The p53 Gene and Protein in Human Brain Tumors

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INTRODUCTION

The p53 gene is the most frequently mutated gene in many common human malignancies, including tumors of the colon, breast, lung, esophagus, liver and brain (1–4). Because p53 gene alterations are commonplace in human tumors and because p53 protein is involved in a number of important cellular pathways, p53 has become a topic of intensive investigation, both by basic scientists and clinicians.

p53 was initially identified by two independent laboratories in 1979 as a 53 kilodalton (kD) protein that complexes with the large T antigen of SV40 virus (5, 6). Shortly thereafter, it was shown that the E1B oncoprotein of adenovirus also binds p53 (7). The binding of two different oncogenic viral tumor proteins to the same cellular protein suggested that p53 might be integral to tumorigenesis. The human p53 cDNA and gene were subsequently cloned in the mid-1980s (8, 9), and analysis of p53 gene alterations in human tumors followed a few years later (3). During these 10 years, researchers grappling with the vagaries of p53 first characterized the gene as an oncogene, then as a tumor suppressor gene, and most recently as both a tumor suppressor gene and a so-called “dominant negative” oncogene (10). The last few years have seen an explosion in work on this single gene and its protein product. A review of a computerized medical database revealed approximately 650 articles on p53 in 1992 alone.

p53 has assumed importance in neuro-oncology because p53 mutations and protein alterations are frequent in the common diffuse, fibrillary astrocytic tumors of adults. p53 mutations in astrocytomas were first described in 1989 (9) and were followed by more extensive analyses of gene mutations (11–19) and protein alterations (11, 18, 20–24) in adult astrocytomas. The gene has also been studied in less common brain tumors (25–28). Elucidating the role of p53 in brain tumorigenesis will not only enhance understanding of brain tumor bi-

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THE p53 GENE

The p53 gene, designated TP53, is located on the short arm of chromosome 17 (17p) at band 13.1, approximately 15–20 centimorgans from the telomere. The gene spans 20 kilobases (kb) of genomic DNA (9), and the genomic structure appears highly conserved: in all studied species, p53 consists of 11 exons, with the first exon being a non-coding exon that is followed by a large first intron (10 kb in human) (9) (Fig. 1, top). The gene contains five domains in which the nucleotide sequence is highly conserved among species: domain I, codons 13–19; II, codons 117–142; III, codons 171–181; IV, codons 234–258; and V, codons 270–286 (1–4). While the specific functions of these highly conserved domains are undetermined, their high degree of sequence homology among species suggests that the regions are vital to protein function. This is strongly supported by the finding that most oncogenic mutations occur in these domains (1, 3). Since these conserved domains lie within exons 5, 7 and 8, exons 5 through 8 have been the most extensively studied in human tumors.

Relatively little is known about transcriptional control of the p53 gene. Attempts to map promoter regions in the gene have demonstrated regulatory sites in the 5' upstream region, in exon 1 and in intron 1 (29, 30). In addition, a region of intron 4 appears to be important for expression of the gene (31). The p53 mRNA transcript is 2.8 kb in length (8) and can be detected in most human cells, with the exception of cells in G0 (32, 33). Stimulation of quiescent cells results in increased mRNA synthesis that reaches highest levels in late G1/early S phase (34). Once cells are proliferating, however, there is relatively little variation in the levels of either p53 mRNA or protein. In most instances, substantial elevations in p53 protein levels are not a result of increased gene expression, but occur at the level of protein stabilization (35).

