Part III

Genetics and Epidemiology of Primary Tumors

9

Genetic and Molecular Basis of Primary Central Nervous System Tumors

C. DAVID JAMES, JUSTIN S. SMITH, AND ROBERT B. JENKINS

Our understanding of the genetic etiology of nervous system tumors has advanced considerably during the last decade. During this time, investigators studying genetic alterations in these tumors were able to make several successful transitions from cytogenetic observations to the identification of specific genes that are targeted for mutation. The new information associated with the results of their investigations will benefit patients with nervous system cancer in at least two ways. One benefit involves the predictive value associated with the identification of specific gene alterations, that is, it is clear that certain mutations are consistently associated with specific clinical behaviors. A second benefit concerns the ability of molecular genetics to provide insights into the fundamental mechanisms associated with tumor development and, in so doing, provide information about potential therapeutic targets. In the near future, it seems likely that concepts that have evolved from this area of study will allow for the application of individualized treatments that will extend the length and quality of life for people afflicted with nervous system cancer.

MOLECULAR AND CYTOGENETIC METHODS

Cytogenetics

Cytogenetic studies provided the earliest clues concerning the genomic locations of genes whose alterations are associated with nervous system tumor development. Many of the karyotypic abnormalities described in the literature are based on the study of glioblastoma multiforme, the most common and malignant central nervous system tumor. Anomalies that occur frequently in glioblastoma include gain of chromosome 7, loss of chromosomes 10 and 22, and structural alterations of chromosomes 1p, 9p, 11p, 12q, and 13q (Bigner et al., 1984). In addition, double-minute chromosomes are often observed in these tumors; their presence suggests the occurrence of gene amplification (Bigner et al., 1987). Alterations associated with other types of glial tumors include loss of chromosomal arms 1p and 19q in tumors with oligodendroglial differentiation (Ransom et al., 1992) and loss of chromosome 22 in ependymomas (Ransom et al., 1992; Weremovicz et al., 1992).

Whereas the most frequent chromosomal anomalies in gliomas involve numerical deviations, medulloblastomas exhibit predominantly structural chromosomal abnormalities that often involve chromosomes 1, 3, 6, 10, 17, and 20 (Bigner et al., 1988; Biegel et al., 1989); among these, isochromosome 17q, with associated 17p loss, appears to be the most frequent. With regard to mesodermal tumors, loss of chromosome 22 occurs frequently in meningiomas and has also been reported in a significant proportion of schwannomas (Zang, 1982; Stenman et al., 1991).

Fluorescence In Situ Hybridization

In the past few years, molecular techniques have been combined with conventional cytogenetic methods to

develop new procedures for identifying chromosomal alterations in brain tumors. 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. The first of these to be discussed is referred to as FISH (fluorescence in situ hybridization). Although FISH was initially applied to the study of chromosome structure in 1986 by Pinkel et al., the widespread use of this technique in a clinical setting has not been achieved until recently.

The FISH method involves the fluorescent labeling, either directly or indirectly (e.g., biotin labeling followed by fluorescence-labeled avidin detection), 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 (Color Fig. 9–1). By labeling different probes with different fluorochromes it is possible to examine multiple chromosomes for alterations, and, in fact, there is a derivative of FISH known as spectral karyotyping (SKY) (Schrock et al., 1997) in which 23 chromosome-specific probes, each labeled with a different fluorochrome or combination of fluorochromes, are simultaneously hybridized to metaphase preparations. Although yet to be extensively applied to the study of brain tumors, this technique may prove useful for the analysis of complex karyotypes that are typical of many nervous system malignancies.

Comparative Genomic Hybridization

An additional molecular cytogenetic method that has been efficacious in identifying chromosomal and gene alterations in solid tumors is comparative genomic hybridization (CGH) (Kallioniemi et al., 1992). This method is based on the competition between two different DNAs, one from a tumor and one from a normal tissue, for hybridization to normal metaphase chromosomes. Before hybridization, the normal and tumor DNAs are labeled with different fluorochromes (usually red and green, respectively), and as a result most regions of hybridized metaphase chromosomes show a fluorescence color combination that is equally balanced between the two labeled DNAs. When the tumor DNA shows either a gain or loss of genetic material, there is either an increase or decrease in green fluorescence, respectively, at points on the normal chromosomes where the gene alteration has occurred (Color Fig. 9–2).

This technique has provided information beyond that available through conventional cytogenetics for a variety of brain tumors (Kim et al., 1995; Reardon et al., 1997; Weber et al., 1997) and has suggested several chromosomal locations of genetic alterations for which there are no established mutation targets. Findings obtained through the application of this technique have provided an effective springboard from which to launch positional cloning projects and/or from which to examine databases containing the chromosomal locations for thousands of genes so that candidate sequences associated with specific chromosomal alterations can be examined.

Molecular Genetics

Linkage Analysis

Linkage analysis, as studied with molecular genetic methods, relies on subtle DNA sequence variations (called *polymorphisms*) between chromosome homologues that allow one to "track" the segregation pattern of a disease-predisposing locus (gene) through multiple generations of an affected family (White and Lalouel, 1988). In the study of such families, the chromosomal proximity of a DNA marker (probe) to a cancer-predisposing gene is indicated by the consistency of the marker's co-segregation with the occurrence of cancer within the family. This approach has been useful in identifying and/or associating tumor suppressor genes (TSGs) such as *TP53, NF2,* and *VHL* with their respective cancer syndromes: Li-Fraumeni, neurofibromatosis type 2, and von Hippel-Lindau.

Loss of Heterozygosity (LOH) Analysis

DNA polymorphisms have also been utilized to locate TSGs in sporadic tumors through a process known as *deletion mapping.* A chromosomal deletion map is obtained through the application of loss of heterozygosity (LOH) analysis (Lasko et al., 1991) in which the patterns of restriction enzyme or polymerase chain reaction (PCR) DNA fragments are compared in a patient's normal and tumor DNAs. Loss of a restriction or PCR fragment-length allele in a tumor DNA sample is indicative of 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 alterations and tumor histopathology.

GENES IMPORTANT TO NERVOUS SYSTEM TUMORS

Classification of Cancer Genes

As in all human cancers, two families of genes appear to be involved in the pathogenesis of brain tumors: oncogenes and tumor suppressor genes (Nagane et al., 1997). The protein products of oncogenes promote cell proliferation, and oncogenes can be activated by increasing the synthesis of their corresponding protein, in its normal form, or by alteration of corresponding protein function through gene mutation. In general, oncogene alterations only involve one of the two copies of a specific oncogene within a cell. In contrast, both copies of a specific tumor suppressor gene (TSG) must be inactivated through deletion or mutation for a cell to gain a growth advantage. As might be suspected from their name, proteins encoded by TSGs inhibit cell growth. Table 9–1 summarizes the oncogene and TSG alterations important to the development of various types of nervous system tumors, and following are brief discussions of specific genes that are frequently altered during the development of specific types of tumors.

