Cytogenetics and molecular genetics of childhood brain tumors¹

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Considerable progress has been made toward improving survival for children with brain tumors, and yet there is still relatively little known regarding the molecular genetic events that contribute to tumor initiation or progression. Nonrandom patterns of chromosomal deletions in several types of childhood brain tumors suggest that the loss or inactivation of tumor suppressor genes are critical events in tumorigenesis. Deletions of chromosomal regions 10q, 11 and 17p, for example, are frequent events in medulloblastoma, whereas loss of a region within 22q11.2, which contains the INI1 gene, is involved in the development of atypical teratoid and rhabdoid tumors. A review of the cytogenetic and molecular genetic changes identified to date in childhood brain tumors will be presented. *Neuro-Oncology* 1, 139–151, 1999 (Posted to *Neuro-Oncology* [serial online], Doc. 98-30, April 30, 1999. URL <neuro-oncology.mc.duke.edu>)

Introduction

Combined cytogenetic and molecular genetic approaches, including preparation of karyotypes, FISH,³ CGH, and loss of heterozygosity studies have led to the identification of regions of the genome that contain a variety of novel tumor suppressor genes and oncogenes. Linkage analysis in families, which segregate the disease phenotype, and studies of patients with constitutional chromosomal abnormalities have resulted in the identification of many of the disease genes for which affected individuals have an inherited predisposition to brain tumors (Table 1). The frequency of mutations of these genes in sporadic tumors, however, is still relatively low. Although tumor development is influenced both by genetic and environmental factors, the strongest case for genetic predisposition to the development of malignancies can be made for the youngest patients, particularly for those children diagnosed in the first year of life. Identifying tumor-specific and tumor-associated genes thus becomes critical on multiple levels. From a genetic standpoint, identifying a germ-line mutation in a family may have great implications for the planning of future pregnancies. In a clinical oncology setting, identifying an acquired chromosomal deletion or gene mutation may necessitate very aggressive approaches, or may allow the administration of less toxic therapy. As we unravel the genetic pathways that lead to childhood brain tumors, treatments will be targeted to those genetic alterations that are present in a given tumor.

Medulloblastoma

The most common malignant brain tumor in children is medulloblastoma, the prototype PNET. Primitive neuroectodermal tumors that arise in the cerebellum are generally classified as medulloblastoma, whereas similar histologic entities that arise in other locations are referred to

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³Abbreviations used are as follows: FISH, fluorescence in situ hybridization; CGH, comparative genomic hybridization; PNET, primitive neuroectodermal tumor; AT/RT, atypical teratoid/rhabdoid tumor; AA, anaplastic astrocytoma; GBM, glioblastoma multiforme.

⁴Feuerstein, B., and Biegel, J.A. (1999) Unpublished data.

⁵Feuerstein, B. (1999) Personal communication.

⁶Biegel, J.A. (1999) Unpublished data.

⁷James, C.D. (1999) Personal communication.

Table 1. Genetic diseases that predispose to the development of brain tumors

Disease	Gene	Tumor type
Li-Fraumeni syndrome	TP53 (17p13)	Glioma, ependymoma, choroid plexus tumor
Neurofibromatosis-1	NF1 (17q11)	Glioma
Neurofibromatosis-2	NF2 (22q12)	Vestibular schwannoma, ependymoma, meningioma
Nevoid basal cell carcinoma syndrome	PTC (9q22)	Medulloblastoma
Tuberous sclerosis	TSC1 (9q34) TSC2 (16p13)	Sub-ependymal giant-cell tumor
Turcot's syndrome	APC (5q21) hMLH1 (3p21) hPMS2 (7p22)	Medulloblastoma Glioblastoma
Von-Hippel Lindau disease	VHL (3p25)	Hemangioblastoma

as PNETs. For the purposes of this review, the term "medulloblastoma" will refer to tumors in the posterior fossa and will not distinguish between tumors without evidence of differentiation versus those with glial, neuronal, or ependymal differentiation. It should be noted that, regardless of location, CNS PNETs are distinct from peripheral PNETs (Ewing's sarcoma or peripheral neuroepithelioma), which are characterized by specific translocations involving the Ewing's sarcoma region (EWS) locus on chromosome 22q12.

Among all of the pediatric brain tumors, the most comprehensive cytogenetic studies have been reported for medulloblastoma. Bigner et al. (1997) and Bhat-tacharjee et al. (1997) have recently published large series on pediatric brain tumors subjected to standard cytogenetic analyses, and detailed findings of a large number of medulloblastomas as well as pediatric gliomas may be found in those reports.

Early cytogenetic studies demonstrated that the most frequent cytogenetic abnormality among medulloblastomas was an isochromosome 17q [i(17q)] (Biegel et al., 1989; Bigner et al., 1988a; Griffin et al., 1988). This results in loss of most of the short arm of chromosome 17 (17p) as well as duplication of the long arm (17q). Molecular genetic studies that used restriction length polymorphism analysis and, more recently, polymerase chain reaction-based microsatellite analysis, have confirmed the frequent loss of 17p in these tumors (Biegel et al., 1992; Thomas and Raffel, 1991), as shown in Fig. 1. Attempts to define a small common region of deletion in 17p, based on the loss of 17p13.3 in a limited number of tumors (Biegel et al., 1992), have been largely unsuccessful. Interphase FISH (Biegel et al., 1995) and microsatellite analysis have shown that the breakpoints in chromosome 17 cluster between the centromere and the proximal region of 17 (17p11.2), a region commonly deleted in patients with the Smith-Magenis syndrome (Scheurle et al., 1998; Wilgenbus et al., 1997). The tendency for chromosome 17 to undergo breakage in this region, with subsequent isochromosome formation, may be related to the repeat sequences present in the region around the centromere; therefore, sequences that are interrupted by the rearrangements may not contain a tumor suppressor gene. Deletion of most or all of 17p in medulloblastoma, instead of mitotic recombination (leading to loss of heterozygosity) or interstitial deletions within 17p13, sug-