THE p53 PROTEIN

The p53 protein is a 53 kD, 393 amino acid phosphoprotein. The protein can be divided into three regions (Fig. 1, bottom): an acidic, proline-rich, predicted α-helical amino-terminus which contains the transcriptional
activation domain and a heat shock protein-binding domain; a hydrophobic, β-pleated central region that has the highly conserved domains II–V; and a basic, α-helical carboxyl-terminus that contains numerous functional sites, including three nuclear localization signals, the oligomerization region, specific and nonspecific DNA binding domains, the TATA binding protein interaction site, a heat shock protein-binding domain, and the phosphorylation sites for cdc2 kinase, cdk2 kinase and casein kinase II (4, 36). Specific functional domains have not been identified in the highly conserved central portion of the molecule, but this region appears necessary for sequence-specific DNA binding (36). Numerous antibodies have been raised to different regions of the p53 protein. Some of the common antibodies include: PAb 1801, a human-specific monoclonal antibody that recognizes an epitope near the amino-terminus of wild-type and mutant p53; PAb 240, a monoclonal antibody that reacts with an epitope in the middle of only mutant forms of mouse and human p53; PAb 421, a monoclonal antibody to a carboxyl-terminal epitope in both wild-type and mutant forms of mouse and human p53; and CM-1, a polyclonal antiserum that binds to wild-type and mutant p53 (37, 38).

The wild-type protein is found in the nucleus and is typically present as a homodimeric (36) or tetrameric complex (39). Oligomerization appears to enable p53 to bind to a symmetrical DNA consensus sequence (36, 39, 40). Wild-type p53 protein is found at low levels in all normal mammalian cells but has a short half-life of 20–30 minutes (33). The protein is stabilized and its half-life is elongated, however, by a variety of viral and cellular proteins that bind to p53 (see below). In addition, p53 can be post-translationally modified by phosphorylation, predominantly on carboxyl-terminal serine residues by a number of cell-cycle-dependent kinases (36, 41, 42). Such phosphorylation is probably important in modulating p53 protein function (36, 43).

THE CELLULAR ROLES OF p53

Wild-type p53 acts as a “tumor suppressor,” a normal cellular protein that represses abnormal cell proliferation and growth. For instance, the addition of wild-type p53 to transformed cells, including glioblastoma multiforme (GBM) cell lines (44), can reverse their malignant phenotype (2). In recognition of this rather awesome protective role, p53 has been dubbed the “guardian of the genome” (45).

p53 probably accomplishes its monumental task through its participation in at least four critical cellular pathways (Fig. 2, left): 1) arrest of the cell in G1 phase of the cell cycle; 2) initiation of DNA repair; 3) induction of apoptosis or programmed cell death; and 4) promotion of cellular differentiation. Wild-type p53 accumulates in the nucleus in response to either DNA damage (46, 47) or deregulated proliferation (35, 48). Accumulation of p53 results in arrest of the cell in G1 (49), serving to prevent both deregulated proliferation and replication of damaged DNA. In this regard, it is interesting to note that patients with ataxia telangiectasia, who have a defect in DNA repair, do not show wild-type p53 accumulation and G1 arrest following irradiation (50). Once arrested in G1, a cell must either repair its DNA prior to S phase or, if DNA damage is irreparable, instigate a suicidal, apoptotic response. p53 appears to be necessary for the induction of the GADD (growth arrest and DNA damage-inducible) DNA repair enzymes, particularly GADD45, which may in turn stimulate a DNA repair cascade (50).

In addition, p53 has been implicated as a crucial factor in programmed cell death (51). For example, apoptosis in response to certain types of radiation and chemotherapy does not occur in the absence of wild-type p53 (52, 53). Further evidence for the role of p53 in these “protective” cellular pathways is provided by the finding that gene amplification is more likely to occur in cells with
Fig. 3. p53 can be inactivated by a variety of mechanisms at the gene or protein level (see text). Mutant p53 = mutant p53 acting as a "dominant negative"; MDM2 = MDM2 oncogene product; SV40 LT = large T antigen of SV40 virus; Ad E1B = E1B oncoprotein of adenovirus; HPV E6 = E6 oncoprotein of human papillomavirus; sequestration = cytoplasmic sequestration of p53.

mutant or absent p53 than in cells with wild-type p53 (54, 55). Finally, in at least some cell types, wild-type p53 promotes differentiation. For instance, wild-type p53 can induce differentiation of pre-B cells into more mature B cells (56). Furthermore, metastases of Saos-2 osteosarcoma cells that have wild-type p53 consist of differentiated bone, and erythroleukemia cell lines with wild-type p53 express hemoglobin, while their counterparts with mutant p53 do not show these signs of differentiation (36). In summary, p53 occupies a pivotal position in various cellular processes (G1 arrest, DNA repair, apoptosis, and differentiation) that dissuade a cell from undergoing oncogenesis. At the same time, it is important to realize that p53 is not essential to early development or normal cell division, since mice lacking p53 appear to develop normally (57).