Table 9–1. Gene and Chromosomal Alterations Associated with the Development of Central Nervous System Tumors

Tumor Types	*Genes/Chromosomes*	*Frequency*
Gliobastoma 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)
	RB (d, m)	10%–15%
Astrocytoma	TP53 (d, m)	30%–40%
Oligodendroglioma	Chromosome 1p (d)	40%–90%
	Chromosome 19q (d)	50%–80%
Ependymoma	Chromosome 22 (d)	25%–50%
Medulloblastoma	MYCN (a)	5%–10%
	CMYC (a)	5%–10%
	PTCH (d, m)	10%–20%
	Chromosome 17p (d)	30%–50%
Pilocytic astrocytoma	Chromosome 17q (d)	20%–30%
Meningioma and schwannoma	NF2 (d, m)	50%–60%
Hemangioblastoma	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. GBM, gliobastoma multiforme; AA, anaplastic astrocytoma.

*Frequency in glioblastoma series strongly influenced by proportion of primary and secondary tumors.

Oncogenes

EFGR. In nervous system tumors, specifically gliomas, oncogene activation appears to exclusively result from 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. The vast majority of brain tumor gene amplifications occur in astrocytomas, and of the oncogenes involved the most frequently amplified is the epidermal growth factor receptor *(EGFR)* gene (Libermann et al., 1985; Wong et al., 1987; Ekstrand et al., 1991). The *EGFR* gene encodes a transmembrane tyrosine kinase that is activated by its binding of the growth factors epidermal growth factor (EGF) and transforming growth factor-α. With regard to cytogenetics, this gene alteration is manifested as double-minute chromosomes that contain the amplified genes and are observed in nearly 40% of glioblastomas (Bigner et al., 1987). *EGFR* amplification occurs much less frequently in lower grade gliomas and has not been reported in nervous system tumors other than gliomas.

In approximately 50% of the patients having *EGFR* amplification, the amplified genes undergo intragene deletion rearrangements that result in the overexpression of a mutant receptor that lacks a portion of the extracellular domain (Sugawa et al., 1990). This mutant has been shown to have constitutive 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 (Ekstrand et al., 1994; Nishikawa et al., 1994; Ekstrand et al., 1995). Gene alterations affecting the receptor's intracellular domain have also been reported (Eley et al., 1998).

EGFR amplification and/or overexpression have been evaluated as prognostic indicators in multiple glioma series, and the majority of these studies indicate that increased *EGFR* gene expression and gene dosage are not predictive of patient survival once tumor grade is taken into account (Olson et al., 1998). However, a recent report suggests that analysis of this gene alteration may be a useful prognostic variable if also considered in the context of patient age (Smith et al., 2001). The associated study showed that *EGFR* amplification was significantly correlated with shorter survival among glioblastoma patients younger than 40 years of age, while in patients aged 60 years or more amplification was associated with extended survival.

Other Oncogenes. Additional oncogenes whose amplification have been observed in patients with malignant gliomas include *MYCN* (Bigner et al., 1987), *CDK4* and *MDM2* (Reifenberger et al., 1994a; He et al., 1994), *CCND1* (He et al., 1994), *PDGFRA* (Fleming et al., 1992), and *MET* (Fischer et al., 1995), the latter two of which, like *EGFR,* are members of the family of tyrosine kinase growth factor receptors. The reported amplification frequencies for these genes are lower than that for *EGFR,* with the highest being 10% to 15% for *CDK4* in anaplastic astrocytomas and glioblastomas. There is a positive correlation between amplification and increasing glial tumor malignancy grade for each of the genes mentioned above.

Tumor Suppressor Genes (TSGs)

TP53. Loss of heterozygosity analysis was instrumental in identifying the TSG whose inactivation is most frequently involved in tumor development. The gene, *TP53,* is at chromosomal location 17p13.1, a site that is often deleted in astrocytomas (James et al., 1989). The remaining copy of *TP53* in an affected cell is usually inactivated by a subtle mutation, most of which results in amino acid substitutions that occur in four "hot spots" in exons 5 through 8 (Hollstein et al., 1991). No type of brain tumor other than those with predominant astrocytic differentiation have been shown to have appreciable *TP53* mutation rates, and 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, while a decreased mutation rate occurs in the glioblastomas (grade IV). Although *TP53* mutations have most often been observed in sporadic astrocytomas, inherited mutations of the *TP53* gene have been identified in the majority of brain tumor patients with Li-Fraumeni syndrome, an inherited condition that additionally confers an elevated risk for the development of sarcomas, breast cancer, and leukemia (Malkin, 1993).

Normal p53 protein induces the transcription of genes that promote cell cycle arrest and apoptosis, including p21 and bax, respectively. Because of p53's role in regulating these cell responses, the loss of p53 function has been shown, in independent studies, to promote the accelerated growth and malignant transformation of astrocytes (Bogler et al., 1995; Yahamada et al., 1995). In these investigations, primary cortical astrocytes isolated from mice bred to lack

any p53 function (−/−), but not from mice homozygous for normal p53 (+/+), were shown to grow rapidly in culture and with limited contact inhibition. With continued passaging, p53 null (−/−) astrocytes exhibited a multistep progression to a transformed phenotype indicated by their ability to form large, well-vascularized tumors in nude mice. Cells heterozygous (+/−) for wild-type p53 generally did not grow well in culture, but on occasion would form colonies that expanded rapidly. In such instances it was determined that these colonies had arisen from cells that had inactivated their single, normal *TP53* gene, causing them to become p53 (−/−). These results, which suggest p53 inactivation as an early event in astrocytoma development, may be consistent with clinical data indicating that constitutional (germline) *TP53* mutations predispose individuals to the development of astrocytic tumors at a relatively young age (Chen et al., 1995).

Results from a few studies suggest that *TP53* mutation status is of prognostic relevance to astrocytoma patients. In a study of 66 similarly treated anaplastic astrocytomas (Smith et al., 2001), *TP53* mutations were a strong univariate predictor of increased survival. In another study, 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 (Korkolopoulou et al., 1997). In the majority of investigations, however, *TP53* mutations have not been shown to correlate with survival regardless of whether the gene alterations are examined with respect to tumor histologic grade.