gests that loss of more than one gene on 17p may be important in tumorigenesis. Furthermore, the identification of tumors with extra copies of the long arm of chromosome 17, in the absence of a 17p deletion, suggests that the duplication of sequences on 17q may confer a selective growth advantage to tumor cells. The whole-arm deletions and duplications of chromosome 17 have made the positional cloning of a chromosome 17 medulloblastoma gene extremely difficult. Several known genes, most notably *p53* (Biegel et al., 1992; Raffel et al., 1993; Saylor et al., 1991), have been ruled out as the chromosome 17 medulloblastoma tumor suppressor gene, and a novel candidate has not yet been reported.

The frequency of a deleted 17p13 region in medulloblastoma is estimated to be between 30 and 50% of cases, depending on the individual series analyzed. Although i(17q) or 17p deletion is not specific for medulloblastoma, it is seen in this tumor at a higher frequency than in any other tumor type. Furthermore, the finding of an i(17q) as a single structural abnormality in karyotypes suggests that it is a primary cytogenetic event, and not a cytogenetic change associated with clonal evolution (Biegel et al., 1989). For this reason, there has been great interest in determining if deletion of 17p is associated with clinical outcome. Batra et al. (1995) and Cogen and McDonald (1996) suggested that 17p deletion confers a poor prognosis, whereas Emadian et al. (1996) and Biegel et al. (1997) did not find a statistically significant difference in outcome in patients with or without tumor-associated 17p deletions. The difference in results regarding 17p deletion and outcome may be biased by the patient population included in the cytogenetic and molecular studies, especially if the treating institution sees a higher-risk group of patients. In recent studies reported by Scheurle et al. (1998), 30 primary medulloblastoma—PNETs and 6 metastasis specimens were analyzed for loss of heterozygosity for 17p13, as well as amplification of the *MYCC* oncogene. Loss of 17p13 was found in 14 of 30 (47%) tumors and 6 of 6 cerebrospinal fluid specimens and was found to be associated with a poor outcome. However, *MYCC* amplification was also shown to predict a poor response to therapy, and every case that demonstrated amplification of *MYCC* also had loss of 17p. It is clear that multivariate statistical analyses on large numbers of patients, preferably in conjunction with clinical trials conducted through a cooperative

group mechanism, will be required to determine whether the loss of 17p, as well as a variety of other genetic markers, may be used to predict prognosis for patients with medulloblastoma.

In addition to the loss of 17p, there are numerical and structural changes that involve several other chromosomes and that are nonrandomly associated with medulloblastoma and PNET. An extra copy of chromosome 7, often seen in tumors with an *i*(17q), is the second most common cytogenetic abnormality in medulloblastoma (Bhattacharjee et al., 1997; Biegel et al., 1989; Bigner et al., 1997; Griffin et al., 1988). Trisomy 7 is seen in association with a wide variety of solid tumors, most notably malignant gliomas (Bigner et al., 1988b; Jenkins et al., 1989). The fact that it is seen in association with other structural chromosome abnormalities in medulloblastoma suggests that it is a secondary change and not an initiating event. Loss of a sex chromosome is also a frequent secondary change, in contrast to malignant gliomas where it may present as the only cytogenetic alteration (Bigner et al., 1988b; Jenkins et al., 1989). Unbalanced translocations leading to extra copies of the long arm of chromosome 1 are frequent in medulloblastoma, and while this results in a relative loss of 1p sequences compared with 1q sequences, it is unclear if this is related to the loss of 1p36 seen in another common neural tumor of childhood, neuroblastoma. In the latter, loss of 1p36 and amplification of the *MYCN* oncogene are associated with a poor prognosis.

Unbalanced translocations or deletions of chromosomes 8, 10q, 11p and 11q, and 16q, in addition to 17p deletions, are the most frequent structural abnormalities in medulloblastoma, suggesting that several different tumor suppressor genes are involved in the initiation or progression of this disease. Loss of 9q, which may involve deletion and mutation of the *PTC* gene (Raffel et al., 1997), is seen in a relatively low percentage of cases, but may specify a subset of tumors with poor prognosis (Scheurlen et al., 1998). Medulloblastomas or PNETs with 9q deletions do not have 17p deletions, suggesting alternate pathways for tumor initiation.