The most likely mechanism by which p53 executes these varied activities is through its ability to act as a transcription factor, by binding to DNA and activating transcription (2, 4, 40, 58, 59). For instance, p53 binds to a promoter sequence in the above-mentioned GADD45 gene (50) to upregulate expression of this gene in response to DNA damage. In addition, a number of cellular and viral genes are either activated or repressed by the action of p53 (60). Of particular interest is the ability of p53 to downregulate expression of proliferating cell nuclear antigen (PCNA) (61), an auxiliary protein to DNA polymerase-δ that is used as an immunohistochemical proliferation marker. In addition to transcriptional control of such DNA repair and proliferation pathways, p53 presumably regulates expression of genes involved in apoptosis and differentiation.

Inactivation of the p53 gene may thus lead to dire cellular consequences (Fig. 2, right). Lack of G1 arrest and the inability to induce DNA repair can lead to the replication of damaged DNA and to genomic instability. This may translate into oncogenic events such as tumor suppressor gene loss or oncogene amplification. Furthermore, the inability to induce differentiation or apoptotic pathway serves to bypass essential cellular checkpoints against neoplastic transformation.

MECHANISMS OF p53 INACTIVATION

p53 can be inactivated at either the gene or the protein level (Fig. 3), but in human tumors, including astrocytomas, gene inactivation appears to be the most common mechanism. A mutation in one copy of the p53 gene is usually accompanied by loss of a portion of chromosome 17p that bears the second gene copy. Allelic loss of 17p can be detected on a Southern blot by comparing normal and tumor DNA at a 17p polymorphism (P) that can distinguish the maternal and paternal chromosomes.

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At the protein level, it is important to point out that a single mutant p53 can complex with the wild-type p53 and negate the function of the wild-type protein; this is known as a "dominant negative" means of inactivation (62–64). In this scenario, only one p53 mutation is necessary for the functional inactivation of both alleles, although loss of the second, wild-type allele may further contribute to oncogenesis. Other proteins can also bind to and inactivate p53. The p90 product of the MDM2 oncogene is a cellular protein with transforming properties that complexes with wild-type and mutant p53 protein (65). MDM2 binds to the transactivating domain of p53, thereby preventing p53 from initiating transcription (66). MDM2 amplification has been noted in approximately one-third of human sarcomas (67) but not in those sarcomas with p53 gene mutations (68), implying that MDM2 amplification is an alternative inactivating mechanism to p53 mutation. p53 may also be bound and inhib
activated by a variety of viral proteins, such as the SV40 large T antigen, adenovirus E1B protein, and human papilloma virus E6 protein. In human cervical carcinomas, for instance, human papilloma virus E6 binds to p53 and leads to its rapid degradation (69). Finally, sequestration of p53 in the cytoplasm may inactivate the protein by preventing it from reaching its nuclear site of action (70).

In human brain tumors, p53 gene mutation, with or without loss of the corresponding normal 17p allele, is by far the most common mechanism for p53 inactivation and is discussed in detail below. Other mechanisms of p53 inactivation will probably be shown to be important but are at present ill-defined. For instance, investigations of the MDM2 oncogene have produced conflicting results: three groups have not detected MDM2 amplification in astrocytomas (18, 19, 24), while one group has reported MDM2 amplification in 8–10% of malignant astrocytomas (71). Furthermore, cytoplasmic sequestration of p53 is not a feature of human astrocytomas (11, 18, 22) and evidence for a viral etiology of human brain tumors remains preliminary (72).

