

Molecular Genetics of Tumors of the Central Nervous System

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1. INTRODUCTION

1985 marked the first reporting of a specific gene alteration in a human central nervous system (CNS) tumor: epidermal growth factor receptor (EGFR) gene amplification in glioblastoma (43). Since that time, a relatively short period by most standards, neuro-oncology research has revealed many genetic abnormalities that indicate consistent genotype-phenotype associations for the various cancers that are collectively referred to as CNS tumors. This chapter reviews the established CNS tumor genotype associations, and discusses resulting molecular biologic consequences as well as the clinical implications of these genetic alterations.

2. TYPES OF GENE ALTERATIONS IN CANCER

2.1. *Oncogenes*

As is the case for all human cancers, the genes that are altered in CNS tumors can be grouped into two general categories: (1) oncogenes and (2) tumor suppressors (37). The protein products of oncogenes promote cell proliferation and/or promote other characteristics important to tumor growth, such as invasion, angiogenesis, and resistance to apoptosis. Oncogenes can be activated by increasing the synthesis of their corresponding protein, in normal form, or by alteration of corresponding protein function through gene mutation.

In nervous system tumors, oncogene activation occurs almost entirely by gene amplification. Gene amplification causes an increase in number of a specific gene within a cell, and invariably results in a corresponding increased expression of the gene's encoded protein. In nearly all instances, CNS tumor gene amplifications have been revealed by Southern analysis; a technique in which DNA probes for specific genes reveal elevated gene copy number in tumor DNAs (83).

2.2. *Tumor Suppressor Genes*

As might be suspected from their name, proteins encoded by tumor suppressor genes (TSGs) inhibit cell growth. Their identification has resulted largely through the application of two molecular genetic methods. One of these is linkage analysis that relies upon subtle DNA sequence variations (polymorphisms) between chromosome homologs that allow one to "track" the segregation pattern of a disease-predisposing gene through multiple generations of an affected family (96). In a study of such families, the chromosomal proximity of a polymorphic variant to a cancer-predisposing gene is indicated by the consistency of its co-segregation with the occurrence of cancer within a family. This approach has been useful in identifying and/or associating tumor suppressor genes, such as *TP53*,

(*NF2*), and (*VHL*), with their respective cancer syndromes: Li-Fraumeni (*TP53*), neurofibromatosis type 2 (*NF2*), and von Hippel-Lindau (*VHL*) disease (see Subheading 3.3).

The other approach that has been extensively used for TSG identification is deletion mapping. Deletion mapping is performed through loss of heterozygosity (LOH) analysis (41), in which the patterns of DNA fragments from restriction enzyme digestions or polymerase chain reactions (PCRs) are compared in a patient's normal and tumor DNAs. Loss of a restriction or PCR fragment-length allele in a tumor DNA sample indicates a genetic alteration directed at the deletion of a TSG. By applying a battery of mapped probes (markers) from a chromosome of interest, one can limit the chromosomal location of a TSG by determining the smallest common region of deletion among a panel of similar tumors. This type of analysis has been applied extensively to brain tumors and has revealed several associations between detectable chromosome losses and tumor histopathology.

3. MALIGNANT ASTROCYTOMAS: A DETAILED GENETIC DESCRIPTION

Given the combined concerns of malignant astrocytoma frequency and mortality, it is perhaps to be expected that the details of genetic alterations in these tumors would be the most extensive among the CNS cancers. Although it is likely that additional gene alterations of importance will be discovered in malignant astrocytomas, it is also possible that most and perhaps all of the high-frequency activation/inactivation targets have been identified. Regardless of the possible discovery of additional high-frequency gene alterations, there is sufficient information on hand to provide a reasonably thorough account of genetic events that promote the development of these tumors.

3.1. *Oncogene Alterations in Malignant Astrocytoma: EGFR*

The vast majority of CNS tumor oncogene alterations have been identified in malignant astrocytomas, and in most instances oncogene activation is accomplished through gene amplification. The most frequent oncogene alteration in CNS tumors is amplification of the, *EGFR* (12,43,98). *EGFR* encodes a transmembrane tyrosine kinase that is activated by its binding of epidermal growth factor (Egf), transforming growth factor alpha (TGF- α), as well as other growth factor ligands. The aforementioned discovery as well as specificity of *EGFR* amplification in glioblastoma, or grade IV astrocytoma, has stood up well over the years, although this gene alteration is also observed in grade III anaplastic astrocytoma at a lesser frequency (12,98). Occurrences of *EGFR* amplification in other types of CNS tumors are at best rare events, and consequently the detection of this gene alteration is predictive of high malignancy grade astrocytoma.