Gene Amplification in Medulloblastoma

CGH is an extremely sensitive method for detecting genomic amplification and is currently the optimal method to determine sites of amplified genes in genome-wide screens of medulloblastoma–PNET. CGH is based on the differential labeling and in situ hybridization of tumor and reference DNA to normal metaphase chromosomes (Kallioniemi et al., 1992). A gain or loss of DNA in the tumor is reflected by an altered ratio of fluorescent signal at a specific location on the chromosome, as shown in Fig. 2. The sensitivity of CGH is limited by the resolution achieved at the cytogenetic level. For example, deletions within a chromosomal band may not be detected. Although the technique cannot be used to detect balanced rearrangements, such as inversions and translocations, it is extremely sensitive for detecting amplification events. Two recent CGH studies of primary medulloblastoma–PNETs suggest a very low frequency of amplification events in these tumors. Reardon

Fig. 1. Polymerase chain reaction-based microsatellite analysis demonstrating loss of heterozygosity for marker D17S559 in chromosome band 17p13 in patients with medulloblastoma. DNA was isolated from peripheral blood or a lymphoblastoid cell line (N) and tumor (T). Loss of one allele in the tumor compared with the normal DNA, consistent with a 17p deletion, is shown in cases 98-02 and 98-03.

et al. (1997) identified amplified regions in only 3 of 27 tumors, including 2 amplifications at 5p13 and 1 at 11q22.3. Similarly, we observed 2 amplified regions, 1 at the locus on 2p that contains the *MYCN* gene, and another near the centromere on chromosome 7 in 32 medulloblastomas analyzed by CGH.⁴ In contrast, Bigner et al. (1988a, 1997) reported a high frequency of double-minute chromosomes, a cytogenetic hallmark of gene amplification, in medulloblastoma karyotypes. In their most recent study, 7 of 17 medulloblastomas with abnormal karyotypes contained double-minute chromosomes. Interestingly, at least 5 of the 7 cases were seen in tumors with one or more copies of an *i*(17q). Although genes that were amplified in these tumors were not described, it is possible that the poor prognosis for patients with a 17p deletion—noted by these authors in other publications (Batra et al., 1996)—may be related to the high frequency of gene amplification present in these tumors.

Monosomy 22 in Medulloblastoma

The identification of medulloblastomas with monosomy 22 has generated considerable debate among investigators in this field. Monosomy 22 was first reported by Vagner-Capodano et al. (1988). Bigner et al. (1997) and Bhattacharjee et al. (1997) recently reported monosomy 22 as a primary abnormality in approximately 10% of medulloblastomas. In contrast, we have observed monosomy or deletion of chromosome 22 in medulloblastomas with more complex karyotypes, especially in tumors with an *i*(17q) (Biegel et al., 1989). Furthermore, CGH analysis of three large series of medulloblastoma–PNET cases has not shown monosomy 22 to be a primary change (Reardon et al., 1997; Schutz et al., 1996).⁴ Most medulloblastoma biopsies with monosomy 22 have been obtained from patients who were diag-

A

Fig. 2. Comparative genomic hybridization analysis of a medulloblastoma specimen. A. Regions that appear red indicate a loss of copy number in the tumor compared with normal DNA, and areas that appear green indicate a gain.

nosed within the first two years of life, raising the possibility that some cases have been misdiagnosed and are in fact atypical teratoid tumors (see below). In some situations, this may also be due to a sampling problem in which only the primitive neuroepithelial component has

been biopsied. Regardless of how the tumors may be classified from a pathologic perspective, however, it will be important to determine if monosomy 22, as a single change or in combination with other alterations, predicts a poor prognosis for these patients.

Fig. 2, continued. Comparative genomic hybridization analysis of a medulloblastoma specimen. B. A histogram of the fluorescence readings for the same tumor shows gain of 7q, loss of distal 8p, gain of distal 9p, loss of 11p and 11q, and loss of 17p and gain of 17q (consistent with an isochromosome 17q).

Cytogenetic and Molecular Genetic Alterations in Supratentorial PNETs

Supratentorial PNET refers to those tumors with histologic features that are similar to medulloblastoma, but arise in the cerebrum, suprasellar region, or pineal gland. They may also be referred to as cerebral neuroblastoma, pineoblastoma, or ependymblastoma. Although some pathologists classify the tumors based on location, others have based their diagnosis on the presence of cellular differentiation as determined by light microscopy or immunohistochemical characterization.

Compared with the infratentorial PNETs or medulloblastomas, there is very little known regarding the cytogenetic and molecular genetic alterations in supratentorial PNETs. Burnett et al. (1997) analyzed 8 supratentorial PNETs from patients ranging in age from 19 days to 16 years. The tumors were located in different parts of the brain and were of varied histology. None of the 8 tumors demonstrated a loss of heterozygosity for markers that map to 17p13.3, in contrast to approximately one-third of the medulloblastomas that did show loss. CGH analysis of 10 supratentorial PNETs demonstrated similar findings.⁵ Although nonrandom cytogenetic gains and losses were present, none of the tumors had a deletion of 17p or i(17q). Kees et al. (1994)

reported a pineoblastoma cell line with a deletion of 17p. However, the cell line was established after the patient had been treated, and it is possible that the structural abnormality was treatment-induced. Pineoblastomas have been reported with monosomy 22 (Bigner et al., 1997) or structural rearrangements of chromosome 11 (Sreekantaiah et al., 1989), suggesting that there may be some overlap with atypical teratoid tumors or medulloblastomas. Although a variety of other abnormalities have been identified in supratentorial PNETs (Griffin et al., 1988), the number is still too small to conclude that the abnormalities in supratentorial PNETs are different from their infratentorial counterparts.

