The impact of p53 tumor suppressor gene on glioma biology

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The tumor suppressor gene, p53, is important in glioma biology. The authors of this paper review its role in cell physiology, epidemiology, glioma progression, prognosis, and therapeutic advances.

Key Words * p53 * tumor suppressor gene * glioma * epidemiology * molecular biology

Central nervous system (CNS) gliomas remain one of the most difficult lesions to treat. Despite significant technological advances and new surgical techniques, the outcome for patients with CNS gliomas has not changed substantially in many years. Researchers have worked to determine the molecular underpinnings of cell physiology and genetic alterations that lead to abnormalities in cell function in the hope that they can understand how these tumors arise and then develop therapies to treat them. A series of oncogenes and their protein products have been identified as being involved in the neoplastic transformation of normal cells. One of the most important oncogenes is p53, a well-established tumor suppressor gene. In this article we will discuss the role of p53 in the cell cycle, glioma biology, and oncogenesis as well as outline its epidemiological characteristics and the techniques that have been used to investigate them. We will also discuss the implications of p53 for the therapy of gliomas.

THE P53 GENE AND TUMORIGENESIS

The tumor suppressor gene, p53, is located on the short arm of chromosome 17. All cells normally have two alleles of the gene that codes wild-type p53. The p53 gene is thought to be recessive;[55] that is, if only one allele of wild-type p53 is present, then the cell will function normally. In a variety of malignancies, including carcinomas of the colon, breast, lung, esophagus, bladder, and liver, sarcomas and lymphomas, and leukemias, a loss of heterozygosity (LOH) on chromosome 17 is observed.[5,33,69,87] In these neoplasms, only one allele of p53 is present. Frequently that single allele has mutated and the protein product is a dysfunctional p53.

The p53 gene is a cell cycle regulator with a short half-life.[85] Basically the cell cycle consists of four phases. During the G1 phase the cell machinery prepares for DNA synthesis, which occurs in the S phase. In the G2 phase the cell prepares for mitosis by repairing any DNA damage. Mitosis occurs during the M phase. Cells not undergoing cell division are in the G0 phase. The p53 gene acts as a check point
regulator at the G₁ to S phase (Fig. 1). When p53 is activated it upregulates protein p21, which in turn binds to a variety of cyclin dependent kinases (CDKs). When p21 is bound to the CDKs, they inactivate the cyclins by preventing their phosphorylation. Phosphorylated cyclins allow the cell to enter the S phase.[9,17,40,41,48] Thus, through a cascade of events, p53 activity arrests entry of the cell from the G₁ phase to the S phase, resulting in cessation of cell division and proliferation.

Fig. 1. A schematic diagram depicting the role of p53 in cell cycle regulation. a: Wild-type p53 upregulates p21. b: p21 binds CDK to prevent its phosphorylation. c: In the absence of p21 binding, CDK is phosphorylated. d: Phosphorylated CDK permits the cell to progress from the G₁ to S phase.

How does p53 serve as a tumor suppressor? Several possible mechanisms have been proposed. By serving as a check point regulator, p53 is able to stop the cell cycle in G₁ if DNA damage is identified.[49,85,99] The DNA can then be repaired so that mutations producing subsequent abnormal cell physiology leading to neoplastic growth are not promulgated. The p53 gene also regulates the cell cycle through another pathway by regulating the growth-arrest-and-DNA-damage-inducible (GADD-45) gene.[9] In response to DNA damage or genotoxic stress, GADD-45 expression is induced in p53-dependent fashion. Inducement of GADD-45 will lead to growth arrest. Additionally, p53 has been shown to induce apoptosis (programmed cell death) if overexpressed.[47,61,62,84,85,100] This inhibition of cell proliferation can also prevent neoplastic growth.