In approximately two-thirds of the tumors having *EGFR* amplification, amplified genes undergo intragene deletion rearrangements that result in the overexpression of mutant Egf receptors (20). The most common *EGFR* mutant, Egfr-vIII, is known to have constitutive, ligand-independent tyrosine kinase activity, as well as an extended half-life that stimulates cell proliferation and enhances the tumorigenicity of human glioma cells in nude mice (13,14,54). Furthermore, the activity of this mutant has been shown to promote tumor angiogenesis (17), as well as to confer tumor resistance to programmed cell death by increasing Bcl-XL expression (51).

EGFR amplification and/or overexpression have been evaluated as prognostic indicators in multiple glioma series, and the majority of these studies suggest that increased *EGFR* gene dosage and high level Egf receptor expression are not predictive of patient survival for glioblastoma patients (58,92). However, a recent report indicates that analysis of this gene alteration may be a useful if also considered in the context of patient age (78), whereas another study suggests that detection of Egf receptor mutants in glioblastoma may help predict their differential clinical behavior (16).

3.2. *Other Oncogene Activations in Astrocytomas*

Additional oncogenes whose amplification have been observed in patients with malignant astrocytomas include *MYCN* (4), *CDK4* and *MDM2* (27,67), *CCND1* (27), and *MET* (19), the latter of which, like *EGFR*, is a member of the family of tyrosine kinase growth factor receptors. The reported

amplification frequency for these genes is lower than that for *EGFR*, with the highest being 10–15% for *CDK4* in anaplastic astrocytomas and glioblastomas. There is a positive correlation between amplification and increasing glial tumor malignancy grade for each of these genes.

3.3. Tumor Suppressor Gene Alterations in Malignant Astrocytomas

3.3.1. *TP53*

LOH analysis was of fundamental importance towards identifying the TSG whose inactivation is most frequently involved in the development of malignant astrocytomas, as well as for human cancer in total. The gene, *TP53*, is located at chromosomal region 17p13.1 and is often deleted in astrocytomas (34). The remaining *TP53* copy in a cell with a *TP53* deletion is usually inactivated by a subtle mutation, most of which result in amino acid substitutions that occur in four coding sequence “hot spots” that are located in exons 5 through 8 (29). CNS tumors other than those with predominant astrocytic differentiation do not have an appreciable incidence of *TP53* mutation. With regard to malignancy, studies in which large series of astrocytomas have been examined for *TP53* mutations indicate that similar mutation rates are observed in grade II and grade III tumors, whereas a decreased mutation rate occurs in the glioblastomas (35; see Subheading 3.4.). Although *TP53* mutations are most often observed in sporadic astrocytomas, inherited mutations of the *TP53* gene have been identified in CNS tumor patients with Li-Fraumeni syndrome, an inherited condition that confers an elevated risk for the development of several types of cancer (47).

Results from a few studies support *TP53* mutation status as being of prognostic relevance to astrocytoma patients. In a study of 66 similarly treated anaplastic astrocytoma patients (81), *TP53* mutation was a strong univariate predictor of increased survival. For another investigation, the analysis of p53 expression in a series of 51 astrocytic gliomas, most of which were glioblastoma, showed a statistically significant association between increased p53 expression and disease-free survival (38). Although not examined in the latter study, elevated p53 immunohistochemical reactivity is known to be highly predictive of *TP53* mutation.

3.3.2. *CDKN2A*

The existence of at least two more astrocytoma TSGs, in addition to *TP53*, had been predicted by results from cytogenetic and loss of heterozygosity studies conducted during the 1980s (3,33), but these genes were slow to be discovered as a result of, primarily, the relative lack of available human genome sequence during that time (see Subheading 7.1). One of the TSGs was believed to reside on the short arm of chromosome 9 that is frequently deleted in astrocytomas. Although the 9p deletions were initially localized to a relatively large region that generally includes the centromere-proximal end of the interferon alpha gene cluster, it is now clear that the *CDKN2A* gene, which resides close to the interferon genes, represents the primary chromosome 9p deletion target (36,56). However, there are two additional genes within as well as near *CDKN2A* that are also thought to have a growth-suppressive function: *CDKN2B* and *p14^{ARF}*. Deletion of both copies of all these genes, along with *CDKN2A*, occur in a variety of cancers, including malignant astrocytomas. Unlike *TP53*, however, for which cancer mutations are common, mutations of the three 9p TSGs have been reported in very few instances. However, a lack of *CDKN2A*-encoded protein, p16, has been shown in a significant fraction of astrocytomas having intact *CDKN2A* genes, indicating that loss of p16 expression can occur in the absence of a corresponding gene alteration. In at least some of these cases, the loss of expression appears to be associated with *CDKN2A* gene hypermethylation (53). As opposed to *TP53* mutations which are observed at a decreasing frequency with increasing tumor malignancy, *CDKN2A* deletions occur more frequently with increasing glioma malignancy grade; *CDKN2A* alterations are also observed more frequently with increasing astrocytic composition of the tumor (35).