CDKN2A. Identification of a second TSG whose inactivation is important to glioma development stemmed from both cytogenetic and LOH studies, which indicated that the short arm of chromosome 9 was frequently deleted in these tumors. Although the 9p loss was initially localized to a relatively large region that generally included the centromere-proximal end of the interferon-α gene cluster (Ichimura et al., 1994; James et al., 1994), it is now clear that the *CDKN2A* gene, which resides close to the interferon gene cluster, represents the primary chromosome 9p deletion target. There are, however, additional genes within as well as near *CDKN2A* that may also have a growth-suppressive function. Deletions of *CDKN2A* occur in a variety of cancers, including malignant gliomas (Kamb et al., 1994; Nobori et al., 1994). Among the gliomas, deletions of *CDKN2A* have been demonstrated in primary glioblastomas as well as in glioblastoma xenografts and cell lines (He et al., 1994; Jen et al., 1994; Schmidt et al., 1994).

In addition to *CDKN2A* inactivation by gene deletion, the p16 protein, which is encoded by *CDKN2A,* is not expressed in a significant fraction of gliomas having intact *CDKN2A* genes, indicating that loss of p16 expression can occur in the absence of a corresponding gene alteration. In at least a small proportion of such cases, this appears to be associated with *CDKN2A* gene hypermethylation (Nishikawa et al., 1995). As opposed to *TP53* mutations, which are observed at a decreasing frequency with increasing astrocytoma malignancy, *CDKN2A*-inactivating mutations occur more frequently with increasing glial tumor malignancy grade.

PTEN/MMAC1. Genetic changes resulting in inactivation of the *PTEN* gene (also referred to as *MMAC1*), residing at chromosomal location 10q23, represents the TSG alteration most highly associated with advanced-stage glial tumor malignancy (Li et al., 1997; Steck et al., 1997). *PTEN* has been shown to be inactivated in as many as 44% of all glioblastomas and in 60% of glioblastomas having 10q deletions (Wang et al., 1997). These results are consistent with *PTEN* being the primary target of inactivation associated with the loss of chromosome 10 that was originally observed in glioblastoma multiforme by cytogenetic analysis.

Although the encoded protein of *PTEN,* Tep1, has been shown to have a dual-specificity phosphatase activity (tyrosine and serine) (Myers et al., 1997), recent evidence suggests that its biologically relevant targets include inositol phospholipids as well as proteins. Among the phospholipid substrates is phosphoinositol triphosphate (Maehama and Dixon, 1998), which promotes the activity of Akt, a serine/threonine kinase that is an important regulator of cell survival and cell proliferation (Cantley and Neel, 1999). Tep1 additionally modulates cell migration and invasion by negatively regulating the signals generated at the focal adhesions (Tamura et al., 1998) through the direct dephosphorylation and inhibition of focal adhesion kinase (FAK). Finally, Tep1 negatively regulates receptor tyrosine kinase (RTK) signaling through its inhibition of the adaptor protein Shc (Gu et al., 1998).

Results from two recent studies suggest that the genetic status of *PTEN* is an important prognostic variable in malignant glioma. Lin et al. (1998) 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 (Raffel et al., 1999).

NF1. Inheritance of a mutated *NF1* gene predisposes to type 1 neurofibromatosis, a syndrome characterized by the development of neurofibromas, café-au-lait spots, and an increased risk for pheochromocytomas, schwannomas, neurofibrosarcomas, and primary brain tumors such as optic gliomas and pilocytic astrocytomas (Pollack and Mulvihill, 1997). Pilocytic astrocytomas occurring in the absence of *NF1* syndrome may also be due to *NF1*-inactivating mutations as deletions of this gene have been found in as many as 20% of such tumors (von Deimling et al., 1993). *NF1* encodes a GTPase-activating protein, neurofibromin, which has been shown to downregulate the activity of ras, an important effector of RTK signaling (Martin et al., 1990).

NF2. Localized to chromosomal region 22q12, the *NF2* gene is responsible for neurofibromatosis type 2, an autosomal dominant disease characterized by bilateral acoustic neuromas, meningiomas, and spinal schwannomas (Pollack and Mulvihill, 1997). Somatic mutations of the gene are seen in as many as 60% of sporadic (non-*NF2*–associated) meningiomas and schwannomas (Ruttledge et al., 1994; Sainz et al., 1994). The *NF2* gene product, merlin/schwannomin, is a cytoskeleton-associated protein whose function is important to the regulation of cell adhesion (Gonzalez-Agosti et al., 1996).

VHL. The *VHL* gene is located at chromosome 3p25–26, and its germline mutation is responsible for the von Hippel-Lindau syndrome (Decker et al., 1997), characterized by predisposition to the development of hemangioblastomas of the central nervous system and retina, as well as to other malignancies (renal cell carcinomas, pheochromocytomas). Somatic mutations of the *VHL* gene are seen in as many as 40% of sporadic hemangioblastomas (Tse et al., 1997). Functional analyses have indicated that the *VHL* gene product is an inhibitor of transcription elongation (Duan et al., 1995). In addition, the VHL protein has been implicated in controlling the expression of VEGF (Gnarra et al., 1996), a potent angiogenesis factor.

PTCH. The identification of tumor suppressor genes whose inactivation are involved with medulloblastoma development has been an area of active research for several years and only recently has yielded a viable candidate. The gene, *PTCH,* was discovered as a result of the association of its mutation with predisposition to nevoid basal cell carcinoma (NBCC) syndrome, an inherited condition in which there is occasional development of medulloblastoma in addition to the more commonly occurring nevoid basal cell carcinomas (Hahn et al., 1996). The *PTCH* gene has now been shown to be inactivated in approximately 20% of cases of sporadic medulloblastoma, and this gene alteration may be primarily associated with the desmoplastic subtype (Raffel et al., 1997; Wolter et al., 1997). Additional investigations have been conducted to determine whether genes that encode proteins that interact with the *PTCH* gene product, a transmembrane receptor (Marigo et al., 1996), are also altered in medulloblastoma. However, their associated results have failed to indicate that these genes are important mutational targets in this nervous system tumor (Reifenberger et al., 1998).