**P53 GENE MUTATIONS IN HUMAN BRAIN TUMORS**

In most human tumors, p53 mutations are clustered in the central, conserved regions of the gene, but the types and sites of mutations can differ markedly among tumor types. For instance, missense mutations are common in many carcinomas (1, 3), while sarcomas and esophageal carcinomas more commonly suffer nonsense mutations (1, 73). In addition, the location of the mutations within exons 5 through 8 varies among tumor types, with different tumor-specific "hot spots." For example, colon carcinomas have a high incidence of mutations at codons 175, 248, 273 and 282, while hepatocellular carcinomas in areas of hepatitis B infection often have mutations at codon 249 (1, 36). Furthermore, the type of base pair change varies among tumor types. Whereas C to T transitions (a transition is the change of one purine to another purine, or one pyrimidine to another pyrimidine) are common in colon carcinomas, G to T transversions (a transversion of the change of a purine to a pyrimidine or vice versa) predominate in non-small cell lung cancer (1, 36). Because characteristic mutational spectra may suggest specific etiologic agents in the genesis of these tumors, it is important to define the mutational spectrum of each tumor.

**Diffuse, Fibrillary Astrocytomas**

*Types of p53 Mutations:* Our group (11, 12, 15) and others (13, 14, 16–19) have demonstrated p53 mutations in approximately one-third (25–45%) of diffuse, fibrillary astrocytomas. Some authors have examined the entire coding sequence of exons 2 through 11, whereas others have concentrated on the highly conserved exons 5 through 8. Studies of the entire coding sequence have revealed only rare mutations outside of exons 5 through 8: one mutation each in exons 4, 9 and 10, and one mutation each in introns 4 and 6 (12–14, 17). (Exon numbers in Chung et al [12] are based on a previous p53 numbering system and have been changed to the current nomenclature; three mutations detected in Newcomb et al [18] that were not reported in Frankel et al [13] are included here as mutations.) To date, mutations have not been described in astrocytomas in exons 2, 3 or 11, or in the 5' promoter region (M-P. Rubio and DNL, unpublished data). Within exons 5 through 8, approximately two-thirds of mutations occur in the conserved domains II, III, IV.
TABLE 1
Overview of 68 p53 Mutations Reported in 65 Diffuse, Fibrillar Astrocytomas, from References 11–19

<table>
<thead>
<tr>
<th>Types of mutations (total = 68)</th>
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<tr>
<td>Missense</td>
<td>54 (80%)</td>
</tr>
<tr>
<td>Nonsense and frameshift</td>
<td>12 (17%)</td>
</tr>
<tr>
<td>Intrinsic</td>
<td>2 (3%)</td>
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Point mutations (total = 59)

<table>
<thead>
<tr>
<th>Missense vs. nonsense</th>
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<tbody>
<tr>
<td>Missense</td>
<td>54 (92%)</td>
<td></td>
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<tr>
<td>Nonsense</td>
<td>5 (8%)</td>
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<table>
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<tr>
<th>Transitions vs. transversions</th>
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<tbody>
<tr>
<td>Transitions at CpG</td>
<td>50 (85%)</td>
<td></td>
</tr>
<tr>
<td>Transversions</td>
<td>30 (54%)</td>
<td></td>
</tr>
<tr>
<td>Base pair preferences</td>
<td>9 (15%)</td>
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| G:C base pairs                                | 48 (81%) |
| A:T base pairs                                | 11 (19%) |

Nonsense and frameshift mutations (total = 12)

| Deletions                                      | 5 |
| Insertions                                    | 2 |
| Point mutations                               | 5 |

and V, and a full 95% of mutations occur in individual codons that are highly conserved through evolution (Fig. 5).