3.3.3. Phosphatase Tensin Homolog (*PTEN/MMAC1*) Inactivation

The most recent and possibly final of the frequent TSG mutations that has been discovered in association with astrocytoma development involves the phosphatase tensin homolog (*PTEN*) gene

(also referred to as *MMAC1*) (42,85) that resides at chromosomal location 10q23. As for *TP53*, *PTEN* inactivation is often accomplished through deletion with mutation of the remaining allele. *PTEN* has been shown to be inactivated in up to 44% of all glioblastomas, and in 60% of glioblastomas having 10q deletions (93). These results are consistent with *PTEN* being the primary target of inactivation associated with chromosome 10 loss that was originally observed in glioblastoma multiforme by cytogenetic analysis and subsequently by deletion mapping (3,33).

The encoded protein of *PTEN*, *Tep1*, has been shown to have dual-specificity phosphatase activity (tyrosine and serine) (50), and recent evidence suggests that its biologically relevant targets include inositol phospholipids and proteins. Among the phospholipid substrates is phosphoinositol triphosphate (46), that promotes the activity of Akt, a serine/threonine kinase that is an important regulator of cell survival and cell proliferation (8). Additionally, *Tep1* modulates cell migration and invasion by negatively regulating the signals generated at the focal adhesions (87) through the direct dephosphorylation and inhibition of focal adhesion kinase. *Tep1* can also act as a negative regulator of receptor tyrosine kinase signaling through its inhibition of the adaptor protein Shc (25), and in this regard the activity of *Tep1* can be viewed as being antagonistic to Egf receptor function.

PTEN genetic alterations represent the TSG inactivation most highly associated with advanced-stage astrocytoma malignancy, and results are accumulating that suggest that the genetic status of *PTEN* is an important prognostic variable in malignant astrocytoma. Lin et al. (44) used LOH analysis to examine the *PTEN* locus in 110 such tumors and showed that *PTEN* LOH was a significant predictor of shorter survival. A similar conclusion was reached from the analysis of *PTEN* mutations in pediatric malignant astrocytomas (63). More recently, *PTEN* status in anaplastic astrocytomas has been shown to be an important independent variable in predicting survival, with reduced survival being associated with detectable *PTEN* mutation (81).

3.4. Molecular Genetics Studies Suggest Two Types of Malignant Astrocytoma

Current thinking regarding the genetic pathogenesis of malignant astrocytoma views these tumors as arising through one of two mechanisms: (1) either from a series of genetic steps, with each step conferring an additional, incremental growth advantage to a tumor becoming progressively more malignant, or (2) as a result of a key gene alteration that “spontaneously” produces a tumor of high grade malignancy (55). The major genetic determinants that distinguish the two types of GBM are *EGFR* amplification and *TP53* mutation, with the first being predominantly associated with the spontaneous variant, and the latter being primarily associated with GBMs arising from astrocytoma malignant progression (Fig. 1). In one study, it was shown that the incidence of *TP53* mutations was approximately sixfold less in *de novo*, primary glioblastomas than in secondary glioblastomas that had undergone malignant progression from a lower malignancy precursor (95). The significance of glioblastoma classification is related to potential differences in clinical behavior between the two glioblastoma multiforme (GBM) types, and, consequently, their genetic classification will continue to be of interest. From the cumulative data that has been published on the genetic origin of glioblastoma, it is possible to construct a model that shows the characteristic alterations giving rise to each type of this tumor (Fig. 2).

4. ONCOGENE ACTIVATIONS IN OTHER CENTRAL NERVOUS SYSTEM TUMORS

Oncogene alterations have not been shown to occur frequently in other types of CNS tumors. Although several reports have revealed *CMYC* and *MYCN* amplification in primitive neuroectodermal tumor (PNET)/medulloblastoma, the cumulative data from such studies suggest that these gene alterations are infrequent, and have a combined incidence of less than 10% (64,94). Presumed activating mutations of β -catenin, a regulator of T-cell-specific transcription factors (TCF)-mediated transcription, have also been shown in a minor proportion of PNET/medulloblastoma (100). The only