Although p53 gene mutation occurs as a late event in human colorectal, lung, and liver cancers, it occurs early, even in precancerous lesions, in tumors of the esophagus, breast, and brain.[21,27,88] Loss or
mutation of \textbf{p53} is especially common in glial tumors and is reported to be the earliest detectable event in their development.[57,76] Further evidence that loss of normal \textbf{p53} gene expression is important in brain tumorigenesis is illustrated by experiments in which administration of a mutagen to pregnant mice carrying one inactivated \textbf{p53} gene resulted in rapid development of primary brain tumors in over 70% of homozygous \textbf{p53}-null offspring.[71]

If a mutation in the \textbf{p53} gene occurs, mutant proteins will be formed. These mutant proteins have a longer half-life than wild-type \textbf{p53}, leading to protein accumulation within the cells.[55] These mutant proteins can form oligomeric complexes that do not bind DNA, thereby preventing the cessation of the cell cycle.[55] Furthermore, through a dominant negative effect, mutant \textbf{p53} protein can oligomerize to wild-type \textbf{p53}, preventing any normal \textbf{p53} from binding to DNA and compromising its "watch dog" effect.[40,54,95] Loss of \textbf{p53} function results in genomic instability and the occurrence of aneuploidy.[31,58]

Some effects of \textbf{p53} inactivation might be more indirect. For instance, wild-type \textbf{p53} represses expression of the basic fibroblast growth factor (\textbf{bFGF}) gene at the transcriptional level and its mutant activates it in vitro, suggesting that another way in which the mutant \textbf{p53} protein may facilitate tumor progression is through increasing growth factor transcription.[90] Similar suppression of transforming growth factor-beta (TGF-b), potent mediator of tumor growth, by wild-type \textbf{p53} has also been shown.[23]

Other proteins can also bind to and inactivate \textbf{p53}, offering yet another indirect way in which \textbf{p53} effects can be subverted. The p90 product of the \textbf{MDM2} oncogene is a cellular protein with transforming properties that complexes with both the wild-type and mutant \textbf{p53} proteins. Thus, \textbf{MDM2} amplification could be an alternative method of inactivating \textbf{p53}. Investigations in various brain tumor types suggest that, for the most part, \textbf{MDM2} amplification is infrequent,[68,78,81] although one investigator reported amplification of this gene in 8 to 10% of malignant astrocytomas.[77]

\section*{INVESTIGATIONAL TECHNIQUES}

The biology of \textbf{p53} in brain tumors has been investigated using mainly two techniques. A variety of molecular biological techniques have been used to identify abnormalities of the \textbf{p53} gene in astrocytomas and other brain tumors. Southern blotting, in conjunction with restriction fragment length polymorphism, has been used to identify LOH on chromosome 17, but it does not reveal specific gene mutations.[12,19,24,25,37,57] A DNA mutation can be detected by using the polymerase chain reaction-single strand conformational polymorphism technique, which identifies bands on an electrophoretic gel corresponding to the conformation of the secondary structure of the DNA.[13,24,32,37,64,80,93,97] If a shift in the banding patterns occurs, then a mutation in \textbf{p53} within the tumor is present. This technique has been used on both paraffin-fixed tissue and frozen tissue,[80] but as with Southern blotting, it does not identify the specific mutations present. Specific mutations within the \textbf{p53} gene are determined by gene sequencing.[11,22,24,57,60,64,68,81,87,93,96] The sequence derived from the brain tumor can be compared with the genetic sequence of the wild-type \textbf{p53} gene to identify specific mutations. In situ hybridization has been used to investigate clonal expansion of \textbf{p53}-mutated cells in brain tumor progression.[86] In this technique, an oligonucleotide probe corresponding to the section of mutated \textbf{p53} DNA is radioactively labeled and then hybridized to DNA in clones in cell culture. This technique, although not widely used, allows the determination of which specific cell clones have the designated mutations.
A second widely used technique of examining tumors for abnormal p53 is immunohistochemistry. Immunohistochemical staining is based on the long half-life of the mutant p53 when compared with wild-type p53.[68] An antibody to epitopes on the p53 protein is used to stain tumor cells. Those cells in which p53 protein has accumulated, whether it be mutated or wild-type, will show positive immunostaining. Although intended to detect mutated p53, a number of studies have shown that between 10% and 29% of immunohistochemically positive tumors do not have p53 mutations within them.[46,60,96] This discordance is thought to be related to stabilization of wild-type p53 by mechanisms that are not completely understood. Cell injury, physiological response to DNA damage, deregulated proliferation, or other mechanisms that alter cell physiology are thought to be involved in creating a wild-type p53 with a longer half-life and subsequent positive immunostaining without an underlying genetic mutation.[30,60,68,78,96]