Mutations in PTC and β -Catenin in Medulloblastoma

Several inherited genetic diseases predispose affected patients to the development of medulloblastoma, including the nevoid basal cell carcinoma syndrome, also known as Gorlin's syndrome. Nevoid basal cell carcinoma syndrome is an autosomal dominant disorder that is characterized by multiple basal-cell carcinomas, keratocysts of the jaw, palmar and plantar pits, and skeletal abnormalities. The gene for nevoid basal cell carcinoma syndrome is located on chromosome band 9q22.3 and is the human homologue of the *Drosophila* patched gene,

PTC (Hahn et al., 1996; Johnson et al., 1996). Somatic mutations in *PTC* have been demonstrated in approximately 10% of patients with sporadic medulloblastomas (Raffel et al., 1997). Raffel and coworkers hypothesized that alterations in other genes upstream or downstream of *PTC*, including *sonic hedgehog* (*SHH*) and *smoothened* (*SMO*), would have the same functional effect as inactivation of *PTC*, resulting in tumor development. Chiappa et al. (1999) screened 27 medulloblastoma-PNETs for mutations in *SHH* and *SMO* and in other genes in this pathway and found no evidence for mutations in any genes other than *PTC*.

Medulloblastoma is also seen in association with Turcot's syndrome. Mutations in the adenomatous polyposis coli gene (*APC*) have been demonstrated in patients with Turcot's syndrome and medulloblastoma, but not in patients with isolated malignancies (Hamilton et al., 1995; Mori et al., 1994). The *APC* gene is part of a pathway that regulates β -catenin, a key component in cell-cell adhesive junctions and transduction of wingless-Wnt signaling, and mutations in β -catenin have been identified in a small percentage of sporadic medulloblastomas (Zurawel et al., 1998). Ongoing mutation and functional studies of the genes in these pathways will lead to a better understanding of how they contribute to tumorigenesis.

Altered Expression of Genes Involved in Neural Growth and Differentiation

Neurotrophins and neurotrophin receptors are involved in differentiation, cell growth, and apoptosis in the developing cerebellum. Medulloblastomas express one or more of the neurotrophins (NGF, BDNF, NT3) as well as their receptors (p75, TrkA, TrkB, TrkC). Pomeroy et al. (1999) have shown that high levels of *TrkC* expression, as determined by Northern blot analysis, are associated with improved prognosis in patients with medulloblastoma (Segal et al., 1994). Tumors with functional TrkC receptors may undergo apoptosis in the presence of NT-3, which may explain why patients with medulloblastoma that demonstrate high levels of *TrkC* have longer survival times than patients with tumors with low levels of *TrkC*. Because it has not yet been possible to develop an antibody specific for TrkC, reverse transcriptase-polymerase chain reaction-based studies or in situ hybridization analysis of tissue sections using antisense probes to the *TrkC* message may be good alternatives to Northern blot analysis for determining expression levels of *TrkC* at the time a patient is diagnosed (Pomeroy et al., 1999). These continuing studies may ultimately provide a basis for developing biologically based treatment strategies.

The *NeuroD* family of basic helix-loop-helix transcription factors regulates transcription of genes involved in neuronal differentiation, which are expressed at distinct times during development. Expression of the *NeuroD* genes was determined in a series of brain tumors and shown to be restricted to medulloblastomas and PNETs (Rostomily et al., 1997). Interestingly, expression of *NeuroD3* was observed primarily in tumors from patients who had metastatic disease at diagnosis or who rapidly progressed. Furthermore, expression of *achaete scute*, another neurogenic transcription factor with homology to

NeuroD genes, was expressed in 3 of 5 supratentorial PNETs but 0 of 13 medulloblastomas examined. These results suggest that the cells that give rise to supratentorial PNETs may be different from those that give rise to medulloblastomas. If confirmed in a larger series of patients, the expression of *NeuroD* family members may provide another prognostic variable for patients with medulloblastoma-PNET. Altered expression of genes in this pathway could also be exploited for biologically based treatments.

Atypical Teratoid/Rhabdoid Tumor

Rhabdoid tumors may occur in all locations of the body, although the kidney and brain are the most common sites of presentation. They are seen almost exclusively in the pediatric population, and although they may account for only 2% of pediatric brain tumors, the complicated histologic appearance and rapidly fatal course make rhabdoid tumors one of the major clinical challenges in pediatric oncology today. In the CNS, these tumors, which Rorke has designated atypical teratoid tumors (Rorke et al., 1996), may consist purely of rhabdoid cells, may contain areas of rhabdoid cells juxtaposed to mesenchymal and epithelial tissue, and may contain regions that resemble PNET. AT/RTs are often misdiagnosed by pathologists as medulloblastomas or PNETs because of the presence of the primitive neuroepithelial component (Burger et al., 1998; Rorke et al., 1996). The tumors may present in any location within the CNS, although the most common regions include the cerebellum, cerebrum, cerebellopontine angle, and pineal gland. AT/RT can best be distinguished from medulloblastoma-PNET by using antibodies to epithelial membrane antigen, which will test positive in all cases (Burger et al., 1998; Rorke et al., 1996).

The median age at presentation for patients with AT/RT is 20 months, and there is a slight male predominance (M:F, 1.6:1) (Burger et al., 1998; Rorke et al., 1996). In contrast to medulloblastomas, with which there is up to a 75% survival rate when combined modality treatment is given, rhabdoid tumors in all sites are extremely aggressive and usually fatal. Because these children are usually diagnosed in the first 2 years of life when cranial radiation therapy is not recommended, it is imperative that the correct diagnosis be made so that they can be treated aggressively. The finding of monosomy or deletion of chromosome 22 in AT/RT tumors (Fig. 3) has proven to be a useful adjunct to pathologic classification in the diagnosis of these patients.

