Molecular genetics of supratentorial primitive neuroectodermal tumors and pineoblastoma

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The supratentorial primitive neuroectodermal tumors (PNETs) are a group of highly malignant lesions primarily affecting young children. Although these tumors are histologically indistinguishable from infratentorial medulloblastoma, they often respond poorly to medulloblastoma-specific therapy. Indeed, existing molecular genetic studies indicate that supratentorial PNETs have transcriptional and cytogenetic profiles that are different from those of medulloblastomas, thus pointing to unique biological derivation for the supratentorial PNET. Due to the rarity of these tumors and disagreement about their histopathological diagnoses, very little is known about the molecular characteristics of the supratentorial PNET. Clearly, future concerted efforts to characterize the molecular features of these rare tumors will be necessary for development of more effective supratentorial PNET treatment protocols and appropriate disease models. In this article the authors review existing molecular genetic data derived from human and mouse studies, with the aim of providing some insight into the putative histogenesis of these rare tumors and the underlying transforming pathways that drive their development. Studies of the related but distinct pineoblastoma PNET are also reviewed.

KEY WORDS • supratentorial primitive neuroectodermal tumor • pineoblastoma • molecular genetics

OVERVIEW

The supratentorial PNET and pineoblastoma are high-grade, relatively undifferentiated tumors arising from the cerebral hemispheres and the pineal regions, respectively. Although they are histologically very similar to medulloblastoma, supratentorial PNETs have markedly different clinical behavior and are generally considered to represent a more aggressive tumor group than medulloblastoma, with a frequently massive tumor burden and a higher incidence of disseminated disease at diagnosis, as reviewed in Jakacki.⁸⁵ Because cerebral supratentorial PNETs may often be deep-seated in paraventricular, thalamic, and hypothalamic sites,⁴⁴ they pose significant challenges to safe resection and the delivery of limited radiation. Thus, despite their smaller numbers, supratentorial PNETs are a significant therapeutic problem because these highly aggressive, therapy-resistant tumors primarily affect younger children, who are the most likely to suffer long-term damage due to radiation.

Existing data support the suggestion that supratentorial

PNETs have molecular features that are unique from those of medulloblastoma. Nevertheless, because of the rarity of supratentorial PNETs, only limited molecular studies of small numbers of supratentorial PNETs have been conducted to date. Clearly, comprehensive knowledge of the molecular genetic features of supratentorial PNETs will be essential in future studies to advance diagnosis, therapy, and prognosis in this rare but challenging tumor group. We review existing molecular studies of supratentorial PNETs with a view to improving our current understanding of putative signaling defects and molecular abnormalities in supratentorial PNETs. We also review molecular studies of pineoblastomas, a WHO⁹⁴ Grade IV pineal parenchymal tumor that has often been considered collectively with the supratentorial PNET in molecular and therapeutic studies. Nevertheless, existing data suggest distinct histogenesis and unique molecular genetics for the pineoblastoma, and they are therefore discussed separately.

SUPRATENTORIAL PNETS

Collectively, supratentorial PNETs are relatively rare, representing approximately one tenth the frequency of medulloblastoma, and 3 to 7% of pediatric CNS tumors.^{91,97} They

Abbreviations used in this paper: cdk = cyclin-dependent kinase; CGH = comparative genomic hybridization; CNS = central nervous system; mRNA = messenger RNA; PNET = primitive neuroectodermal tumor; Shh = Sonic hedgehog; WHO = World Health Organization.

are diagnosed at a younger median age than medulloblastomas; more than 65% of supratentorial PNETs are reported in patients younger than 5 years of age with no significant sex predominance (reviewed in Jakacki, et al.⁸⁶). In a Pediatric Oncology group trial for delayed radiation in children younger than 3 years of age, supratentorial PNETs represented the second most common tumor (13 of 19).⁴⁴ Supratentorial PNETs can present as congenital tumors.^{39,64,113} Although supratentorial PNET is generally considered almost exclusively a pediatric tumor, rare adult series have been described.^{91,155} There is currently insufficient literature to judge whether supratentorial PNETs in adults differ from those in children. It is notable, however, that one study reported a 3-year survival rate of 75% for a series of 12 patients, a rate far superior to any reported in pediatric supratentorial PNETs.⁹¹ These observations, together with reports of a high frequency of p53 mutations in adult supratentorial PNETs,⁷⁴ suggest they are likely to represent distinct diseases.