**EPIDEMIOLOGICAL CHARACTERISTICS OF P53**

**Mutation of p53 and Protein Overexpression in Astrocytoma**

The localization of the p53 gene to the short arm of chromosome 17 is notable because of the frequent loss of one of these alleles in human astrocytoma.[19,25,37] p53 mutations in adult astrocytoma were first described in 1989[69] and were followed by more extensive analyses of gene mutations[14,22,24,60,64,68,81,86,93] and protein alterations.[10,20,28,39,60,68,78] In studies that have examined both p53 gene mutations and LOH on chromosome 17p, 70% of tumors with p53 mutation have corresponding loss of chromosome 17p.[12,14,22,24,64,81,86,92]

Although p53 can be inactivated at either the gene or the protein level, gene inactivation appears to be the most common mechanism in astrocytoma formation.[59] In astrocytoma, p53 gene mutation, with or without loss of the corresponding normal 17p allele, is by far the most common mechanism for p53 dysfunction. Mutations of p53 occur in approximately 25 to 45% of diffuse fibrillary astrocytomas.[14,22,24,60,64,68,81,86,98] Mutations occur with approximately equal frequency in both low-grade and progressive (anaplastic and glioblastoma multiforme) astrocytomas.[14,59,94] Approximately 95% of mutations occur in those individual codons that are highly conserved. Most mutations are missense; nonsense (frameshift) and intronic mutations occur much less frequently.[60] These mutations result in mutant or truncated p53 proteins that lack transcriptional activating ability.

Sequestration of p53 in the cytoplasm is another potential way in which protein may be inactivated because it will be prevented from reaching its nuclear site of action; however, this aberration is not a feature of human astrocytomas.[59,68] Nevertheless, gliomas with mutations in the same codon of p53 can display different intracellular distributions, suggesting that in addition to the genotype the intracellular microenvironment may be augmenting the effects of a mutation by causing cytoplasmic sequestration.[3]

Immunohistochemical studies that have focused primarily on diffuse fibrillary astrocytomas indicate that the high rates of p53 protein expression occur in gliomas. The p53 protein is detected in approximately 15 to 40% of lower-grade astrocytomas, 35 to 60% of anaplastic astrocytomas, and 45 to 70% of glioblastomas (Table 1).[2,6,10,14,20,28,39,60,68] Because the rate of p53 mutation is essentially equivalent among the three astrocytoma grades, the higher immunopositivity in glioblastoma multiforme probably reflects a physiological accumulation of wild-type p53 protein in response to deregulated proliferation or DNA damage.
Mutations of \( p53 \) in Pediatric Astrocytoma

Initial studies examining gliomas occurring in the pediatric population have suggested a lower incidence of \( p53 \) mutation than occurs in adults.[57] In one large series of gliomas collected from patients in all age groups, \( p53 \) mutations occurred much more frequently in those between the ages of 18 and 45 years at diagnosis compared with those in pediatric (and older) patients.[76] Subsequent studies, however, suggest that the rates of \( p53 \) mutation and protein overexpression in pediatric astrocytomas are not significantly different from those seen in adults.[52,53,74,83] Furthermore, cases in which mutations had occurred showed the highest proliferation indices, suggesting similarities with adults.[83]

Incidence of \( p53 \) Mutation and Protein Overexpression in Other Primary Brain Tumors

The high incidence of \( p53 \) mutation and expression in diffuse astrocytomas raises the question of whether such abnormalities exist in other brain tumors (Table 1). One phenotypically glial tumor in which the incidence of \( p53 \) gene abnormality appears much less is the juvenile pilocytic astrocytoma: only a single gene mutation has been reported and 17p loss is rare.[34,38,53,57,72,83,94] Interestingly, \( p53 \) protein accumulation is not uncommon.[53] These observations suggest that there may very well be a different molecular basis for this very benign-behaving childhood neoplasm.