Chromosomal Regions Harboring Putative Tumor Suppressor Genes

Chromosome 19q. Allelic loss of 19q occurs in approximately 50% to 80% of oligodendroglial tumors and, with rare exception, involves the entire 19q chromosomal arm (von Deimling et al., 1992; Reifenberger et al., 1994a; Bello et al., 1995; Kraus et al., 1995; Smith et al., 1999). However, multiple investigations have progressively narrowed the chromosome 19q deletion region to an interval within 19q13.3 (Rosenberg et al., 1996; Smith et al., 1999), allowing for several candidate genes within the 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 (Reifenberger et al., 1994b; Bello et al., 1995; Kraus et al., 1995), a finding that contrasts with the 19q loss observed in astrocytic gliomas that is generally restricted to the high-grade cases (Smith et al., 1999).

Chromosome 1p. Deletion of chromosome 1p is another frequent event in oligodendrogliomas, occurring in 40% to 90% of these tumors (Bello et al.,

1994; Reifenberger et al., 1994b; Kraus et al., 1995; Smith et al., 1999). 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 oncogenesis. Data from a recent report showed two distinct deletion regions on 1p, D1S76-D1S253 at 1p36.3 and D1S482-D1S2743 at 1p34-35, that contain potential TSGs whose inactivation may be important to the development of these tumors (Husemann et al., 1999).

Evaluation of chromosomal arms 1p and 19q are of prognostic utility for the oligodendroglioma patient. Cairncross et al. (1998) examined 39 anaplastic oligodendroglioma patients, 37 of whom had received PCV chemotherapy. Allelic loss of 1p was a statistically significant predictor of chemosensitivity, and combined loss of 1p and 19q was significantly associated with both chemosensitivity and longer recurrence-free survival following chemotherapy. Moreover, Smith et al. (2001) have demonstrated that the association of 1p and 19q loss with prolonged survival is also evident in low-grade oligodendroglioma patients, and that this association may be independent of PCV chemotherapy.

CANCER GENE ALTERATIONS AND CELL CYCLE DYSFUNCTION

p53–MDM2–p14^{ARF}–p21

The mammalian cell cycle is divided into four distinct phases: G1, S, G2, and M. Unrestricted cell multiplication represents a hallmark feature of cancer, and this process is associated with continued cell cycle progression, a process that is usually kept under control by a complex system of positive and negative regulators that constitute a series of cell cycle checkpoints. One of the most important of these checkpoints involves the p53, MDM2, p14^{ARF}, and p21 proteins, which regulate the progression of cells through the G1 cell cycle phase.

Initially this checkpoint was thought to be abrogated only by inactivation of p53 through gene deletion or mutation. However, a considerable amount of information has emerged during the past few years concerning the regulation of p53 function by other cellular proteins. Important among the relevant studies are those dealing with MDM2, which binds to, destabilizes, and inactivates p53 (Oliner et al., 1993). Significantly, amplification of the *MDM2* gene has been demonstrated as an alternative mechanism to inactivating mutations of *TP53* in astrocytomas (Reifenberger et al., 1993). *MDM2* gene amplification has been reported in as many as 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 (Reifenberger et al., 1993).

MDM2-mediated destabilization of p53 is inhibited by p14^{ARF} (Pomerantz et al., 1998), whose gene resides partly within the coding sequence of the gene for p16 (Mao et al., 1995), *CDKN2A.* Because 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. 9–3).

The activity of wild-type p53 is known to promote the synthesis of the universal cyclin–cdk inhibitor p21 (el-Deiry et al., 1994), and this is thought to prevent the replication of altered DNA in normal cells that have incurred DNA damage (Di Leonardo et al., 1994). Because the synthesis of p21 is stimulated by wild-type p53 activity, *TP53* gene inactivation, *MDM2* amplification, or *p14^{ARF}* gene deletion could contribute to reduced p21 synthesis and thus promote the accumulation of gene alterations in tumor cells due to 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, thus far there is no evidence that the p21 gene itself is mutated in human cancers (Shiohara et al., 1994; Tenan et al., 1995).

p16–cdk4–pRb–cyclin D

Another important G1 checkpoint is constituted by the p16, pRb, 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 (Serrano et al., 1993). The primary substrate of this complex is the retinoblastoma protein (Lukas et al., 1995), pRb. In its hypophosphorylated form, pRb

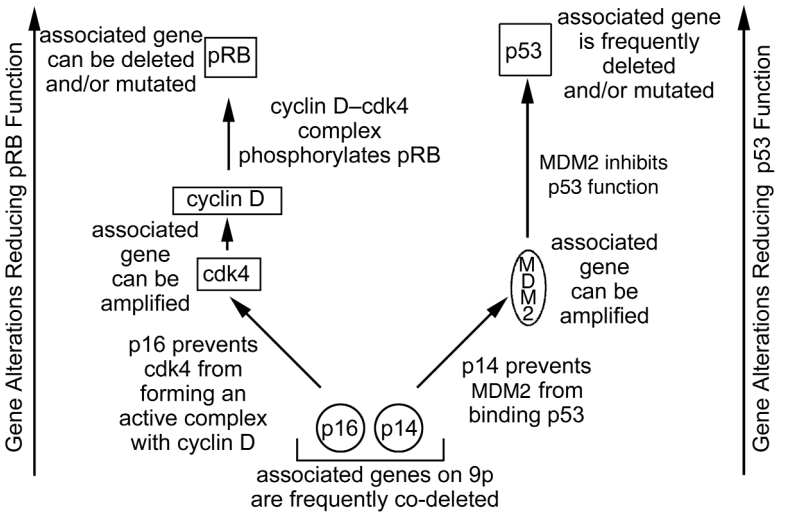

Figure 9–3. Gene alterations reducing pRb- and p53-associated cell cycle regulation.

arrests cells at the G1/S cell cycle checkpoint. This checkpoint is abrogated when pRb is phosphorylated, and cyclin D1–cdk4 has been shown to phosphorylate most 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. 9–3): *(1)* inactivation of the positive upstream regulator p16; *(2)* increased expression of the negative upstream regulator cdk4; or *(3)* inactivation of the retinoblastoma protein through mutation of its gene. 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 (He et al., 1994; Schmidt et al., 1994). Furthermore, it has been shown that loss of pRb 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 (He et al., 1995; Ueki et al., 1996).

Although there are inconsistencies between the results of studies that examined the prognostic significance of detecting *CDKN2A, CDK4,* or *RB* gene alterations, it is generally thought that a member of this pathway is altered during the malignant transformation of nearly every astrocytoma (Ichimura et al., 1996). Interestingly, the results of investigations that support this concept show a genetic change to one and only one member of the *CDKN2A–CDK4–RB* triad in each malignant astrocytoma studied, suggesting that a single alteration within this pathway is sufficient to disrupt its regulatory function. Although the prognostic significance of this checkpoint's alteration in malignant astrocytoma is unclear, a recent study indicates that detection of *CDKN2A* deletions in tumors from patients with oligodendroglial tumors, albeit an infrequent event in oligodendroglioma, is significantly associated with decreased survival. Additionally, these deletions occur in tumors having intact copies of chromosomes 1 and 19 (Cairncross et al., 1998).