The combined cytogenetic and molecular genetic characterization of CNS AT/RTs (as well as rhabdoid tumors of the kidney and other extrarenal sites) has recently led to the identification of a rhabdoid tumor suppressor gene in chromosome band 22q11.2. We first described 3 AT/RT tumors of the brain that had monosomy 22 as the only cytogenetic change (Biegel et al., 1990), thus implicating it as a primary genetic event in tumor development. Subsequently, a commonly deleted region of chromosome 22 was defined in a series of primary renal rhabdoid tumors (Schofield et al., 1996) and a variety of rhabdoid tumor cell lines, implicating the same gene in the development of both renal and extrarenal rhabdoid tumors

(Biegel et al., 1996). A positional cloning approach was initiated to isolate candidate genes from the minimally deleted region in tumors, ultimately leading to the identification of the *INI1* gene as a candidate gene for renal and extrarenal rhabdoid tumors (Versteeg et al., 1998) and CNS AT/RTs (Biegel et al., 1999).

The *hSNF5/INI1* gene is the human homologue of the yeast *SNF5* gene and is one of at least 12 proteins in the SWI/SNF complex (Wang et al., 1996). The SWI/SNF complex is thought to function in an ATP-dependent manner to cause a conformational change in the nucleosome that alters histone-DNA binding (Schnitzler et al., 1998), thereby facilitating transcription factor access. Studies of the mammalian SWI/SNF complex have suggested that different cells may contain distinct subunits, and thus may function to remodel chromatin in a cell-specific manner (Wang et al., 1996). *INI1* appears to be an invariant component of all complexes. *INI1* was also isolated through its interaction with human immunodeficiency virus integrase and is thought to mediate retroviral integration of human immunodeficiency virus into the genome (Kalpana et al., 1994).

Studies of the *INI1* *Drosophila* homologue, *snr1*, have shown that *snr1* is expressed at high levels during early embryogenesis and, in later development, is expressed most highly in the CNS (Dingwall et al., 1995). *Snr1* homozygous mutants die during embryogenesis, also supporting its role as a tumor suppressor gene. Dingwall et al. (1995) reported that *snr1* interacts with trithorax, an activator of homeotic gene transcription. Rozenblatt-Rosen et al. (1998) have recently shown that the human homologue of trithorax, *ALL-1*, also interacts with *INI1*, and propose that the SWI/SNF complex is recruited to *ALL-1* target genes through its interaction with *INI1*. The trithorax-polycomb group of proteins controls expression of a variety of homeotic genes required for normal development.

Deletion and mutation analysis of a large series of CNS, renal, and other extrarenal rhabdoid tumors have shown that alterations in the *INI1* gene are responsible for the development of most rhabdoid tumors, regardless of site (Biegel et al., 1999). Among 40 tumors analyzed to date, 6 demonstrated homozygous deletions of the entire *INI1* gene and 15 contained smaller deletions within the coding sequence. Mutations within 1 of the 9 coding exons of the gene have been identified in the remaining 19 tumors. Most mutations are nonsense mutations causing premature truncation of the protein. Single bp deletions have been observed in 3 brain tumors, whereas 2 bp, 7 bp, and 10 bp deletions have been seen in one patient each. A 4 base insertion in exon 2 and a 19 base duplication in exon 6 have each been identified in one CNS AT/RT. Each of these deletions and insertions causes a frameshift and also results in premature truncation of the protein.

The most important finding has been the identification of germline mutations in the *INI1* gene in five patients with rhabdoid tumors (Biegel et al., 1999). The mutations include the 10-bp deletion in exon 7 noted above, and 4 point mutations, all of which were C to T transitions resulting in the coding of a stop codon. As expected, mutations were heterozygous in the DNA from

Fig. 3. Interphase fluorescence in situ hybridization of a rhabdoid tumor cell with probes for the *INI1* gene (red) and control *EWS* probe (green). Only one red signal is present in this cell, consistent with a partial deletion of chromosome 22.

peripheral blood. Tumors demonstrated loss of the wild-type allele or an acquired mutation in the second allele. These findings are consistent with the tumor suppressor gene hypothesis, which predicts homozygous inactivation of both genes through mutation and/or deletion. The germline and acquired (somatic) mutations described above were observed in patients with renal and CNS rhabdoid tumors, supporting our hypothesis that they are a similar biologic entity, regardless of the site of presentation and despite varied histologic appearance.

The specificity of *INI1* mutations for rhabdoid or AT/RTs has not yet been determined, and there are a variety of tumors with deletions of chromosome 22q11 that need to be examined. We have reported one tumor that was histologically confirmed to be a PNET, although a very small biopsy was available for study. The karyotype demonstrated monosomy 22 as the only abnormality, and a homozygous deletion within the *INI1* gene was identified (Biegel et al., 1999). It is possible that deletions or mutations of the *INI1* gene are associated with an aggressive clinical course regardless of the histologic appearance of the tumor. For example, patients who are diagnosed in the first 3 years of life with a medulloblastoma or PNET and have an implied poor prognosis may, in fact, have mutations in *INI1* that could account for their poor clinical outcome. Furthermore, *INI1* mutations in tumors that have other primary abnormalities, such as an *i*(17q), may also help to explain the poor prognosis seen in otherwise good-risk patients. Molecular analysis of *INI1* should thus have diagnostic utility and will be useful for genetic testing and counseling in families who may be predisposed to develop malignancies.