High-grade glial tumors are well recognized as potential late complications of the craniospinal radiation treatment used in childhood leukemias. Interestingly, supratentorial PNETs can also present as secondary malignancies after therapy for hematological as well as primary intracranial neoplasms. To date, seven cases of secondary supratentorial PNET arising after treatment for childhood acute lymphocytic leukemia, acute myeloid leukemia, or lymphoma have been reported.¹⁰² Secondary supratentorial PNETs have been reported in the context of cancer predisposition syndrome in two patients. One child presented with supratentorial PNET 5 years after treatment for unilateral retinoblastomas,⁵⁰ and in another with neurofibromatosis Type 1, supratentorial PNET developed after radiation treatment for a brainstem astrocytoma.¹³² Supratentorial PNETs can also occur as secondary malignancies in both children and adults with no genetic condition. Tumors occurring up to 18 years after radiation treatment for low-grade primary intracranial neoplasms such as cerebellar pilocytic astrocytoma and low-grade astrocytoma have been described.^{34,69}

A supratentorial PNET most commonly arises in the cerebrum, and is seen less frequently in deep paraventricular or midline locations such as the diencephalon and basal ganglia.⁴⁷ Rarely, PNETs can present in the leptomeninges without evidence of primary tumor in the supra- or infratentorial compartments.¹³ These are proposed to arise from heterotopic glial nests within the subarachnoid space;⁴¹ however, their true histogenesis and their relationship to either medulloblastoma or supratentorial PNET is unknown.

The current literature contains reports suggesting that metastatic disease, extent of surgery, and age at diagnosis may be of prognostic relevance in supratentorial PNETs.^{3,75,135} Based on more aggressive clinical features, supratentorial PNETs have traditionally received therapies designed for metastatic, high-risk medulloblastomas, which involves more intensive chemotherapy and higher doses of radiation to the head and spine. Several retrospective^{123,135} and prospective studies^{40,75,86} have consistently reported significantly inferior outcomes for even localized supratentorial PNETs treated with high-risk medulloblastoma therapy. To date, the overall survival rate for supratentorial PNETs is substantially lower than that for medulloblastomas, with an expected 3-year progression-free survival of approximately 50% for localized supratentorial PNETs.^{75,135} These observations sug-

gest intrinsic biological differences between supratentorial PNET and medulloblastoma.

Histopathological Diagnosis of Supratentorial PNET

Histopathological classification of supratentorial PNET remains a contentious issue. Based on the indistinguishable histological features of supratentorial PNET and medulloblastoma, a common histopathological grouping of all PNETs with a presumed common histogenesis was proposed¹⁴⁰ and remains disputed.¹⁰⁹ Current WHO criteria identify supratentorial PNET as a Grade IV tumor with undifferentiated or poorly differentiated neuroepithelial cells, and with the capacity for divergent differentiation along multiple lineages, including neuronal, astrocytic, ependymal, muscular, or melanocytic lines.¹⁴² Because specific marker(s) for supratentorial PNET do not exist, these lesions are still diagnosed primarily on the basis of a supratentorial location and histological features of a predominantly undifferentiated neuroepithelial tumor with focal areas of divergent differentiation. The inclusion of supratentorial neuronal tumors with more distinct neuronal differentiation, such as the cerebral neuroblastoma, or the ganglioblastoma with its distinct ganglionic features, remains controversial.^{12,14} When supratentorial PNETs demonstrate very little morphological or immunohistochemical signs of differentiation, they pose diagnostic challenges with respect to other high-grade supratentorial tumors such as the “round” or “small cell” glioblastoma variants, which may also exhibit a similarly undifferentiated phenotype.⁹⁴ For polyphenotypic supratentorial PNETs presenting in a young child with expression of epithelial or muscular antigens such as epithelial membrane antigen and smooth-muscle actin, the differentiation from the highly aggressive CNS rhabdoid tumors¹⁴¹ may pose significant challenges, because *IN11* mutation has been reported in supratentorial tumors without typical histological “rhabdoid” features.¹⁵⁰

In a recent survey, pathologists identified histopathological diagnosis of supratentorial PNET as an area of significant challenge.⁷ The continuing uncertainties about the precise histological criteria required for diagnosis and the relative rarity of these tumors means that published genetic and molecular genetic data, particularly in small series, may be difficult to interpret, and may have included high-grade neuroglial and neuroepithelial tumors whose histogenetic relationships to supratentorial PNETs were uncertain. Nonetheless, molecular cytogenetic data compiled so far suggest that supratentorial PNETs collectively exhibit changes that differ from those of the infratentorial medulloblastoma,^{115,146} and support the development of supratentorial PNETs from biological pathways distinct from those involved in medulloblastoma development.