Because the activity of cdk4 depends on its binding to D-type cyclins, one might predict that increased cyclin D synthesis would contribute to 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 (Peters, 1994), but is uncommon in gliomas. However, it has been shown that cyclin D1 expression is increased by stimulating receptor tyrosine kinase activity (Sherr, 1995), 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 pRb protein inactivation.

HIGH-GRADE MALIGNANCY: SPONTANEOUS VERSUS DEVELOPMENT FROM A LOW-GRADE PRECURSOR

Much has been written and discussed during the past several years concerning the genetic etiology of malignant astrocytoma. The most common hypothesis considers two types of malignant brain tumor: one occurring spontaneously in full-blown malignancy and the other arising through a series of steps, with each step conferring an additional, incremental growth advantage. The former group of tumors appear to be characterized by the more frequent occurrence of *EGFR* amplification, whereas in the latter *TP53*-inactivating mutations are more common (Ng and Lam, 1998). In one study, for instance, it was shown that the incidence of *TP53* mutations was approximately six-fold less in de novo glioblastomas than in recurrent glioblastomas, which had displayed malignant progression (Watanabe et al., 1996).

The significance of the debate involving primary and progressive glioblastoma is related to potential differences in clinical behavior between the two groups of tumors, and their accurate genetic classification will continue to be of interest. Regardless of one's position on this issue, it is possible to construct a reasonably detailed model that describes the molecular genetic changes that are associated with different stages of malignant astrocytoma (Fig. 9–4). It is because of these associations between specific gene alterations and astrocytoma malignancy, as well as cellular differentiation, that genetic analysis will play an increasingly important role in the diagnosis and treatment of these tumors.

THE FUTURE OF CANCER GENETICS RESEARCH

It is anticipated that within the next few months to, at most, the next few years it will be possible to perform comprehensive analyses of gene expression patterns in human tissues and thereby obtain extensive detail regarding the identities of genes that are consistently overexpressed or underexpressed in specific types of tumors (DeRisi et al., 1996). This will result from combining microarray technological advances (Brown and Botstein, 1999) with by-products of the Human Genome Project (Sinsheimer, 1990).

Microarrays represent solid support templates on which single-stranded DNAs, representing coding sequences of thousands of different genes, can be placed. These arrays or "chips" are used for competitive hybridizations of normal-tumor cDNA or ge-

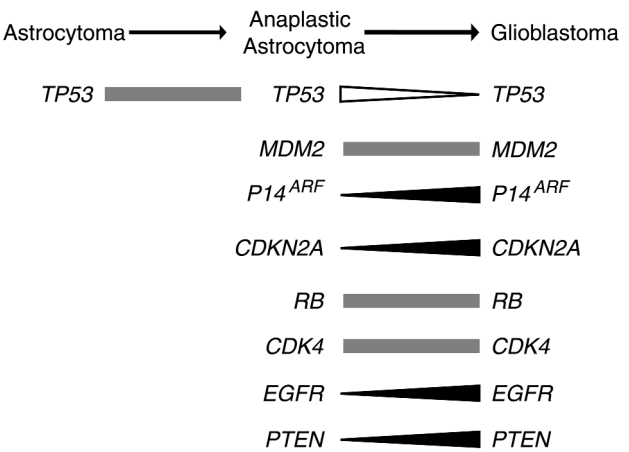

Figure 9–4. Gene alterations associated with astrocytoma malignancy stages. Gray, horizontal bars indicate that there are similar frequencies of alteration in different stages of malignancy. Black and white sloped bars indicate increasing and decreasing alteration frequencies, respectively, in association with increasing malignancy grade. This model, suggesting an accumulation of gene alterations with increasing astrocytoma malignancy, may be applicable to other types of nervous system tumors, but for all other tumor types there are insufficient data with which to propose specific gene alterations as associated with specific stages of malignant progression.

nomic DNA pairs that have been labeled with different fluorochromes. The principle on which this works is entirely analogous to that described previously for comparative genomic hybridizations (Fig. 9–2). Overrepresentation of the tumor cDNA or genomic DNA fluorochrome at a specific coordinate on the array indicates overexpression or overrepresentation, respectively, of the gene whose nucleotide sequence was spotted onto that coordinate. From a time-cost perspective the potential efficiency of this process for providing extensive information on gene expression patterns in tumors is astounding and will allow for the development of databases containing expression profiles for all common cancers. Such databases are already being developed (Kuska, 1996). Microarray technology is being extended to the detection of gene sequence alterations, and a first generation model for *TP53* mutation detection has already been marketed (Ahrendt et al., 1999). Chips for the detection of gene amplification and gene deletion are also being developed.

Developments in microarray technology have been largely driven by the progress and imminent completion of the Human Genome Project. 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 datasets containing the genetic and physical locations of all genes on each human chromosome, plus the complete nucleotide sequence of the genome for humans. This initiative now appears to be entering its final phase and will result, as early as 2002, in a complete and accurate whole genome DNA sequence representing the genetic blueprint of the human species. With respect to the gene identification and localization aspect of this initiative, the most recent report indicates that more than 30,000 genes have been localized to specific chromosomal regions with a high degree of accuracy (Deloukas et al., 1998).

The total gene content within our cells is not currently known but most estimates place it at approximately 50,000. The map that currently exists can already be applied to the identification and isolation of genes that either directly cause human ailments or increase our susceptibility to disease. It is obvious that this initiative when combined with emerging technologies, some of which have been described here, will allow for the rapid and in many cases complete diagnosis of specific genetic lesions in individual brain tumors.

THERAPEUTIC IMPLICATIONS

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 (Sausville et al., 1999), farnesyltransferase inhibitors (Ferrante et al., 1999), and inhibitors of EGF receptor-associated tyrosine kinase activity (Fry, 1999). Knowing whether a tumor has a gene alteration that would affect 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 critically important to ascertain the manner in which cells respond to radiation-induced DNA damage (Kuerbitz et al., 1992). Consequently, information regarding a tumor's *TP53* gene status may help determine a patient's response to radiation treatment; a recent report suggests this is the case for glioblastoma patients (Tada et al., 1998).

The identification of specific genetic lesions in combination with exciting new therapeutic strategies that depend on the knowledge of tumor genotypes should greatly facilitate the development of effective, individualized therapies for patients with nervous system 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.