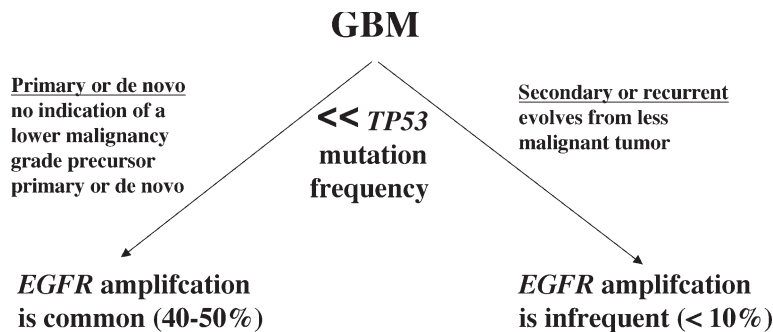


Fig. 1. Association of *EGFR* amplification and *TP53* mutation with primary (*de novo*) and secondary (recurrent) glioblastoma, respectively. *EGFR* amplifications are as much as 5 times more common in primary GBM, whereas *TP53* mutations occur much more frequently in recurrent GBM.

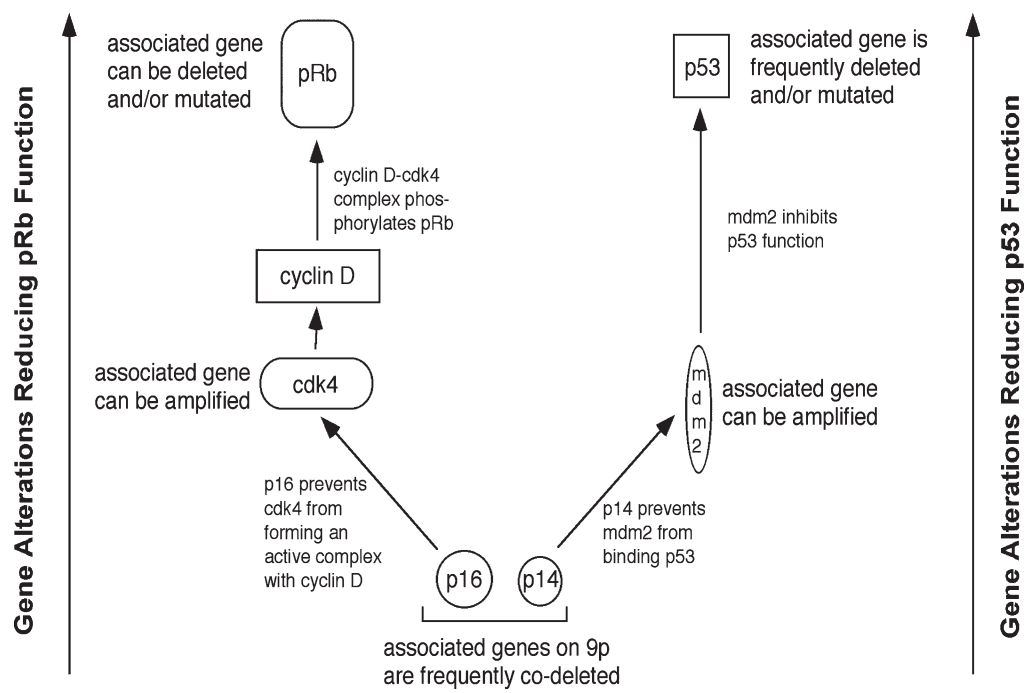


Fig. 2. Gene alterations effecting retinoblastoma (Rb) and/or p53 function. Chromosome 9p deletions occur frequently in malignant astrocytomas, and simultaneously inactivate both *CDKN2A* (encoding the p16 protein) and *p14^{ARF}* genes. *CDK4* and/or *MDM2* gene amplifications are often observed in malignant astrocytomas having intact chromosome 9p tumor suppressor genes. In malignant astrocytomas without alterations of *CDKN2A*, *p14^{ARF}*, *CDK4*, or *MDM2*, inactivating mutations of *RB* and *TP53* are found in nearly all instances.

other gene alteration that has been suggested to be anything more than a rare event in any group of CNS tumor involves the amplification of *PDGFRA* in highly malignant oligodendrogliomas (vascularization and/or necrosis), of which some 20% may be affected (82).