The data on mutations in diffuse, fibrillar astrocytomas are summarized in Table 1. Of a total of 68 reported mutations (in 65 tumors), 54 (80%) have been missense mutations, 12 (17%) nonsense/frameshift, and 2 (3%) intrinsic. Most exonic mutations have been point mutations (59 of 66, 90%), but deletions and insertions have also been reported (see below). Of the 59 point mutations, 50 (85%) have been transitions and only 7 (15%) have been transversions. These point mutations occur far more commonly at G:C pairs (81%) than at A:T pairs (19%). While the reasons for the striking predilection for transition mutations at G:C pairs are not entirely clear, one explanation is that mutations are particularly common at so-called CpG dinucleotides (in which a cytosine is 5' to a guanine) (Fig. 6, top) (1). CpG dinucleotides are the site at which 5-methylcytosine ("the fifth base") is present in the human genome. Such methylation is believed to be important in such processes as genomic imprinting and in transcriptional control of some promoters. Spontaneous deamination of 5-methylcytosine results in a normal thymine base (Fig. 6). It is estimated that such spontaneous deamination occurs frequently in the genome (74). Unfortunately, while deamination of cytosine, guanine and adenine result in non-DNA bases (uracil, xanthine and hypoxanthine, respectively) that are readily recognized and repaired by DNA repair enzymes, the thymine produced by deamination of 5-methylcytosine is less readily recognized and repaired, resulting in a C to T transition at that site (74). In astrocytomas, 53% of all point mutations occur at such CpG dinucleotides. These CpG dinucleotides account for the focal "hot spots" seen in codons 175, 248 and 273 (Fig. 5).

As mentioned earlier, nonsense and frameshift mutations are uncommon in astrocytomas. These can be divided into small (usually single base pair) deletions (5 of 12 nonsense mutations) and small insertions (2 of 12) that cause frameshifts and premature stops, or point mutations (5 of 12) that introduce stop codons. Because the carboxyl-terminal region of the protein contains numerous functional domains, even truncated proteins containing the vast majority of the protein would most likely be functionally inactive. Intrinsic mutations have not been screened for extensively, since most assays only screen intron segments that flank the exons. None of the intrinsic mutations have been in definite splice junction or lariat formation sites, and the functional effects of these mutations remain unclear.

Correlation with Loss of Chromosome 17p: The identification of consistent regions of chromosomal loss in specific tumor types suggests the presence of an inactivated tumor suppressor gene on the remaining allele (Fig. 4). This common mechanism provides a convenient means for identifying potential tumor suppressor genes: loss of heterozygosity (LOH) studies. These studies employ genetic polymorphisms, such as restriction fragment length polymorphisms (RFLP), to distinguish the patient's normal maternal and paternal chromosomes. If a particular RFLP identifies two alleles in a patient, the patient is said to be "heterozygous" or "informative" at that RFLP locus. Alletic chromosomal loss, or "loss of heterozygosity," can then be detected by comparing the polymorphism in normal and tumor tissues (Fig. 4). In human astrocytomas, loss of chromosome 17p has been frequently noted (75–77) and raises the question whether...
p53 is the important astrocytoma tumor suppressor gene on chromosome 17p.

In studies that have examined both p53 gene mutation and LOH of chromosome 17p, 70% of cases with p53 mutation have corresponding loss of chromosome 17p (12–17, 19). In our experience, the correlation between p53 mutation and 17p loss is statistically significant (15). As discussed above, those cases with p53 gene mutation and no 17p loss may represent mutant p53 acting in a “dominant negative” manner; alternatively, later biopsies may disclose LOH of 17p (78). In this regard, it is of interest to note that nonsense and frameshift p53 mutations, which presumably cannot act in a “dominant negative” manner since they lack the carboxyl-terminal oligomerization domain, are usually accompanied by loss of chromosome 17p (10/12 cases). Of those astrocytomas with 17p loss, however, approximately 30% do not have detectable p53 mutations (13–15, 19). It seems unlikely that such cases harbor exonic mutations, since some cases have been completely sequenced (19) and mutations in nonconserved regions are rare, but some of these cases may have intronic mutations (12, 27, 79). Alternatively, a second astrocytoma tumor suppressor gene may be present on chromosome 17p. For some tumor types, such as breast and hepatocellular carcinoma, a more telomeric tumor suppressor gene has been suggested by loss of chromosome 17p distal to p53 with maintenance of both 17p copies at the p53 locus. For astrocytomas, however, only two cases have been reported with LOH telomeric to the p53 gene and maintenance of both p53 alleles, but these cases also had mutations in the p53 gene (19). Using multiple highly informative markers telomeric to the p53 gene on 17p, we have been unable to find astrocytomas with only telomeric 17p loss (15). Thus, while p53 is clearly an important astrocytoma tumor suppressor on chromosome 17p, there is as yet no firm evidence to suggest a second astrocytoma tumor suppressor gene on 17p.