Cytogenetic and Molecular Genetic Alterations in Human Supratentorial PNETs

In contrast to medulloblastomas, very few cytogenetic and molecular genetic studies of human supratentorial PNETs are available. To date, karyotypic studies of only 22 pediatric supratentorial PNETs arising in the cerebrum have been reported as part of larger collective series on medulloblastomas and PNETs.^{1,11,15,21,30,32,61,139,162} (Table 1). Supratentorial PNETs frequently show complex karyotypes, with evidence of double minutes structures associated with high-

TABLE 1
Literature review and summary of published karyotypes for supratentorial PNETs*

Authors & Year	Tumor No.	Age (yrs), Sex	Location	Karyotype
Chadduck, et al., 1991	16	<1		normal
	17	<1		normal
	18	<1		normal
	19	<1		45,XY,-22
Fujii, et al., 1994	7	9, F	normal	normal
	8	14, M		43-44;XY;+2, -6; der(10)t(10;11)(q26;q21), del(11)(q210,-12,-13,=mar, (8)/84-90, XXYY, der(10)t(10;11)(q26:21), der(10)t(10;11)(q26;q21), del(11)(q21), del(11)(q21), +2mar(8)
Agamanolis & Malone, 1995	1	9, F	frontal	normal
Bhattacharjee, et al., 1997	6			parietal 46,XY,i(1)(q10);-9,t(9;11)(q34;q13)<8?/90; idemx2, -X,Y ¹ /46;XY
Bigner, et al., 1997	2	2, M		46,XY,t(6;9)(q21;q13),del(10)(q22) ² /45. idem, t(11;13)(q15;q11)-13 ³
	3			normal
	4			normal
	5			normal
Burnett, et al., 1997	21	7	parietal	49,XX, add(3)(q23or q24), +5, +8, dup(11)(q12q22.3 or q13q23), del(16)(q22q24), add(19)p13, +21[8]/49, idem, i(1)(q10)[1]/49.idem, add(13)(q340[1]
	22†	16	parietal	90,XX, add (X)(p22)x2, dic(1;9)(q42;p21)x2, dic(4;9)(q3?5;p2?2), -6, add(6)(p24, -9, add(11)(p15)x2, -15x2, add(16)(q2?2), +mar1, +mar2[11]
Bayani, et al., 2000	10	3		70-103 chromosome, double ring, and dmins
	11	2		46,XY, t(6;13)(q25;q14)
	12	3.5		46,X, rea(X), ? Rea(10p), ?(14q), add (19q), ?ass (22q), 22q 55-57,XX, -X, del(1)(p22), i(4)(p10), -5, +6, +add(7)(q36), add(9)(p21), -11, -13, -17, add(18)(q23), -19, -19, +13mars, +dmins
Roberts, et al., 2001	13		cerebral	46,XX,del(2)(p22.2-2p23.1) del(5)(q33.35)/46, idem, del(17)(q21.3q21.3),
	14		parietooccipital	der(9;15)(q10;q10)x2, +11, +13, +18, +20, +2056~59, Xc,+X, +1,+1, +1, add(1)(p?), add(1)(q?), +2, del(2)(p24), +7, +8, add(8)(q?),
	15		parietal	69~75XX, -X, add (10(q42)x2,-4,-4,add(4)(q3?), -10, -11, del(11)(q2?), -13,-16,-18,+7~13mar, dmin(cp7)dmins
Uematsu, et al., 2002	20	7	insular cortex	52,XX,+1X2, add(3)(q25)+7X2, add(11)(q25)X2, +21X2

* Dmin = double minute.

† Secondary malignancy after acute lymphocytic leukemia therapy.

level gene amplification. Interestingly, eight of 19 reported cases had normal karyotypes. In one series of four infants with supratentorial PNET, three cases had normal karyotypes, whereas one case had monosomy²² as the sole cytogenetic abnormality,³² indicating that subtle genetic changes may be characteristic of a substantial proportion of supratentorial PNETs. Complex karyotypes in 10 of 19 cases involved mostly structural changes with interstitial deletions, partial chromosome gains, and translocations involving a number of different chromosomes. Recurrent regions of cytogenetic alterations included deletions or translocations involving chromosome 10q22-26 in three of 10 cases, and translocations involving chromosome 6q21-25 in two of 10.