Other phenotypically glial tumors probably have rates of \( p53 \) mutation and \( p53 \) protein accumulation similar to those of the diffuse astrocytomas. For example, mutations occurring in all grades of oligodendrogliaoma totalled 19% in one study, a figure not appreciably different from that of diffuse astrocytomas.[29] Similarly, a mutation in exon 5 of \( p53 \) was detected in one of three anaplastic ependymomas examined.[89]

Other primary brain tumors have also been examined for \( p53 \) mutations. In a study of 60 astrocytic and 60 nonastrocytic types of human brain tumors, \( p53 \) gene mutations were observed only in those of astrocytic origin (six of 59, all in the conserved regions).[98] Furthermore, mutations in exons 5 to 8 in medulloblastomas, meningiomas, and hemangiopericytomas rarely occur.[1,72] This has led investigators to question whether such an association exists in other brain tumors. Interestingly, in both medulloblastoma and cerebral primitive neuroectodermal tumors, loss of chromosome 17p is noted approximately 50% of the time without identifiable mutations being present, suggesting that there is another 17p tumor suppressor gene.[7,73,75,82]

Mutations of \( p53 \) in Familial Brain Tumor Syndromes

Most often, \( p53 \) mutations in glioma are tumor specific and do not represent a germline mutation; in a
reported series of 120 brain tumors, not one mutation was germline in nature. Furthermore, the observation of tumor-specific, but not germline, \textit{p}53 \textit{mutations in two patients with glioma polyposis (Turcot's syndrome)} suggests a role for \textit{p}53 in progression rather than initiation of gliomas. Nevertheless, the known association of brain tumors in certain familial predispositions to cancer would seem to suggest a relationship. Brain tumors resulting from germline \textit{p}53 mutations have been reported, although they are apparently infrequent. Germline \textit{p}53 mutations were detected in one of 80 unselected glioma cases and in three of 15 cases selected for an unusual personal or familial history of cancer, and it has been noted that germline \textit{p}53 mutations are more frequent in patients with multifocal glioma, glioma and another primary malignancy, and/or glioma associated with a family history of cancer, particularly if these risk factors are combined. A recent report examining a family with predisposition to brain tumor also reported a novel germline deletion of the \textit{p}53 tumor suppressor gene, which considering the unusual accumulation of CNS tumors in family members, may have resulted from a certain organ-specific effect of this particular \textit{p}53 mutation.

**GLIOMA PROGRESSION AND \textit{p}53**

It is well established that gliomas, particularly the astrocytic type, can become increasingly anaplastic over time. The \textit{p}53 gene is thought to play a significant role in this progression. Sidransky, et al., have shown that low-grade tumors with \textit{p}53 mutations undergo a clonal expansion of the \textit{p}53-mutated tumor cells when those tumors progress to a higher grade. Other studies have shown that although a \textit{p}53 mutation does not predict that a tumor will progress, tumors that do progress tend to have \textit{p}53 mutations early in their course, and the \textit{p}53 mutations may be associated with the reduced time to progression. In \textit{p}53 knock-out mice (mice that do not have a wild-type \textit{p}53 gene) astrocytes have been shown to be more susceptible to transformation stimuli, further indicating that a mutated \textit{p}53 gene may play a significant role in tumor progression.

Not all gliomas that progress have \textit{p}53 mutations. For example, one study indicates two independent paths of progression. In low-grade tumors in which \textit{p}53 mutation was present, tumors progress through an intermediate stop to becoming anaplastic astrocytomas before culminating as glioblastomas multiforme. Those with wild-type \textit{p}53, however, progress directly to becoming glioblastomas multiforme.

Differences in \textit{p}53 expression also occur between recurrent gliomas progressing to glioblastomas and de novo glioblastomas seen primarily in older patients. Those patients harboring a de novo glioblastoma have a relatively low rate of \textit{p}53 mutation. On the other hand, those patients with secondary glioblastoma arising from a previously diagnosed lower-grade glioma have a high rate of mutation, again suggesting a role for \textit{p}53 in tumor progression. This difference is also reflected in studies showing that younger adult patients with glioblastoma are more likely to have \textit{p}53 mutations. These patients are usually more likely to have progressive secondary glioblastoma rather than de novo glioblastoma and tend to survive longer.