Ependymoma

Ependymomas are the third most common type of childhood brain tumor, accounting for approximately 10% of cases. The tumors arise from the layer of epithelial cells

lining the ventricular walls and spinal canal. Most tumors are intracranial and have a greater propensity to arise in the posterior fossa in young children. Four major histologic subtypes of ependymoma include ependymoma, malignant or anaplastic ependymoma, subependymoma, and myxopapillary ependymoma. The latter two types are rarely seen in children. As with gliomas, ependymomas may show a range of anaplastic features, including high mitotic indices, necrosis, cellular pleomorphism, and vascular proliferation. In contrast to diffuse astrocytomas, however, correlations between histologic features and clinical outcome in patients with ependymomas have been inconclusive (Hamilton and Pollack, 1997). Extent of surgical resection is currently the best predictor of outcome (Pollack et al., 1995; Sutton et al., 1990–1991). Similar to patients with medulloblastoma, children <3 years of age may also have a significantly worse prognosis, although the basis for this is not yet known (Pollack et al., 1995).

The molecular and cytogenetic studies of ependymoma are quite limited, and the accumulated data has been based on case reports and small series of patients. There has been one report of a patient with a germline p53 mutation who developed an ependymoma (Metzger et al., 1991), although this is not one of the brain tumors typically seen in Li-Fraumeni families with germline p53 mutations. Fink et al. (1996) analyzed 31 ependymomas, including 6 anaplastic ependymomas and 22 pediatric cases, for mutations in exons 5–8 of the p53 gene. None of the tumors demonstrated any mutations.

Patients with neurofibromatosis type 2 (NF2) have an increased propensity to develop ependymomas and meningiomas, and mutations in the *NF2* gene have been observed in tumors isolated from such patients (Rubio et al., 1994; Rutledge et al., 1994). Sporadic ependymomas, however, do not demonstrate mutations in the *NF2* gene, even in cases where there is loss of heterozygosity for markers in the 22q11–q12 region (Rubio et al., 1994; Slavic et al., 1995). Park et al. (1996) reported a child with an ependymoma and a constitutional t(1;22)(p22;q11), implicating a possible ependymoma locus in chromosome 1p22 or 22q11. The chromosome 22 breakpoint in this case is located proximal to the *NF2* locus and maps to the 22q11 translocation breakpoint region associated with the supernumerary t(11;22)(q23;q11) syndrome. The tumor did not demonstrate loss of the remaining chromosome 22 homologue, and the authors have therefore postulated that a locus on chromosome 1 may have been interrupted by the translocation (Rhodes et al., 1997).

Cytogenetic studies of pediatric ependymomas have shown a varying degree of complexity ranging from normal karyotypes or monosomy 22 to complex karyotypes with a variety of structural alterations (Hamilton and Pollack, 1997; Kramer et al., 1998; Neumann et al., 1993). Chromosomes that appear to be nonrandomly involved include chromosomes 1, 11, 17, and 22. FISH and molecular genetic studies have confirmed the loss of chromosome regions 17p and 22q in both ependymomas and anaplastic ependymomas (Kramer et al., 1998; von Haken et al., 1996), although the frequency of loss of heterozygosity for chromosome 22 appears to be lower than that predicted based on the earlier cytogenetic reports.

Reardon et al. (1999) recently reported a study using CGH to analyze 23 primary pediatric ependymomas. Among the 23 samples analyzed, 10 tumors did not demonstrate any regions of gain or loss. This is similar to what has been reported previously in standard cytogenetic studies of large series of patients (Kramer et al., 1998). The remaining 13 tumors showed relatively simple patterns of gain and loss, which did not appear to be associated with the degree of malignancy. Only one of the 23 cases, the single spinal cord myxopapillary tumor, demonstrated a total of 18 gains and losses. The remaining 12 abnormal cases were relatively simple, with no more than 3 regions of chromosomal gain, and/or 3 regions of loss. Three ependymomas demonstrated loss of the X chromosome as the only change, whereas an additional 3 tumors had loss of an X in the presence of other imbalances. One benign ependymoma contained monosomy 22 as the only detectable alteration, whereas in 3 tumors the loss of chromosome 22 was seen with other gains or losses. Two tumors demonstrated deletions of chromosome 17, including a case with monosomy 17 and a case with a 17p deletion. None of the cases appeared to have an i(17q). Five tumors contained monosomy 6 or a smaller deletion of 6q. The most frequent region of chromosomal gain in the series of tumors involved the long arm of chromosome 1 and chromosome 9, present in 5 cases and 4 cases, respectively. Interestingly, there were no regions of amplification detected in any of the tumors.

Based on what is currently known regarding the cytogenetic and molecular alterations in ependymomas, we can conclude the following. First, a large percentage of tumors (up to 50%) may have submicroscopic alterations that are not detected by standard cytogenetic studies, CGH, or random screening for regions of loss of heterozygosity. When present, the number of alterations does not appear to be associated with the malignancy of the tumor as determined by histology, or at present, by clinical course. Numerous studies have demonstrated loss of alleles on chromosome 22 by cytogenetics, FISH, loss of heterozygosity studies, or CGH. Although the number of pediatric tumors with chromosome 22 deletions is still small, they will be the most likely targets for mutation screening of candidate tumor suppressor genes that map to chromosome 22. It has now become evident that loss of a region on 6q may be important in the development of pediatric ependymomas. Among the 22 ependymomas reported by Kramer et al. (1998), 5 tumors had alterations of chromosome 6, a frequency similar to that noted in the study by Reardon et al. (1999). Neumann et al. (1993) and Rogatto et al. (1993) reported the involvement of chromosome 6 in ependymomas, and two ependymoma xenografts established by McLendon et al. (1996) demonstrated deletion or loss of chromosome 6 in the karyotypes. These xenografts may provide useful models for studies of tumor cell growth as well as the analysis of candidate tumor-associated genes.