Notably, one patient (Case 11 in Table 1) had a translocation involving chromosome 6q25 and chromosome 13q14 as the sole cytogenetic abnormality (this was a child with no history of retinoblastoma).¹¹ The finding of this sole abnormality, also confirmed by the authors using high-resolution spectral karyotyping analyses, implies involvement of the RB1 locus on chromosome 13q14, and a putative novel supratentorial PNET oncogene or tumor suppressor gene at chromosome 6q21-25. No data were available on the constitutional DNA of this patient. Double minutes, indicative of high-level gene amplification, were seen in three

of 18 cases, a frequency comparable to that reported for medulloblastoma.^{16,21}

Comparative Genomic Hybridization Studies

The CGH profiles of 16 supratentorial PNETs (five of which were found in adults but not separately identified)^{8,11,30,115,146} are summarized in Fig. 1. Not surprisingly, these higher-resolution analyses indicate a greater frequency of genomic imbalances than appreciated on karyotypic studies, with only one of 16 supratentorial PNETs being reported as normal based on results of CGH. Existing data indicate that supratentorial PNETs differ from medulloblastomas in the pattern and frequency of DNA copy number changes. Most significantly, chromosome 17p loss, which characterizes many medulloblastomas,^{19,30,131} is rare in supratentorial PNETs. In up to 40% of medulloblastomas, a characteristic isochromosome 17, which results from concurrent gain of chromosome 17q and loss of 17p, is observed.²⁰ In contrast, although gain of 17q or the entire chromosome 17 is seen in supratentorial PNET, isochromosome 17 has only been reported in one case of supratentorial PNET.^{146,175} So far, allelotyping studies have disclosed loss of chromosome 17p in only one of 36 primary supratentorial PNETs^{11,23,30,99,131} and one supratentorial PNET cell line.⁶² In addition, epigenetic changes at a breakpoint cluster

Fig. 1. Ideograms showing DNA copy number profiles of supratentorial PNETs. (The DNA copy number profiles of 16 supratentorial PNETs as determined based on CGH studies are shown.) These include profiles of three pineal tumors that are not separately identified in the original report by Russo, et al. Green and red vertical lines represent copy number gains and losses, respectively, in tumor samples. Regions of high-level copy number gains or amplification reported in supratentorial PNETs are indicated by black boxes and include chromosomes 2p24 (N-myc); 9p (Bayani, et al.); 4q12-q13 (Russo, et al.); 7p11.2-p12 and 7q21.3-q22 (Nicholson, et al.); 12q21, 20q, 1q, and 6p (Prebble, et al.); and the hTERT locus on chromosome 5p15 (Fan, et al.).

region on chromosome 17p11.2, which is presumed to inactivate a putative tumor suppressor in approximately 30% of medulloblastomas, is not seen in supratentorial PNETs.⁶⁰

Higher frequency and greater complexity of DNA copy number changes are reported in supratentorial PNETs compared with medulloblastomas. In one of the largest studies of 17 pediatric supratentorial PNETs, in which high-resolution CGH was used,¹³⁰ genomic imbalance was reported in 70% of tumors, with a median of four changes per tumor. Most changes (63%) involved whole chromosomes, with partial chromosome imbalance seen in 37% of tumors.

Collectively, supratentorial PNETs exhibit a greater frequency of chromosome loss than gain. Distal chromosome 4q loss is the most frequent change, and is observed in up to 50% of the supratentorial PNETs studied.

Other regions of loss observed at significant frequency include chromosome 9 centering around distal 9p, and chromosomes 13q, and 14q. Less frequently, losses involving all or part of chromosomes 5, 6, 10q, 12, 18q, and 19q are reported. Interestingly, an isolated 3p21 interstitial deletion has been reported in one tumor;¹¹⁵ it remains to be determined whether the *hMLH1* DNA repair gene mapping to this site is involved. Losses of chromosome 10q observed in approximately 25% of supratentorial PNETs appear to involve the known tumor suppressors *BTRC*,¹⁷³ *PTEN*, or *DMBT1* only rarely, with only one mutation reported in 21^{81,98} and nine supratentorial PNETs⁸¹ for *PTEN* and *DMBT1*, respectively. The *DLC-1*, a putative tumor suppressor on chromosome 8p22, is epigenetically inactivated in one of three supratentorial PNETs.¹²¹ Clearly, fur-

ther studies are needed to determine the nature of putative supratentorial PNET tumor suppressor loci associated with DNA copy number losses and their mechanisms of gene inactivation in supratentorial PNETs.