5. TUMOR SUPPRESSOR GENE ALTERATIONS IN OTHER CENTRAL NERVOUS SYSTEM TUMORS

5.1. PNET/Medulloblastoma

The identification of tumor suppressor genes whose inactivation are involved with the development of PNET/medulloblastoma has been an area of active research for several years, but one that has only recently yielded an accepted TSG target. The gene, patched homolog (*PTCH*), was discovered as a result of its mutation being associated with predisposition to nevoid basal cell carcinoma (NBCC) syndrome, an inherited condition in which there is occasional development of medulloblastoma as well as the more commonly occurring nevoid basal cell carcinomas (26). The *PTCH* gene is thought to be inactivated in approx 20% of sporadic medulloblastoma, and mutation/deletion of *PTCH* appears to preferentially occur in the desmoplastic subtype of this tumor (65,97). Additional investigations have been conducted to determine whether genes that encode Ptc-interacting proteins are mutated in medulloblastoma, but their results have yet to support this speculation (69).

5.2. Oligodendroglioma

TSG alterations that are frequently or specifically associated with oligodendroglial tumors have yet to be identified, although cytogenetic and deletion mapping studies support the existence of two such genes. Allelic losses of 19q occurs in 50–80% of oligodendroglial tumors and, with rare exception, involve the entire 19q chromosomal arm (68,91). The chromosome 19q deletion region has been progressively narrowed to an interval occupying a portion of 19q13.3 (70,79), allowing for candidate genes within this region to be examined for mutation. The incidence of 19q deletion is not significantly different between low- and high-grade oligodendrogliomas, suggesting that this alteration is an early event in the neoplastic development of these tumors (68). This finding contrasts with the 19q loss observed in astrocytic gliomas that is generally restricted to the high-grade cases (79).

Deletion of chromosome 1p is another frequent event in oligodendrogliomas, occurring in 40–90% of these tumors (2,68). Interestingly, nearly all cases of oligodendroglioma studied with deletion of 1p also exhibit deletion of 19q, suggesting that inactivation of one or more genes on each of these chromosomal arms is an important event in oligodendroglioma development. Data from a recent report showed two distinct deletion regions on 1p, D1S76-D1S253 at 1p36.3 and D1S482-D1S2743 at 1p34-35, and these contain several candidate TSG targets (30).

Evaluation of chromosomal arms 1p and 19q are of prognostic significance for the oligodendroglioma patient. Cairncross et al. (7), examined 39 anaplastic oligodendroglioma patients, 37 of which had received procarbazine, lomustine, and vincristine (PVC) chemotherapy. Allelic loss of 1p was a statistically significant predictor of chemosensitivity, and combined loss of 1p and 19q were significantly associated with both chemosensitivity and longer recurrence-free survival following chemotherapy. Moreover, Smith et al. (80) have demonstrated that the association of 1p and 19q loss with prolonged survival is also evident in low grade oligodendroglioma patients, but that this association may be independent of PCV chemotherapy.

5.3. Pilocytic Astrocytoma

Inheritance of a mutated *NF1* gene (chromosome location 17q11) predisposes to type 1 neurofibromatosis, a syndrome characterized by the development of neurofibromas, cafe-au-lait-spots, and an increased risk for pheochromocytomas, schwannomas, neurofibrosarcomas, and primary brain tumors such as optic gliomas and pilocytic astrocytomas (61). Pilocytic astrocytomas occurring in the absence of NF1 syndrome may also be from *NF1*-inactivating mutations as deletions of this gene have been found in up to 20% of such tumors (90). *NF1* encodes a GTPase-activating protein, neurofibromin, that has been shown to down-regulate the activity of ras, an important effector of receptor tyrosine kinase signaling (49).

5.4. Acoustic Neuromas and Schwannomma

Frequently, acoustic neuromas and schwannomas are observed in patients with neurofibromatosis type 2, and inherited *NF2* gene (chromosomal region 22q12) defects are responsible for these tumors (61). It is therefore not unexpected that somatic *NF2* gene mutations have been observed in a majority (> 60%) of sporadic schwannomas (32,71). The *NF2* gene product, merlin/schwannomin, is a cytoskeleton-associated protein whose function is important to the regulation of cell adhesion (24).

5.5. Hemangioblastoma

VHL syndrome is a consequence of germline *VHL* gene mutations and is characterized by predisposition to the development of hemangioblastomas of the CNS and retina, as well as to other malignancies (renal cell carcinomas, pheochromocytomas, etc.) (9). In addition to its association with hemangioblastoma in VHL patients, somatic mutations of the *VHL* gene are seen in up to 40% of sporadic hemangioblastomas (88). Functional analyses indicate that the *VHL* gene product is an inhibitor of transcription elongation (11). In addition, the VHL protein has been implicated in controlling the expression of vascular endothelial growth factor (VEGF) (23), a potent angiogenesis factor.