REFERENCES

Ahrendt SA, Halachmi S, Chow JT, et al. 1999. Rapid p53 sequence analysis in primary lung cancer using an oligonucleotide probe array. Proc Natl Acad Sci USA 96: 7382–7387.

Bello MJ, Leone PE, Vaquero J, et al. 1995. Allelic loss at 1p and 19q frequently occurs in association and may represent early oncogenic events in oligodendroglial tumors. Int J Cancer 64:207–210.

Bello MJ, Vaquero J, de Campos JM, et al. 1994. Molecular analysis of chromosome 1 abnormalities in human gliomas reveals frequent loss of 1p in oligodendroglial tumors. Int J Cancer 57:172–175.

Biegel JA, Rorke LB, Packer RJ, et al. 1989. Isochromosome 17q in primitive neuroectodermal tumors of the central nervous system. Genes Chromosomes Cancer 1:139–147.

Bigner SH, Mark J, Mahaley MS, Bigner DD. 1984. Patterns of

the early gross chromosomal changes in malignant human gliomas. Hereditas 101:103–113.

Bigner SH, Mark J, Friedman HS, Biegel JA, Bigner DD. 1988. Structural chromosomal abnormalities in human medulloblastoma. Cancer Genet Cytogenet 30:91–101.

Bigner SH, Wong AJ, Mark J, et al. 1987. Relationship between gene amplification and chromosomal deviations in malignant human gliomas. Cancer Genet Cytogenet 29:165–170.

Bogler O, Huang HJ, Cavenee WK. 1995. Loss of wild-type p53 bestows a growth advantage on primary cortical astrocytes and facilitates their in vitro transformation. Cancer Res 55:2746–2751.

Brown PO, Botstein D. 1999. Exploring the new world of the genome with DNA microarrays. Nat Genet 21:33–37.

Cairncross JG, Ueki K, Zlatescu MC, et al. 1998. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst 90:1473–1479.

Cantley LC, Neel BG. 1999. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. Proc Natl Acad Sci USA 96:4240–4245.

Chen P, Iavarone A, Fick J, Edwards M, Prados M, Israel MA. 1995. Constitutional p53 mutations associated with brain tumors in young adults. Cancer Genet Cytogenet 82:106–115.

Decker HJ, Weidt EJ, Brieger J. 1997. The von Hippel-Lindau tumor suppressor gene. A rare and intriguing disease opening new insight into basic mechanisms of carcinogenesis. Cancer Genet Cytogenet 93:74–83.

Deloukas P, Schuler GD, Gyapay G, et al. 1998. A physical map of 30,000 human genes. Science 282:744–746.

DeRisi J, Penland L, Brown PO, et al. 1996. Use of a cDNA microarray to analyse gene expression patterns in human cancer. Nat Genet 14:457–460.

Di Leonardo A, Linke SP, Clarkin K, Wahl GM. 1994. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. Genes Dev 8:2540–2551.

Duan DR, Pause A, Burgess WH, et al. 1995. Inhibition of transcription elongation by the VHL tumor suppressor protein. Science 269:1402–1406.

Ekstrand AJ, James CD, Cavenee WK, Seliger B, Petterson RF, Collins VP. 1991. Genes for epidermal growth factor receptor, transforming growth factor alpha, and epidermal growth factor and their expression in human gliomas in vivo. Cancer Res 51:2164–2172.

Ekstrand AJ, Liu L, He J, et al. 1995. Altered subcellular location of an activated and tumour-associated epidermal growth factor receptor. Oncogene 10:1455–1460.

Ekstrand AJ, Longo N, Hamid ML, et al. 1994. Functional characterization of an EGF receptor with a truncated extracellular domain expressed in glioblastomas with EGFR gene amplification. Oncogene 9:2313–2320.

el-Deiry WS, Harper JW, O'Connor PM, et al. 1994. WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. Cancer Res 54:1169–1174.

Eley G, Frederick L, Wang XY, Smith DI, James CD. 1998. 3′ end structure and rearrangements of EGFR in glioblastomas. Genes Chromosomes Cancer 23:248–254.

Ferrante K, Winograd B, Canetta R. 1999. Promising new developments in cancer chemotherapy. Cancer Chemother Pharmacol 43:S61–68.

Fischer U, Muller HW, Sattler HP, Feiden K, Zang MD, Meese E. 1995. Amplification of the MET gene in glioma. Genes Chromosomes Cancer 12:63–65.

Fleming TP, Saxena A, Clark WC, et al. 1992. Amplification and/or overexpression of platelet-derived growth factor receptors and epidermal growth factor receptor in human glial tumors. Cancer Res 52:4550–4553.

Fry DW. 1999. Inhibition of the epidermal growth factor receptor family of tyrosine kinases as an approach to cancer chemotherapy: progression from reversible to irreversible inhibitors. Pharmacol Ther 82:207–218.

Gnarra JR, Zhou S, Merrill MJ, et al. 1996. Post-transcriptional regulation of vascular endothelial growth factor mRNA by the product of the VHL tumor suppressor gene. Proc Natl Acad Sci USA 93:10589–10594.

Gonzalez-Agosti C, Xu L, Pinney D, et al. 1996. The merlin tumor suppressor localizes preferentially in membrane ruffles. Oncogene 13:1239–1247.

Gu J, Tamura M, Yamada KM. 1998. Tumor suppressor PTEN inhibits integrin- and growth factor–mediated mitogen-activated protein (MAP) kinase signaling pathways. J Cell Biol 143:1375–1383.

Hahn H, Wicking C, Zaphiropoulous PG, et al. 1996. Mutations of the human homolog of *Drosophila* patched in the nevoid basal cell carcinoma syndrome. Cell 85:841–851.

He J, Allen JR, Collins VP, Allalunis-Turner MJ, Godbout R, Day RS 3rd, James CD. 1994. CDK4 amplification is an alternative mechanism to p16 gene homozygous deletion in glioma cell lines. Cancer Res 54:5804–5807.

He J, Olson JJ, James CD. 1995. Lack of p16INK4 or retinoblastoma protein (pRb) or amplification-associated overexpression of cdk4 is observed in distinct subsets of malignant glial tumors and cell lines. Cancer Res 55:4833–4836.

Hollstein M, Sidransky D, Vogelstein B, Harris CC. 1991. p53 mutations in human cancers. Science 253:49–53.

Husemann K, Wolter M, Büschges R, Boström J, Sabel M, Reifenberger G. 1999. Identification of two distinct deleted regions on the short arm of chromosome 1 and rare mutation of the CDKN2C gene from 1p32 in oligodendroglial tumors. J Neuropathol Exp Neurol 58:1041–1050.