Similar to observations in medulloblastomas,^{8,17} gains at chromosome 7 (six of 16), followed by 1q, occur most frequently in supratentorial PNETs. Gains of chromosomes 9qter, 13, and 17 have been reported in more than one case. Consistent with detection of double minutes in karyotypic studies, CGH analyses of supratentorial PNETs have identified high-level DNA amplification at chromosomes 2p24, 4q12-13, 7q21.3, and 7p11.2. Additional amplicons at chromosomes 1q;6p, 12q21, 6p, and 20q have been reported.¹³⁰ With the exception of the chromosome 2p24 amplicon, which has been shown to be related to N-myc gene amplification,¹¹ the majority of putative oncogenic loci indicated by cytogenetic findings in supratentorial PNETs remain unknown. Studies with higher-resolution single gene mapping tools (such as the recently developed array-based CGH technology)⁴⁵ and with larger tumor numbers will be very valuable for defining the true spectrum and frequency of genomic imbalances, and to locate oncogenes and tumor suppressor genes that underlie supratentorial PNET development.

Developmental Signaling Pathways in Supratentorial PNETs

Based on the relatively primitive histological features of these tumors, it has long been postulated that PNETs of the CNS (supratentorial PNET and medulloblastoma), arise

from transformation of periventricular germinal matrix cells.¹⁵⁹ Nevertheless, studies of murine models and primary human tumors have now demonstrated that some medulloblastomas can arise from germinal cells in the external granular layer of the cerebellum.⁶⁵ Recently, a microarray-based study in which the expression profiles of eight supratentorial PNETs were compared with those of normal human cerebellum and medulloblastoma, showed that supratentorial PNETs have transcriptional signatures distinguishable from those of normal fetal cerebellum and medulloblastoma. In particular, medulloblastoma and supratentorial PNET differentially express cerebellar granule cell-specific transcription factors like *ZIC1* and *NSCL*,¹²⁷ supporting a cerebellar granule cell origin for medulloblastomas but not supratentorial PNETs. Although the cell of origin in supratentorial PNETs is not known, their putative derivation from cells of periventricular germinal matrix is suggested by the frequent clinical presentations of these tumors from deep periventricular locations. Histopathological studies of human pineal and midbrain tumors¹⁰⁶ as well as studies of transgenic mice with midbrain PNET,¹⁰⁷ also indicate that subependymal cells of the diencephalon are likely to be targets of transformation in supratentorial PNETs.

There is now overwhelming evidence that developmental signaling pathways that control normal brain development are dysregulated by tumor-specific mutations in medulloblastomas.¹⁵⁶ Current evidence suggests that the Shh–Gli, Notch, and to a lesser extent Wnt (or Wingless) signaling pathways may also be involved in supratentorial PNET development.

The Shh–Gli Pathway. Extensive studies have now established a clear role for Shh signaling in normal and malignant cerebellar development. In addition, studies of human tumors and mouse models have shown that abnormal Shh signaling contributes to development of a proportion of medulloblastomas. Specifically, mutations in the *PTCH*, *SMO*, and *SUFU* genes, leading to pathological Shh pathway activation have been demonstrated in a proportion of syndrome-associated and sporadic medulloblastomas. The Shh–Gli pathway is reviewed in detail in the article by Taylor, et al.,¹⁵⁶ and thus only salient features are outlined here.

The role of Shh signaling in development of the cerebral cortex and the corresponding malignant tumors, such as supratentorial PNET and gliomas, has not been extensively investigated. The *Ptch* mutant mice, even in a p53-deficient background, have not been reported to develop supratentorial PNETs.¹⁶⁹ Nevertheless, recent studies indicate that Shh may also serve important mitogenic functions in the developing mouse cortex. Specifically, Dahmane, et al.,⁴³ demonstrated expression of all three Gli proteins (Gli1–Gli3), which are downstream effectors of Shh signaling, in the ventricular and subventricular zone of the perinatal murine cerebral cortex and midbrain. They observed that Shh had mitogenic effects on nestin-positive neocortical precursors and cells of the ventricular zone, and could induce Gli1 in the latter cell population. These findings thus indicate that in addition to its role in cerebellar development, the Shh–Gli pathway may also control the development of dorsal brain/cerebral cortical growth, and contribute to the development of corresponding tumors, such as supratentorial PNET.