6. RELATIONSHIPS BETWEEN GENE ALTERATIONS IN ASTROCYTOMAS AND CELL CYCLE REGULATION

6.1. *p53-mdm2-p14^{ARF}-p21*

Unrestricted cell multiplication represents a hallmark feature of cancer, and this process is a result of continued cell-cycle progression. In normal cells, cell cycling is kept under control by a complex system of positive and negative regulators that constitute a series of checkpoints. One of the most important of these checkpoints consists of the p53, mdm2, p14^{ARF}, and p21 proteins that regulate progression of cells through the G1 cell-cycle phase.

The loss of p53 function is known to promote accelerated growth and malignant transformation of astrocytes both in vitro and in vivo (5,99). In human cancer, it was initially believed that p53 protein function could only be compromised through *TP53* gene deletion or mutation. However, a considerable amount of information has emerged during the past few years that indicates that p53 function is effected by other cellular proteins. Important among these is mdm2 that binds to, destabilizes, and inactivates p53 (57). Significantly, amplification of the *MDM2* gene has been demonstrated as an alternative mechanism to inactivating mutations of *TP53* in astrocytomas (66). *MDM2* gene amplification has been reported in up to 10% of anaplastic astrocytomas lacking *TP53* mutations, and the combined frequency of *TP53* and *MDM2* gene alterations indicates the inactivation of p53 function in approximately one-half of these tumors (66).

Mdm2-mediated destabilization of p53 is inhibited by p14^{ARF}(62), the corresponding gene for which resides mostly within the coding sequence of the p16 gene, *CDKN2A* (48). As a result of its overlapping localization with *CDKN2A*, both copies of the *p14^{ARF}* gene are often deleted in astrocytomas. Consequently, the 9p alterations, that are so common in these tumors, contribute to aberrant p53 function by promoting increased interaction between p53 and mdm2 (Fig. 3). Because of the relationships between p53, p14^{ARF} and mdm2, it can be argued that nearly all malignant astrocytomas have compromised p53 function from an alteration of one of the corresponding genes for these proteins (22).

The activity of wild-type p53 is known to promote the synthesis of the universal cyclin-cdk inhibitor p21-waf1-cip1 (15), and this is thought to prevent the replication of altered DNA in normal cells that have incurred DNA damage (10). Because the synthesis of p21 is stimulated by wild-type p53 activity, *TP53* gene inactivation, *MDM2* amplification, or *p14^{ARF}* gene deletion can contribute to reduced p21 synthesis, and thus promote the accumulation of gene alterations in tumor cells as a result of the reduced function of a checkpoint preventing the synthesis of damaged DNA. Although reduced p21 expression appears to play an important role in tumor development, there has been no demonstration of the p21 gene itself as a mutagenic target in human cancers (77).

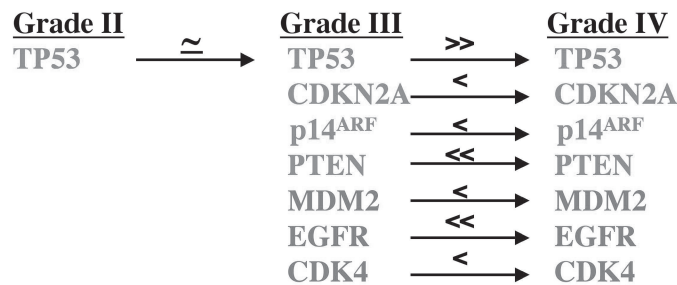


Fig. 3. Associations between gene alterations and astrocytoma malignancy grades. Similar frequencies of *TP53* mutations are observed among grade II and grade III astrocytoma, with their incidence being much higher than is observed among glioblastomas. All other astrocytoma signature gene alterations are most common among glioblastomas, with *EGFR* amplifications and *PTEN* mutations being most highly associated with grade IV malignancy.

6.2. *p16-cdk4-Rb-Cyclin D*

Another important G1 checkpoint is constituted by the p16, Rb, cdk4, and cyclin D proteins. The protein encoded by the *CDKN2A* gene, p16, acts as a negative regulator of cell growth and proliferation through its binding to cdk4 protein kinase and preventing it from forming an activated complex with cyclin D proteins (75). The primary substrate of this complex is the retinoblastoma protein (45), Rb. In its hypophosphorylated form, Rb arrests cells at the G1/S cell-cycle checkpoint. This checkpoint is abrogated when Rb is phosphorylated, and cyclin D1-cdk4 has been shown to phosphorylate most of the retinoblastoma sites in vitro that are phosphorylated in vivo during late G1.