Ichimura K, Schmidt EE, Goike HM, Collins VP. 1996. Human glioblastomas with no alterations of the CDKN2A (p16INK4A, MTS1) and CDK4 genes have frequent mutations of the retinoblastoma gene. Oncogene 13:1065–1072.

Ichimura K, Schmidt EE, Yamaguchi N, James CD, Collins VP. 1994. A common region of homozygous deletion in malignant human gliomas lie between the IFN-alpha/omega gene cluster and the D9S171 locus. Cancer Res 54:3127–3130.

James CD, Carlbom E, Nordenskjold M, Collins VP, Cavenee WK. 1989. Mitotic recombination of chromosome 17 in astrocytomas. Proc Natl Acad Sci USA 86:2858–2862.

James CD, He J, Collins VP, Allalunis-Turner MJ, Day RS 3d. 1993. Localization of chromosome 9p homozygous deletions in glioma cell lines with markers constituting a continuous linkage group. Cancer Res 53:3674–3636.

Jen J, Harper JW, Bigner SH, et al. 1994. Deletion of p16 and p15 genes in brain tumors. Cancer Res 54:6353–6358.

Kallioniemi A, Kallioniemi OP, Sudar D,, et al. 1992. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258:818–821.

Kamb A, Gruis NA, Weaver-Feldhaus J, et al. 1994. A cell cycle regulator potentially involved in the genesis of many tumor types. Science 264:436–440.

Kim DH, Mohapatra G, Bollen A, Waldman FM, Feuerstein BG. 1995. Chromosomal abnormalities in glioblastoma multiforme tumors and glioma cell lines detected by comparative genomic hybridization. Int J Cancer 60:812–819.

Korkolopoulou P, Christodoulou P, Kouzelis K, et al. 1997. MDM2 and p53 expression in gliomas: a multivariate survival analysis including proliferation markers and epidermal growth factor receptor. Br J Cancer 75:1269–1278.

Kraus JA, Koopman J, Kaskel P, et al. 1995. Shared allelic losses on chromosomes 1p and 19q suggest a common origin of oligodendroglioma and oligoastrocytoma. J Neuropathol Exp Neurol 54:91–95.

Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. 1992. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 89:7491–7495.

Kuska B. 1996. Cancer genome anatomy project set for take-off. J Natl Cancer Inst 88:1801–1803.

Lasko D, Cavenee W, Nordenskjold M. 1991. Loss of constitutional heterozygosity in human cancer. Annu Rev Genet 25:281–314.

Li J, Yen C, Liaw D, et al. 1997. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275:1943–1947.

Libermann TA, Nusbaum HR, Razon N, et al. 1985. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. Nature 313:144–147.

Lin H, Bondy ML, Langford LA, et al. 1998. Allelic deletion analyses of MMAC/PTEN and DMBT1 loci in gliomas: relationship to prognostic significance. Clin Cancer Res 4:2447–2454.

Lukas J, Parry D, Aagaard L, et al. 1995. Retinoblastoma-protein–dependent cell-cycle inhibition by the tumour suppressor p16. Nature 375:503–506.

Maehama T, Dixon JE. 1998. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 273:13375–13378.

Malkin D. 1993. p53 and the Li-Fraumeni syndrome. Cancer Genet Cytogenet 66:83–92.

Mao L, Merlo A, Bedi G, et al. 1995. A novel p16INK4A transcript. Cancer Res 55:2995–2997.

Marigo V, Davey RA, Zuo Y, Cunningham JM, Tabin CJ. 1996. Biochemical evidence that patched is the Hedgehog receptor. Nature 384:176–179.

Martin GA, Viskochil D, Bollag G, et al. 1990. The GAP-related domain of the neurofibromatosis type 1 gene product interacts with ras p21. Cell 63:843–849.

Myers MP, Stolarov JP, Eng C, et al. 1997. P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. Proc Natl Acad Sci USA 94:9052–9057.

Nagane M, Huang HJ, Cavenee WK. 1997. Advances in the molecular genetics of gliomas. Curr Opin Oncol 9:215–222.

Ng HK, Lam PY. 1998. The molecular genetics of central nervous system tumors. Pathology 30:196–202.

Nishikawa R, Furnari FB, Lin H, et al. 1995. Loss of p16INK4 expression is frequent in high grade gliomas. Cancer Res 55:1941–1945.

Nishikawa R, Ji XD, Harmon RC, et al. 1994. A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. Proc Natl Acad Sci USA 91:7727–7731.

Nobori T, Miura K, Wu DJ, Lois A, Takabayashi K, Carson DA. 1994. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature 368:753–756.

Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris J, Kinzler KW, Vogelstein B. 1993. Oncoprotein MDM2 conceals the activation domain of tumor suppressor p53. Nature 362: 857–860.

Olson JJ, Barnett D, Yang J, Assietti R, Cotsonis G, James CD. 1998. Gene amplification as a prognostic factor in primary brain tumors. Clin Cancer Res 4:215–222.

Peters G. 1994. The D-type cyclins and their role in tumorigenesis. J Cell Sci Suppl 18:89–96.

Pinkel D, Straume T, Gray JW. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83:2934–2938.

Pollack IF, Mulvihill JJ. 1997. Neurofibromatosis 1 and 2. Brain Pathol 7:823–836.

Pomerantz J, Schreiber-Agus N, Liegeois NJ, et al. 1998. The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. Cell 92:713–723.

Raffel C, Frederick L, O'Fallon JR, et al. 1999. Analysis of oncogene and tumor suppressor gene alterations in pediatric malignant astrocytomas reveals reduced survival for patients with PTEN mutations. Clin Cancer Res 5:4085–4090.

Raffel C, Jenkins RB, Frederick L, et al. 1997. Sporadic medulloblastomas contain PTCH mutations. Cancer Res 57: 842–845.

Ransom DT, Ritland SR, Kimmel DW, et al. 1992. Cytogenetic and loss of heterozygosity studies in ependymomas, pilocytic astrocytomas, and oligodendrogliomas. Genes Chromosomes Cancer 5:348–356.

Reardon DA, Michalkiewicz E, Boyett JM, et al. 1997. Extensive genomic abnormalities in childhood medulloblastoma by comparative genomic hybridization. Cancer Res 57: 4042–4047.

Reifenberger G, Liu L, Ichimura K, Schmidt EE, Collins VP. 1993. Amplification and overexpression of the MDM2 gene in a subset of human malignant gliomas without p53 mutations. Cancer Res 53:2736–2739.