Astrocytomas

Astrocytomas account for the largest percentage of solid tumors in children and comprise approximately 40% of

all pediatric brain tumors. The most common histologic subtype of astrocytoma in children is the juvenile pilocytic astrocytoma. Pilocytic astrocytomas are the most common brain tumors seen in patients with neurofibromatosis-1, where they usually involve the optic nerve. Sporadic tumors may present throughout the CNS, and depending on their location, are often amenable to complete surgical resection. The long-term prognosis for children with pilocytic astrocytomas is very good, although some tumors do recur as malignant lesions, which may then be fatal.

Diffuse astrocytomas are histologically similar to astrocytomas seen in adults, although the incidence is much lower in children. The most common location is the cerebral hemispheres. The diffuse astrocytomas in children include the low-grade fibrillary, gemistocytic, and protoplasmic astrocytomas; grade III AAs; and grade IV GBMs. AA and GBM rarely present as primary lesions in children, whereas they account for most of the astrocytomas in adults.

Molecular and Cytogenetic Studies of Pediatric Astrocytomas

Cytogenetic studies of adult gliomas have shown that low- or intermediate-grade astrocytomas contain simple numerical changes, such as trisomy 7 or loss of a sex chromosome, whereas the pattern of chromosomal abnormalities in GBMs is more complex. The consistent abnormalities seen in adult GBMs include extra copies of chromosome 7, loss of 9p, monosomy 10, deletion of 22q, and double-minute chromosomes (Bigner et al., 1988b). Several of the genes associated with these chromosomal deletions have now been identified, such as *CDKN2/p16* deletions in tumors with loss of 9p (Schmidt et al., 1994) and *MMAC1/PTEN* (Li et al., 1997; Steck et al., 1997) or *DMBT1* (Mollenhauer et al., 1997) deletions and mutations in tumors with 10q loss. (For review see Louis, 1997.) Efforts to determine if the molecular genetic alterations seen in adult astrocytomas occur in childhood astrocytomas have been hampered by the low incidence of primary high-grade gliomas in the pediatric population.

Cytogenetic studies of juvenile pilocytic astrocytoma have generally demonstrated normal karyotypes, a finding that has been substantiated by the absence of chromosomal gains or losses as determined by CGH (Schrock et al., 1996). In the large series of pediatric brain tumors reported by Neumann et al. (1993), Bhattacharjee et al. (1997), and Bigner et al. (1997), several low-grade tumors, including some pilocytic astrocytomas, demonstrated abnormal karyotypes with apparently random changes. Furthermore, Ashby et al. (1999) recently analyzed 11 patients with pilocytic astrocytoma and showed that the presence of an abnormal karyotype was correlated with clinical recurrence. White et al. (1995) reported trisomy 7 and trisomy 8 as common findings in pilocytic astrocytomas, but not in fibrillary astrocytomas, as determined by interphase FISH of isolated nuclei from paraffin sections. While it seems intriguing to speculate that the presence of chromosomal imbalances (detected by karyotype, CGH, FISH, or molecular analysis) pre-

dicts a poor prognosis, large studies with independent pathology review and long-term follow-up for these patients will be required to support this hypothesis.

In contrast to the low-grade astrocytomas, karyotypes from high-grade pediatric AAs and GBMs demonstrate a variety of numerical and structural chromosomal changes (Griffin et al., 1988; Neumann et al., 1993).⁶ Although some tumors show abnormalities that are typical of adult malignant gliomas, such as gains of chromosome 7 and loss of chromosome 10, many tumors have a variety of rearrangements that appear to be distinct. Structural changes of chromosomes 1, 7, 9, 17, and 22 have been observed in several cases among the different series, but there are no consistent breakpoints. Similar to adult GBMs, double-minute chromosomes in the karyotypes consistent with *EGFR* gene amplification and molecular alterations of the epidermal growth factor receptor have also been noted in pediatric astrocytomas (Moscatello et al., 1995). Preliminary CGH data suggest that pediatric gliomas may contain a variety of amplified genes, some of which map to chromosomes 2, 8, and 12 (Warr et al., 1999). A much larger series of pediatric tumors from patients who have not been previously treated needs to be examined to identify candidate regions of the genome that may contain unique tumor-related genes.