**Correlation with Astrocytoma Grade:** An important question is whether p53 mutations are early or late changes in the formation of diffuse, fibrillary astrocytomas. In colon carcinoma, for instance, certain genetic changes are associated with the transition from colonic hyperplasia to adenoma, while other genetic changes are associated with the change from adenoma to carcinoma. In diffuse, fibrillary astrocytomas, loss of chromosomes 17p and 22q is seen in all three grades (astrocytoma WHO grade II, anaplastic astrocytoma WHO grade III and GBM WHO grade IV) (15, 75–77, 80, 81). loss of 19q is only seen in anaplastic astrocytoma and GBM (82, 83), and loss of chromosome 10 and epidermal growth factor receptor (EGFR) gene amplification are largely restricted to GBM (84, 85). This has led most investigators to conclude that loss of tumor suppressor genes on chromosomes 17p and 22q are early events in astrocytoma formation, while events such as loss of the chromosome 10 tumor suppressor gene is a late event only seen in the transition from anaplastic astrocytoma to GBM. If 17p loss is an early change, and if p53 mutations are associated with 17p loss, then p53 mutations should be an early event in astrocytoma formation. We have confirmed this by showing equal frequencies of p53 mutation in astrocytoma, anaplastic astrocytoma and GBM (11, 12, 15). Sidransky et al (16) have detected subpopulations of mutant p53 cells in three grade II astrocytomas that progressed to GBM by clonal expansion. This emphasizes that p53 mutations occur relatively early in astrocytoma tumorigenesis and is in agreement with one of our cases, which showed a p53 mutation in the original grade II tumor and in the recurrent GBM (11, 15), and with other analyses of primary and recurrent tumors (13). The discovery of germline p53 mutations in the Li-Fraumeni syndrome, in which affected members develop breast carcinomas, sarcomas and gliomas early in life, further supports a role for p53 early in tumorigenesis (86, 87). We have also described a germline p53 mutation in a patient with neurofibromatosis type 1 and a GBM (12). In summary, p53 mutations appear to be early changes in diffuse, fibrillary astrocytoma formation, occurring before the progression to anaplastic astrocytoma and GBM.

**Other Central Nervous System Tumors and Tumor Syndromes**

**Other Gliomas:** We have studied seven pediatric brain stem GBM for p53 gene and chromosome 17p alterations (27). Four of seven cases had lost portions of chromosome 17p that included the p53 gene. These four cases and one additional case had mutations in the p53 gene. Interestingly, two of these mutations were intronic. These results implicate the p53 gene in pediatric brain stem gliomas and suggest similarities between these tumors and those GBM that occur in younger patients (see below) (88). Because our study did not include lower-grade brain stem astrocytomas, we cannot evaluate whether p53 mutations are early or late changes in these tumors.

Analysis of the p53 gene in pilocytic astrocytomas has not revealed mutations to date (89; A. von Deimling and DNL, unpublished data), and 17p loss is rare in pilocytic astrocytomas (90, 91). More commonly, these tumors show loss of chromosome 17q, including the region of the neurofibromatosis type 1 (NF1) gene (90). Since NF1 patients often develop pilocytic astrocytomas of the optic nerve, this may implicate the NF1 gene, rather than the p53 gene, in pilocytic astrocytoma tumorigenesis.

A study of the p53 gene in 17 oligodendrogliomas detected only two mutations, both missense mutations (26). This low mutation frequency is in agreement with the relatively low frequency of 17p loss in oligodendrogliomas, and other evidence suggests an alternative oligodendroglioma tumor suppressor on chromosome 19q (82).
Studies of the p53 gene in ependymomas have not revealed mutations (26), and allelic loss of 17p is not a feature of ependymoma (91), although a germ-line mutation has been described in a patient with a malignant ependymoma (92).