Reifenberger G, Reifenberger J, Ichimura K, James CD, Wechsler W, Collins VP. 1994a. Amplification of multiple genes from chromosomal region 12q13–14 in human malignant gliomas: preliminary mapping of the amplicons shows preferential involvement of CDK4, SAS, and MDM2. Cancer Res 54:4299–4303.

Reifenberger J, Reifenberger G, Liu L, et al. 1994b. Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. Am J Pathol 145:1175–1190.

Reifenberger J, Wolter M, Weber RG, et al. 1998. Missense mutations in SMOH in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. Cancer Res 58:1798–1803.

Rosenberg JE, Lisle DK, Burwick JA, Ueki K, Mohrenweiser A, Louis DN. 1996. Refined deletion mapping of the chromosome 19q glioma tumor suppressor gene to the D19S412-STD interval. Oncogene 13:2483–2485.

Ruttledge MH, Sarrazin J, Rangaratnam S, et al. 1994. Evidence for the complete inactivation of the NF2 gene in the majority of sporadic meningiomas. Nat Genet 6:180–184.

Sainz J, Huynh DP, Figueroa K, Ragge NK, Baser ME, Pulst SM. 1994. Mutations of the neurofibromatosis type 2 gene and lack of the gene product in vestibular schwannomas. Hum Mol Genet 3:885–891.

Sausville EA, Zaharevitz D, Gussio R, et al. 1999. Cyclin-dependent kinases: initial approaches to exploit a novel therapeutic target. Pharmacol Ther 82:285–292.

Sherr CJ. 1995. D-type cyclins. Trends Biochem Sci 20:187–190.

Schmidt EE, Ichimura K, Reifenberger G, Collins VP. 1994. CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas. Cancer Res 54:6321–6324.

Schrock E, Veldman T, Padilla-Nash H, et al. 1997. Spectral karyotyping refines cytogenetic diagnostics of constitutional chromosomal abnormalities. Hum Genet 101:255–262.

Serrano M, Hannon GJ, Beach D. 1993. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 366:704–707.

Shiohara M, el-Deiry WS, Wada M, et al. 1994. Absence of WAF1 mutations in a variety of human malignancies. Blood 84:3781–3784.

Sinsheimer RL. 1990. Human genome initiative. Science 249:1359.

Smith JS, Alderete B, Minn Y, et al. 1999. Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. Oncogene 18:4144–4152.

Smith JS, Perry A, Borell TJ, et al. 2000. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. J Clin Oncol 18:636–645.

Smith JS, Tachibana I, Passe SM, Huntley BK, Borell TJ, et al. 2001. PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. J Natl Cancer Inst 93:1246–56.

Steck PA, Pershouse MA, Jasser SA, et al. 1997. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 15:356–362.

Stenman G, Kindblom LG, Johansson M, Angervall L. 1991. Clonal chromosome abnormalities and in vitro growth characteristics of classical and cellular schwannomas. Cancer Genet Cytogenet 57:121–131.

Sugawa N, Ekstrand AJ, James CD, Collins VP. 1990. Identical splicing of aberrant epidermal growth factor receptor transcripts from amplified rearranged genes in human glioblastomas. Proc Natl Acad Sci USA 87:8602–8606.

Tada M, Matsumoto R, Iggo RD, et al. 1998. Selective sensitivity to radiation of cerebral glioblastomas harboring p53 mutations. Cancer Res 58:1793–1797.

Tamura M, Gu J, Matsumoto K, Aota S, Parsons R, Yamada KM. 1998. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. Science 280:1614–1617.

Tenan M, Carrara F, DiDonato S, Finocchiaro G. 1995. Absence of mutations and identification of two polymorphisms in the SSCP and sequence analysis of p21CK1 gene in malignant gliomas. Int J Cancer 62:115–117.

Tse JY, Wong JH, Lo KW, Poon WS, Huang DP, Ng HK. 1997. Molecular genetic analysis of the von Hippel-Lindau disease tumor suppressor gene in familial and sporadic cerebellar hemangioblastomas. Am J Clin Pathol 107:459–466.

Ueki K, Ono Y, Henson JW, Efird JT, von Deimling A, Louis DN. 1996. CDKN2/p16 or RB alterations occur in the majority of glioblastomas and are inversely correlated. Cancer Res 56:150–153.

von Deimling A, Louis DN, von Ammon K, Petersen I, Weistler OD, Seizinger BR. 1992. Evidence for a tumor suppressor gene on chromosome 19q associated with human astrocytomas, oligodendrogliomas, and mixed gliomas. Cancer Res 52:4277–4279.

von Deimling A, Louis DN, Menon AG, et al. 1993. Deletions on the long arm of chromosome 17 in pilocytic astrocytoma. Acta Neuropathol 86:81–85.

Wang SI, Puc J, Li J, et al. 1997. Somatic mutations of PTEN in glioblastoma multiforme. Cancer Res 57:4183–4186.

Watanabe K, Tachibana O, Sata K, Yonekawa Y, Kleihues P, Ohgaki H. 1996. Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. Brain Pathol 6:217–223.

Weber RG, Bostrom J, Wolter M, et al. 1997. Analysis of genomic alterations in benign, atypical, and anaplastic meningiomas: toward a genetic model of meningioma progression. Proc Natl Acad Sci USA 94:14719–14724.

Weremovicz S, Kupsky WJ, Morton CC, Fletcher JA. 1992. Cytogenetic evidence for a chromosome 22 tumor suppressor gene in ependymoma. Cancer Genet Cytogenet 61:193–196.

White R, Lalouel JM. 1988. Chromosome mapping with DNA markers. Sci Am 258:40–48.

Wolter M, Reifenberger J, Sommer C, Ruzicka T, Reifenberger G. 1997. Mutations in the human homologue of the Drosophila segment polarity gene patched (PTCH) in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. Cancer Res 57:2581–2585.

Wong AJ, Bigner SH, Bigner DD, Kinzler KW, Hamilton SR, Vogelstein B. 1987. Increased expression of the epidermal growth factor receptor gene in malignant gliomas is invariably associated with gene amplification. Proc Natl Acad Sci USA 84:6899–6903.

Yahamada AM, Bruner JM, Donehower LA, Morrison RS. 1995. Astrocytes derived from p53-deficient mice provide a multistep in vitro model for development of malignant gliomas. Mol Cell Biol 15:4249–4259.

Zang KD. 1982. Cytological and cytogenetical studies on human meningioma. Cancer Genet Cytogenet 6:249–274.