Similar to medulloblastoma, pediatric high-grade gliomas demonstrate loss of alleles on 17p. However, in contrast to medulloblastoma, mutations of the *p53* gene have been seen in a high percentage of both pediatric AAs and GBMs (Felix et al., 1995; Pollack et al., 1997). James has compared the frequency of *p53* mutations, homozygous *p16* deletions, and *PTEN* mutations in pediatric and adult grade II–IV gliomas.⁷ Mutations in *p53* were noted in tumors of all grades, regardless of age. Deletions of *p16* and *PTEN* mutations were observed in grade III and grade IV gliomas in both children and adults, but were not detected in any grade II tumors. Based on these results, it appears that the molecular genetic events that are involved in tumor development may be similar, regardless of age. In contrast to what has been reported for adult malignant gliomas, however, a recent study by Pollack et al. (1997) demonstrated that mutation in exons 5–8 of the *p53* gene (11 of 29 AAs and GBMs) or overexpression of *p53* by immunohistochemical analysis (18 of 29 tumors) was associated with decreased survival when controlled for extent of resection, location, age, and histology. The same group of tumors was subsequently analyzed for the expression of basic fibroblast growth factor, and a strong correlation was seen with low basic fibroblast growth factor and increased progression-free survival (Bredel et al., 1997). If larger studies confirm these findings, it may be possible to use *p53* mutation status and basic fibroblast growth factor expression levels as predictors of outcome for patients with AA and GBM, and ultimately stratify patients for therapeutic trials based on the results of these evaluations.

Choroid Plexus Tumors

Choroid plexus tumors are most frequently seen in children under the age of 10 and may account for as many

Fig. 4. Representative G-banded karyotype from a choroid plexus papilloma. The karyotype is 53, XY, +6, +7, +9, +11, +12, +14, +15, -18, -19, +20, +21. The losses of 18 and 19 were only seen in this cell and thus appear to be random.

as 10–20% of tumors seen in the first year of life (Aguzzi et al., 1997). The most common location is the lateral ventricle, although they may arise in any location where there is choroid plexus. Choroid plexus papillomas are benign epithelial tumors, which are often amenable to complete resection. In contrast, choroid plexus carcinomas show all of the typical signs of malignancy, including high mitotic indices, nuclear pleomorphism, and invasiveness. Bergsagel et al. (1992) reported the presence of SV40 sequences in a high frequency of choroid plexus tumors, as well as ependymomas, implicating a viral etiology for these tumors. A constitutional (X;17)(q12;p13) translocation has been described in a patient with hypomelanosis of Ito and a choroid plexus papilloma (Steichen-Gersdorf et al., 1993), raising the possibility that a gene on 17p13 or Xq12 may be involved in the development of choroid plexus tumors.

Cytogenetic studies of choroid plexus tumors have been limited, and yet a simple pattern of numerical changes appears to be a consistent finding. A representative karyotype from a choroid plexus papilloma is shown in Fig. 4. Punnett et al. (1994) and Donovan et al. (1994) described two additional choroid plexus papillomas with hyperdiploid karyotypes (52 or 56 chromosomes per cell), both of which contained extra copies of chromosomes 7, 11, 12, 15, and 18 but no structural abnormal-

ities. Trisomy for one or more of these chromosomes was demonstrated in 5 of 8 choroid plexus tumors by interphase FISH (Donovan et al., 1994). Abnormal karyotypes were also obtained from 3 of 5 choroid plexus papillomas reported by Bhattacharjee et al. (1997). The karyotypes had 51–61 chromosomes per cell, and in addition to the trisomies noted above, had extra copies of chromosome 20. In contrast, 1 choroid plexus carcinoma reported by Bhattacharjee et al. (1997) and 1 unspecified choroid plexus tumor analyzed by Neumann et al. (1993) were hypodiploid, with 32 or 33 chromosomes per cell and simple structural changes involving chromosomes 9, 19, or 21. At present, the number of cases analyzed is too small to correlate the cytogenetic findings with clinical course.

Germ-Cell Tumors

Germ-cell tumors comprise approximately 3% of childhood brain tumors. Approximately 90% of CNS germ-cell tumors present in those younger than 20 years of age (Rosenbloom and Ng, 1997). The histologic subtypes of these tumors include germinoma, teratoma, yolk sac tumors, embryonal carcinoma, and choriocarcinoma. Similar to atypical teratoid tumors, CNS germ-cell

tumors frequently present with mixed histology. The most common location is the pineal gland.

Although males with Klinefelter's syndrome (47, XXY) have an increased risk of developing germ-cell tumors, most CNS germ-cell tumors are sporadic. There are few reported cytogenetic or molecular genetic studies of CNS germ-cell tumors that highlight areas of the genome likely to contain candidate tumor-associated genes. The characteristic isochromosome 12p found in adolescent testicular germ-cell tumors has been reported in one pineal germinoma (de Bruin et al., 1994), but not in 5 other germ-cell neoplasms (Bhattacharjee et al., 1997; Shen et al., 1990). Yu et al. (1995) reported a variety of sex chromosome abnormalities in pineal germ-cell tumors that are also seen in these tumors at other sites. Bhattacharjee et al. (1997) studied 4 germ-cell tumors in their series of 120 pediatric brain tumors. One had a normal karyotype and the other 3 contained a variety of structural changes. Interestingly, one tumor contained an i(17q), a derivative chromosome 12, and a homogeneously staining region consistent with genomic amplification. Because of the limited number of cytogenetic and molecular studies on intracranial germ-cell tumors, it is too early to determine whether the molecular etiology is

similar to other types of brain tumors or to germ-cell tumors at other sites.

Summary

Cytogenetic and molecular studies of childhood CNS tumors have demonstrated tumor-associated abnormalities that have clinical utility in a diagnostic setting. The differential diagnosis for a child with a malignant tumor often includes tumors with vastly different clinical outcomes, and as such may require aggressive therapeutic strategies when less toxic protocols could be used. Identifying mutations or altered expression of specific genes in different tumor types may ultimately lead to novel therapeutic agents that can be specifically targeted to those defects.

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