al. (40) reported mutations of p53 in malignant neurofibrosarcomas from two NF patients. One mutation was silent; however, the other mutation was a C to A transversion in codon 129 of exon 5. It will be important to extend these observations to determine whether C to A transversions in the p53 gene are common in tumors arising in NF patients.

The majority of mutations identified in our study were G:C to A:T transitions (73%, 11 of 15), as has been reported for other brain tumors (4, 17, 41) and occurred frequently at sites of CpG dinucleotides, which are known hot spots for mutations in the p53 gene in many other types of human cancers (4, 5).

G to T transversions occurred in 25% of the mutations (4 of 16) that we identified in our panel of 40 gliomas (2 G to T; 1 C to A; 1 C to G), a frequency not previously reported for brain tumors (4, 17). The incidence of transversions, mainly G to T, is high for lung cancers (52-100%) (26, 42), hepatocellular carcinomas (84-100%) (27, 28), breast tumors (30%) (43, 44), and esophageal squamous cell carcinoma (25%) (29). One explanation for the high frequency of G to T transversions in lung, esophageal, and liver cancers is exposure to the carcinogens in tobacco and aflatoxin B1, respectively (4, 27-29).

We noted that 4 of the 8 mutations localized to exon 5 (codons 126-186) were clustered within codons 156-159, and 75% (3 of 4) of these mutations were transversions. Chiba et al. (26) noted in their study of non-small cell lung cancer that 6 of 10 mutations in exon 5 occurred between codons 151 and 159, and 67% (4 of 6) were transversions. More recently, Casson et al. (45) reported 2 of 5 mutations in exon 5 at codons 152 and 155 in Barrett's epithelium, a premalignant stage of esophageal carcinoma. This region (codons 143-170) of the p53 gene lies between the two highly conserved domains, II and III, which bind the SV40 large T antigen, and has not been shown previously to have a special function (25). None of the patients studied in this report who had mutations resulting in a transversion showed loss of heterozygosity for 17p alleles, suggesting that these mutations may be dominant-negative or gain-of-function p53 mutations (46-49). These mutations may confer a selective growth advantage such that selective pressure for loss of the second 17p allele is unnecessary. The high frequency of mutations, particularly transversions, recognized in this region of the p53 gene in the gliomas studied here, together with the fact that G to T transversions are increased in cancers from patients exposed to carcinogens such as tobacco and fungal toxins, may point to an environmental carcinogen important in the genesis of some malignant brain tumors. This possibility should be investigated further. Moreover, these data provide evidence for a fifth hot spot in the p53 gene where mutations may disrupt or alter an important function of the p53 protein.

Absence of Rb (13q14) Deletions in Gliomas. Inactivation of the Rb gene by partial deletions has been observed in retinoblastomas, osteosarcomas, malignant gliomas, breast carcinomas, and T- and B-cell lymphoid malignancies (6, 18, 50-53). Losses of chromosome 13, which harbors the Rb gene, have been noted in 15% of brain tumors by RFLP analysis (11, 15). We examined the Rb locus in 40 gliomas and found no evidence for deletions or structural alterations. However, it is still possible that point mutations have occurred within the Rb gene that also cause inactivation of the gene (54).

Genetic Events in Brain Tumors. This report represents the first major study of high-grade gliomas of the astrocytic lineage for mutations of the p53 gene (17p). Loss of 17p is a common event in different subtypes of glial tumors, i.e., astrocytic (astrocytomas) versus nonastrocytic (ependymomas and oligodendrogliomas) (11, 12). Ohgaki et al. (41) recently surveyed nonastrocytic brain tumors for p53 mutations and found a frequency of 11-12% in medulloblastomas and oligodendrogliomas, respectively. These reports, taken together, provide evidence for p53 mutations occurring in tumors of widely divergent histological types within the central nervous system (17, 41), as has been documented for human cancers of many other tissue systems (4, 5).

Several groups have hypothesized a model of progression for the development of low-grade astrocytomas into high-grade gliomas which involves the frequent loss of genes on chromosomes 17, 9, and 10 (13, 15, 16, 34) and amplification and overexpression of genes such as c-myc, gli, and epidermal growth factor receptor (55-59). Chromosome 17p loss occurs in tumors of different grades, whereas losses of chromosome 9 and 10 are confined to the more malignant gliomas, suggesting that the loss of genes on chromosome 17p is an early, perhaps initiating event, whereas the loss of loci on chromosomes 9 and 10 is more closely associated with the transition to malignancy. Similarly, gene amplification, when it occurs, is restricted to tumors of high grade (55, 56, 58, 59). Amplification, often with rearrangement of the epidermal growth factor receptor gene, occurs most frequently and has been observed in approximately 33-50% of high-grade gliomas (58, 59). These genetic changes are also late steps in progression to malignancy (57-59). From this brief discussion of the severe changes known to occur within brain tumors of the astrocytic lineage, it is clear that progression from a low-grade astrocytoma into a high-grade astrocytoma (glioblastoma) is a complex process. To further delineate the genetic steps in the progression to malignancy in astrocytomas, we plan to conduct similar studies in low-grade astrocytomas.

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