Medulloblastomas: Medulloblastomas show frequent allelic chromosomal losses, particularly of chromosomes 6q, 11, 16q and 17p (93). Chromosome 17p is the most common site of allelic loss and therefore probably contains a tumor suppressor gene integral to medulloblastoma formation. This correlates well with the cytogenetic observation that one-third of medulloblastomas have an isochromosome 17q which can result in the loss of one copy of 17p (80). However, while 17p loss occurs in approximately 50% of medulloblastomas, mutations in the p53 gene are rare (25, 26), thus suggesting the presence of another 17p tumor suppressor gene (94, 95). Medulloblastomas have been described with allelic losses of 17p telomeric to p53, with maintenance of heterozygosity at the p53 region, and these cases support the hypothesis that a medulloblastoma tumor suppressor gene lies telomeric to the p53 locus on 17p (94). One study has demonstrated that medulloblastomas with 17p loss may act more aggressively than other medulloblastomas (96). It is possible that such clinical aggressiveness is due to inactivation of the distal 17p tumor suppressor, but is probably independent of the p53 gene.

Hereditary Glioma Syndromes: Hereditary brain tumor syndromes provide important clues to the genetic basis of brain tumorigenesis (97). Germ-line p53 mutations have been noted in the Li-Fraumeni syndrome (86, 87), in a patient with NF1 and a GBM (12), and in a patient from a cancer-prone family who had a malignant ependymoma (92). Germ-line p53 mutations have not been reported, however, in Turcot’s syndrome (colonic polyposis and primary brain tumors) (98) or in the rare families with hereditary astrocytomas that do not have a higher incidence of other cancers (DNL, unpublished data).

p53 PROTEIN ACCUMULATION IN HUMAN BRAIN TUMORS

Studies of the p53 protein in brain tumors have been predominantly immunohistochemical in nature. The availability of antibodies that recognize fixation-resistant epitopes, such as the popular monoclonal antibody PAb 1801 and the polyclonal antiserum CM-1, has led to a plethora of recent papers on p53 in brain tumors (11, 12, 18, 20–23, 99, 100). These studies have focused on diffuse, fibrillary astrocytomas and have detected the p53 protein immunohistochemically in approximately 15–40% of astrocytomas, 35–60% of anaplastic astrocytomas and 45–70% of GBM. In almost all studies, higher-grade astrocytomas are more frequently immunopositive than lower-grade tumors. The number of positive cells, however, varies greatly within grades, and there is no obvious relationship between the number of positive cells per tumor and the tumor grade.

Most papers reporting immunohistochemical results have assumed that only mutant proteins have the elongated half-life and high levels necessary for immunodetection, and have therefore inferred that p53 immunopositivity reflects gene mutation. Numerous lines of evidence, however, are suggesting that this assumption may be too simplistic (101). In astrocytomas, we have shown that immunohistochemistry with the amino-terminal PAb 1801 antibody will not detect nonsense p53 mutations, presumably because the truncated protein cannot oligomerize and is not stable (11). Carboxyl-terminal antibodies such as PAb 1802 will also not detect proteins with a truncated carboxyl-terminus. Even more problematic has been our finding that approximately 30% of astrocytomas are immunopositive with PAb 1801 but are not immunopositive with the mutant-specific antibody PAb 240 and do not have p53 gene mutations (11, 24). Other groups have reported astrocytomas and glioma cell lines with similar discrepancies between genetic and immunohistochemical analyses (18, 102). These cases probably represent examples of wild-type p53 protein accumulation (24). It seems prudent, therefore, to regard immunohistochemical positivity as evidence of p53 protein accumulation that can result from different underlying biological phenomena, and not to infer p53 gene mutations from such immunopositivity. In fact, given the general trend for more immunopositive cases in GBM than in lower-grade tumors, a plausible explanation may be that GBM show more physiological accumulation of wild-type p53 in response to deregulated proliferation or DNA damage than do the less atypical lower-grade tumors.