In association with the proposed model relating the activities of these proteins, one might anticipate the existence of at least three tumor-associated mechanisms for suppression of retinoblastoma function (Fig.3): (1) inactivation of p16; (2) increased expression of cdk4; or (3) inactivation of the retinoblastoma protein. Consistent with this hypothesis, *CDK4* gene amplification and associated overexpression of cdk4 protein has been determined to occur in gliomas with intact and expressed *CDKN2A* genes (28,73). Furthermore, it has been shown that loss of Rb expression, in association with inactivating *RB* gene mutations, generally occurs in glial tumors and cell lines for which there is no evidence of *CDKN2A* or *CDK4* gene alterations (28,89).

It is generally thought that the function of cdk4, p16, or Rb is altered during the malignant transformation of nearly every malignant astrocytoma (31). Interestingly, molecular genetic studies have shown that one and only one of the corresponding genes for these proteins is altered in each tumor, suggesting that a single alteration within the pathway is sufficient to disrupt its regulatory function. Although the prognostic significance of this checkpoint's alteration in astrocytoma is unclear, one study suggests that detection of *CDKN2A* deletions in tumors from patients with oligodendroglioma, albeit an infrequent event, is significantly associated with decreased survival, and additionally occur in tumors having intact copies of chromosomes 1 and 19 (7).

Because the activity of cdk4 is dependent on its binding to D-type cyclins, one might predict that increased cyclin D synthesis would contribute towards oncogenesis by promoting the formation of active cyclin D/cdk4 complexes. Increased cyclin D1 expression in association with gene amplification has been reported in a number of cancers (59), but is uncommon in gliomas. However, it has been shown that cyclin D1 expression is increased by stimulating receptor tyrosine kinase activity (76), and on this basis it is reasonable to speculate that the increased receptor tyrosine kinase activity that commonly occurs in malignant gliomas, usually in association with *EGFR* gene amplification or alteration, may play an important role in promoting cyclin D expression and thereby contribute to Rb protein inactivation.

7. MOLECULAR GENETICS IN THE STUDY AND TREATMENT OF CNS TUMORS: NOW AND THE FUTURE

7.1. Microarrays

At the time of this writing, the ability to perform comprehensive analyses of gene expression patterns in human tumors is nearly at hand, and the application of this analysis, through use of microarrays (6), is already generating substantial information regarding the identities of genes that are consistently overexpressed or underexpressed in specific types of cancer (40).

Microarrays consist of a solid support template upon which thousands of DNAs, representing coding sequences of different genes, are placed. These arrays or “chips” are used for competitive hybridizations of normal and tumor tissue cDNA pairs. Overrepresentation of a tumor cDNA (synthesized from corresponding tumor mRNA) at a specific coordinate on the array indicates overexpression of the gene whose nucleotide sequence was spotted onto that coordinate. Hybridizations can be carried out with DNAs labeled with isotopes or with fluorochromes. From a time-cost perspective the potential efficiency of this process for providing extensive information on gene expression patterns is significant, and is allowing for the development of databases containing expression profiles for many common cancers (Cancer Genome Anatomy Project: CGAP; <http://cgap.nci.nih.gov/>). Microarray technology is being extended to the detection of gene sequence alterations, and a first generation model for *TP53* mutation detection has been marketed (1). In addition, comprehensive genome arrays for the detection of gene amplification and gene deletion are also being developed (84).

Developments in microarray technology have been largely driven by the progress and completion of the Human Genome Project (<http://www.ncbi.nlm.nih.gov/genome/guide/human/>). In 1989, The Department of Energy and the National Institutes of Health began funding this project whose purpose is to provide a series of linked data sets containing the genetic and physical location of all genes on each human chromosome, plus the complete nucleotide sequence of the human genome. This initiative has provided us with a complete and accurate whole genome sequence containing a genetic blueprint of the human species. With respect to its gene identification and localization objectives, more than 30,000 genes have been localized to specific chromosomal regions with a high degree of accuracy. The total gene content within our cells is not currently known, but most estimates place it near 50,000. The human genome map that currently exists can already be applied to the identification and isolation of genes that either directly cause disease, or increase susceptibility to disease. It is obvious that the information being generated by the project, when combined with emerging technologies such as the microarrays, will allow for the rapid and, in many cases, complete diagnosis of specific genetic lesions in individual brain tumors.