Due to the rarity of supratentorial PNETs, the status of

the Shh–Gli signaling pathway in supratentorial PNETs remains incompletely investigated, and has generally only been reported collectively with medulloblastoma. Limited studies of supratentorial PNET tumors to date indicate however, that the Shh–Gli pathway is active, and may be altered, in a proportion of supratentorial PNETs. Specifically, expression of Gli1, Gli3, and *Ptch1* transcript has been reported in one supratentorial PNET.⁴³ Increased expression of *PTCH*, *SMO*, and *GLI1* mRNA has been observed in three to five of five supratentorial PNETs studied.¹³⁶ In addition, all supratentorial PNET tumors in a small series of three were reported to express N-myc, a known downstream target of Shh signaling, according to immunohistochemistry studies.¹¹² Missense mutation¹⁷² as well as loss of heterozygosity¹⁶⁴ involving the *ptch* locus at chromosome 9q22.3 has also been reported in three of eight pediatric supratentorial PNETs studied. The functional implications of these molecular findings need to be further validated in appropriate tumor models. Nonetheless, these limited studies indicate that Shh–Gli1 pathway aberrations are likely to have a pathogenetic role in at least a proportion of supratentorial PNETs. The scope of Shh–Gli1 pathway involvement in supratentorial PNET, and whether they correlate with any specific histopathological features in these tumors must await studies conducted in larger numbers of tumors.

Notch–Hes Signaling. Experimental evidence from murine studies indicates that the Notch signaling pathway plays an important role in regulating normal neurogenesis by restricting neuronal differentiation and promoting maintenance of progenitor cells.⁸³ Notch family genes encode large transmembrane proteins (Notch 1–4) with extracellular endothelial growth factor–like repeats that act as receptors for membrane-bound ligands (Jagged, delta) expressed by neighboring cells (Fig. 2). Current evidence supports the proposition that Notch signaling may act negatively or positively to regulate cellular proliferation or differentiation depending on the cell context. Notch signaling is activated by ligand binding and involves complex cleavage and release of an intracellular Notch fragment (the Notch intracellular domain), which translocates to the nucleus. The Notch intracellular domain heterodimerizes with the DNA binding transcription factor CBF1 to activate target genes of the Hes (1–5) family. The best-characterized Notch effector, Hes 1, negatively regulates basic helix-loop-helix neurogenic transcription activators such as hASH1.^{38,153} The Hes 1 is highly expressed in the ventricular zone of the developing nervous system, and misexpression of Hes 1 has been shown to prevent neural differentiation.^{83,117}

Aberrant Notch signaling has been linked to a spectrum of human malignancies including T-cell leukemia, in which a t(7:9) translocation results in a constitutively active and oncogenic Notch 1 molecule (see review by Allenspach, et al.⁵). Investigations of Notch signaling in PNETs and medulloblastomas of the CNS are at an early stage, with only a few studies reported to date. These studies, however, demonstrate evidence of Notch pathway activation in PNETs of the CNS, and differential expression of Notch pathway components in medulloblastoma and supratentorial PNETs, perhaps reflecting distinct cells of origin for these tumors. In one study of 13 medulloblastomas and five supratentorial PNETs, hASH1 expression was seen in three of five

Fig. 2. Simplified schema of Notch signaling in mammalian cells. Signaling is activated by binding of Notch family receptors (Notch 1–4) to membrane-bound ligands such as Jagged and Delta, on neighboring cells. Ligand binding triggers proteolytic cleavage and release of the signal-transducing Notch intracellular domain. This domain translocates into the nucleus and associates with DNA-binding nuclear proteins of the RBP-Jk family to activate transcription of target genes such as *HES1*. Then, *HES1*, a basic helix-loop-helix protein, acts to repress downstream target genes, such as *hASH1*.

supratentorial PNETs and none of 12 medulloblastomas, whereas expression of *NeuroD* genes appeared to be more prevalent in medulloblastomas.¹⁴³