APPLICATIONS OF P53 ANALYSIS IN NEURO-ONCOLOGY

Analyses of p53 and chromosome 17p may provide means for subdividing brain tumor patients into diagnostic, prognostic or therapeutic groups. For instance, those patients whose tumors have chromosome 17p loss, often with corresponding p53 gene mutations, appear to be a separate genetic subset from those patients whose tumors have EGFR gene amplification (88). We have suggested, based on a small number of patients, that patients with p53 mutations are typically younger and have slightly better prognoses than those without p53 gene mutations (12). In larger groups of patients, we have confirmed that p53 gene mutations (11) and chromosome 17p loss (88) are more characteristic of younger patients with astrocytomas. In addition, p53 gene mutations are particularly common in pediatric brain stem gliomas, further emphasizing the association of p53 gene mutations with the younger age groups (27). These data argue that p53 gene mutations may designate a type of astrocytoma...
more common in younger patients, but the relationship of these genetic changes to prognosis has not yet been fully evaluated.

It is tempting to hypothesize that the presence of p53 gene mutations in younger astrocytoma patients is related to the better prognosis observed in younger patients. If p53 normally acts to monitor the cell and initiate DNA repair, then a tumor with a p53 mutation may not be able to summon its DNA repair mechanisms and may be more susceptible to the DNA-damaging effects of therapeutic radiation and chemotherapy. As a result, patients with p53 mutations would have more complete responses to therapy and longer survivals (4). On the other hand, a tumor with elevated wild-type p53 protein may be actively inducing DNA repair and could possibly evade therapeutic attempts more effectively. This attractive explanation, however, remains speculative and may be complicated by recent studies of p53-induced apoptosis in response to radiation and certain chemotherapeutic agents (52, 53). Further experiments are needed to clarify the relationships between p53 mutation, patient age, and response to therapy.

A number of studies have addressed the possible association of p53 immunohistochemical results and patient prognosis. One report showed a correlation between p53 immunopositivity and poorer prognosis, but this was not significant on multivariate analysis and the study artificially grouped cystic cerebellar grade I astrocytomas with the diffuse, fibrillary astrocytomas (22). Two unpublished investigations have produced conflicting results, one group suggesting that p53 immunopositivity correlates with poor prognosis (F. S. Pardo et al, personal communication) and another showing no prognostic differences between immunopositive and immunonegative cases (T. J. Montine and P. C. Burger, personal communication). Studies of the relationship of proliferation indices to p53 immunopositivity have also resulted in disparate findings: some authors have suggested that p53 immunopositive cases have higher Ki-67 (22) or PCNA (23) labeling indices, while others have not been able to show such correlations (99; Y-S. Chae and DNL, unpublished observations). Because p53 immunopositivity probably reflects different underlying biological phenomena (see above), it is perhaps not surprising that p53 immunohistochemistry does not reliably predict clinical or kinetic tumor characteristics. In this regard, it is of interest that we were unable to show significant age differences when p53 immunopositive cases were added to the p53 mutation cases (11), further emphasizing the heterogeneity of immunopositive cases. The use of mutant-specific antibodies may allow the distinction of mutant from wild-type immunopositive astrocytomas (24) and may more accurately contribute to a classification of these tumors. At present, however, the clinical relevance of p53 analysis, either at the genetic or the protein level, remains an open field.

**SUMMARY**

p53 mutation is most likely an integral early step in the formation of a subset of human diffuse, fibrillary astrocytomas, but it is not a frequent event in other studied brain tumors. In astrocytomas, p53 mutations are clustered in the conserved regions of the gene and are predominantly single base pair transitions, frequently at CpG dinucleotides. These mutations result in mutant or truncated p53 proteins that lack the transcriptional activating ability to induce G1 arrest, DNA repair, apoptosis or differentiation. On the other hand, some astrocytomas without p53 mutations may accumulate wild-type protein, perhaps as a physiological response to DNA damage or deregulated proliferation in the tumor cells. Finally, while data on the p53 gene and protein studies in human brain tumors are accumulating rapidly, the clinical significance of such data remains unclear.

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