7.2. Fluorescence In Situ Hybridization Tissue Arrays

Recently, molecular genetic techniques have been combined with conventional cytogenetic methods to produce new procedures for identifying chromosomal alterations in cancers. The resulting molecular cytogenetic procedures have not only helped to make infrequently used archival material amenable to genetic analysis, but have also provided information leading to the identification of novel gene alterations. One method that is proving to have a significant impact in clinical genetic practices for diagnosing specific types of cancer is fluorescence *in situ* hybridization (FISH). The FISH method involves the fluorescent labeling of relatively large segments of cloned human DNA. The cloned DNA segments, each of which has been previously determined to contain known genes from specific chromosomal regions, can be hybridized to either isolated metaphase chromosomes or to intact interphase nuclei. In many instances the probes can be used to find their target sequence in cells that have been embedded and preserved in paraffin. By labeling different probes with different fluorochromes it is possible to examine multiple chromosomes for alterations. There is, in fact, a

Table 1
Signature Gene/Chromosomal Alterations Associated With CNS Tumor Subtypes

Tumor types	Genes/chromosomes	Frequency
Glioblastoma and anaplastic astrocytoma	EGFR ^{a,m,*}	30–40% ^{GBM} 10–15% ^{AA}
	CDK4 ^a	10–15%
	MDM2 ^a	5–10%
	TP53 ^{d,m,*}	20–30% ^{GBM} 30–40% ^{AA}
	CDKN2A ^d	30–40%
	PTEN ^{d,m}	25–30% ^{GBM} 10% ^{AA}
Astrocytoma	RB ^{d,m}	10–15%
Oligodendroglioma	TP53 ^{d,m}	30–40%
	Chromosome 1p ^d	40–90%
Medulloblastoma/PNET	Chromosome 19q ^d	50–80%
	MYCN ^a	5–10%
	CMYC ^a	5–10%
	PTCH ^{†d,m}	10–20%
	β-catenin ^{†m}	5%
Pilocytic astrocytoma	Chromosome 17p ^d	30–50%
Schwannoma	Chromosome 17q ^d	20–30%
Hemangioblastoma	NF2 ^{d,m}	50–60%
	VHL ^{d,m}	10–20%

Type of gene alterations: ^a, amplification; ^d, deletion; ^m, mutation. Chromosome arms are listed in instances where the corresponding gene alteration is yet to be identified.

*Frequency in different glioblastoma series is strongly influenced by proportion of *de novo* vs secondary tumors.

† Observed primarily in desmoplastic variant.

derivative of FISH known as spectral karyotyping (SKY) (74), in which probes for each chromosome are labeled with a different fluorochrome or combination of fluorochromes, and simultaneously hybridized to normal metaphase chromosome preparations. Although yet to be extensively applied to the study of brain tumors, this technique may prove to be useful for the analysis of complex karyotypes that are typical of many nervous system malignancies.

8. DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS

8.1. CNS Tumor Diagnosis

This chapter has made reference to the specificity of certain gene alterations for CNS tumor malignancy grade and cellular differentiation (Table 1). Whether molecular genetic analysis will become as efficient and cost-effective as conventional histopathologic analysis for the diagnosis of CNS tumors remains to be seen, but there are an increasing number of reports in the literature that indicate important associations between gene alterations and outcome for CNS tumor patients. Because of the increasing detail of the information that can be obtained through molecular genetic analysis, it seems likely that the accuracy of predicting clinical behavior for individual tumors will similarly increase. At a minimum, one would suppose that genetic testing of all tumor types will be viewed as a necessary component of diagnostic services.

8.2. Central Nervous System Tumor Treatment

The characterization of the genetic mechanisms associated with malignant transformation has opened the way to test novel molecular therapeutic modalities, such as the delivery of small molecules that target disrupted growth-regulatory pathways. Examples of small molecules that may be useful in targeting unbalanced pathways include cdk4 inhibitors (72), farnesyltransferase inhibitors (18), and inhibitors of Egf receptor-associated tyrosine kinase activity (21). More recently, there have been indications that tumor cells with *PTEN* mutations show increased sensitivity to growth inhibition by rapamycin, which targets the mammalian target of rapamycin/FKBP rapamycin-associated protein (mTOR/FRAP) protein (52,60). Knowing whether a tumor has a gene alteration that affects the function of a protein that is being used as a therapeutic target could be critical to determining the success of the agent. For example, it is clear that p53 function is fundamentally important to determining the manner in which cells respond to radiation-induced DNA damage (39). Consequently, information regarding a tumor's *TP53* gene status may help determine a patient's response to radiation treatment: at least one report suggests this is the case for glioblastoma patients (86).

The identification of specific genetic lesions, in combination with promising new therapeutic strategies that are dependent upon the knowledge of tumor genotypes, should greatly facilitate the development of effective, individualized therapies for patients with CNS tumors. It is reasonable to suspect that knowledge of tumor genotypes will soon play an important part in the clinical decision-making process for all cancer patients, and that this information will result in improved patient care.

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