**THE PROGNOSTIC ROLE OF \textit{P}53?**

The authors of numerous studies have attempted to address whether the absence or presence of \textit{p}53 mutations has a prognostic role in patients with glioma. Kyritsis, et al., have shown that positive p53 immunoreactivity is associated with a longer survival time in patients with anaplastic astrocytoma and low-grade glioma. They postulated that the tumor cells are unable to correct DNA injury induced by radiation therapy or chemotherapy, leading to better response to treatment. In glioblastoma, however,
they find no relationship between p53 immunoreactivity and patient survival time. The authors of another study found that p53 immunoreactivity was associated with a shorter patient survival time, although no multivariate analysis was performed.[39] Almost all other studies of glioma show no relationship between p53 immunoreactivity and patient survival.[2,6,13,15,16,29,35,67] In an editorial, Dowell and Hall[18] note that studies of larger numbers of patients are more likely to show that p53 has prognostic potential. They point out that this suggests that p53 is at best weakly associated with prognosis and perhaps has no real role in prognostication. It is possible that p53 may be related to other factors that have been correlated with prognosis such as the Ki-67 or proliferating cell nuclear antigen proliferating indices,[28] but those correlations are not always seen.[15] Therefore, at present, the prognostic implication of either a p53 genetic mutation or protein accumulation in gliomas is uncertain.

**THERAPEUTIC IMPLICATIONS OF P53**

Because p53 is instrumental in cell replication and repair processes, its overexpression in glioma is also potentially important from a therapeutic standpoint. For instance, the presence of p53 correlates with both radiation and chemosensitivity. In this regard, p53 is required for the arrest of the G1 phase after ionizing radiation and cells having mutant or no p53 gene fail to exhibit this response.[42,43] Furthermore, inactivation of p53 in glioma cell lines results in a large increase in clonogenic survival when cells are irradiated in the early G1 phase, suggesting a role for G1 arrest in determining cell sensitivity to irradiation.[101] One could envision a role therefore for reintroduction of wild-type p53 into tumor cells as a radiosensitizing measure.

Interestingly, p53 expression may have an opposite role in determining chemosensitivity. Mutations of p53 in glioma may contribute to procarbazine sensitivity by failing to induce arrest at the G1 to S phase cell cycle checkpoint. This in turn could prevent the repair of chemotherapy-induced genetic alterations.[79] Astrocytes with wild-type p53 are also significantly more resistant to 1,3-bis(2-chloroethyl)-1-nitrosourea than astrocytes harvested from animals with inactivated p53.[70] Despite these results suggesting that wild-type p53 is associated with decreased chemosensitivity, when in vitro chemosensitivity testing is performed on freshly excised brain tumors, the mean number of effective chemotherapeutic agents for gliomas without p53 mutation in vitro is much higher than for those with mutations, suggesting that p53 mutation correlates with chemoresistance.[36]

Furthermore, the results of several studies suggest that reconstitution of wild-type p53 can induce gliomas to behave more benignly. For instance, reintroduction of wild-type p53 into p53-deficient glioma cells suppresses tumor cell growth[65,66] and inhibits angiogenesis.[91] This effect of wild-type p53 overexpression even occurs in cells that express the protein; thus, in vitro, the restoration of wild-type p53 encoded protein in mutant cell lines induces apoptosis and in those with wild-type p53, p53 overexpression inhibits cellular proliferation and modifies the neoplastic phenotype.[26] This suggests that inducing p53 overexpression is unlikely to promote tumorigenesis in cells expressing wild-type p53.

Inducing p53 as a therapeutic gene strategy has also been assessed. A p53-specific dose-dependent inhibition of in vitro cellular proliferation in five of six human glioma lines transfected with recombinant adenoviruses encoding wild-type p53 has been demonstrated; growth inhibition was further correlated with gene transfer and expression.[45] Studies in nude mice demonstrated that ex vivo infection with this adenovirus produced tumors that grew more slowly as did direct injection into tumor.[4,45]
CONCLUSIONS

Through its effects on the cell cycle, p53 is an important regulator of cell physiology. Mutation of p53 is an important but not an essential step in tumorigenesis and progression of astroglial neoplasms. Restoring normal p53 function in astroglial tumors may have some therapeutic benefit. Although understanding p53 is not necessarily the key to curing glioma, the study of this gene has already provided useful therapeutic insights.

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