A more recent study⁵⁴ has shown differential expression of Notch1 and Notch 2 in supratentorial PNETs compared with medulloblastomas, with a greater prevalence of high Notch 2 expression in supratentorial PNETs. Indeed, more than 50% of supratentorial PNETs, including a supratentorial PNET cell line, expressed extremely high levels of Notch 2 mRNA, whereas both primary medulloblastomas and two cell lines expressed Notch 2 and Notch 1 at levels comparable to control cerebellar cells. Significantly, three of six tumors with high Notch 2 mRNA had genomic amplification or copy number gains of the Notch 2 locus on chromosome 1p11-13, and Notch 2 knockdown in PFSK, a supratentorial PNET cell line,⁶² resulted in inhibition of cell growth, thus supporting a functional role for Notch 2 in supratentorial PNET. Interestingly, a second study has reported more frequent Notch 1 than Notch 2 overexpression in medulloblastoma, and demonstrates *HES1* and/or *HES5* as Notch effectors in medulloblastoma. Whether these two tumor types can indeed be differentiated by their Notch activation profile remains to be confirmed in studies with a larger number of cases. Further studies will also be necessary to determine the prevalence of Notch 2 dysregulation by gene amplification, or alternate mechanisms in supratentorial PNETs. Intriguingly, recent studies of transgenic

medulloblastoma mice with targeted overexpression of the Shh pathway gene (*Smo*) to the cerebellar external granule layer through a *NeuroD2* promoter⁷⁰ suggest crosstalk between Notch and Shh signaling in medulloblastoma. Whether the same will hold true in supratentorial PNET remains to be seen.

The Wnt Pathway. The Wnt (or Wingless) pathway is a highly conserved signaling pathway with a key role in control of cellular proliferation, differentiation, migration, and adhesion. A role for Wnt signaling in brain tumor development was first recognized through studies of Turcot syndrome, a heritable association of CNS neuroepithelial tumors and adenomatous polyposis of the large intestine caused by germ line mutations of the *APC* gene.⁷¹

The APC protein normally exists as part of a large degradative protein complex that includes Axin, GSK-3 β , and β -catenin. This protein tightly regulates the subcellular location and function of β -catenin, a key transcriptional effector of Wnt signaling.⁹⁰ In the context of appropriate growth or differentiation signals, Wnt signaling is activated by ligand binding to Frizzled transmembrane receptors, which triggers degradation of GSK3 β and consequently inactivates the APC complex. In the absence of degradative signals, accumulated cytosolic β -catenin translocates to the nucleus, and acts with TCF transcription factors to activate target genes, which include the *c-myc* oncogene.⁷³ The β -catenin also acts to regulate cell adhesion by associating

with membrane-bound cadherin proteins. Tumor-specific mutations of Wnt pathway components have been described in medulloblastomas, in which *c-myc* and *N-myc* activation are reported in a substantial proportion of tumors.^{4,52}

The Wnt pathway aberrations have not been extensively investigated in supratentorial PNETs. Single β -catenin mutation was reported in a small study of four supratentorial PNETs.⁹⁵ This mutation, which resulted in a somatic Gly to Val change within a protein degradation targeting signal in exon 3, correlated with β -catenin nuclear accumulation and Wnt activation. Increased expression of *c-myc* and *N-Myc*, a downstream β -catenin target, has been reported in supratentorial PNET cell lines,⁹⁰ suggesting potential etiological roles for the Wnt pathway in supratentorial PNET development. Nevertheless, further studies are needed to assess the prevalence of Wnt pathway aberrations in supratentorial PNET.

Tumor Suppressor Loci in Supratentorial PNETs

Genomic mapping studies indicate a putative supratentorial PNET suppressor loci on chromosomes 4q, 9p, 13, and 14q, in which DNA copy number losses are most often observed.^{115,130,146} The identities of these putative loci remain to be determined. Known tumor suppressor pathways with evidence of involvement in supratentorial PNET development are discussed later.

The *p53* Locus. The *p53* tumor suppressor locus on chromosome 17p13.1 is one of the most frequently mutated genes in human cancers. Nullizygous *p53* mice are viable but highly susceptible to tumor development.⁴⁹ In humans, germ-line *p53* mutations result in the Li-Fraumeni syndrome, a rare heritable cancer syndrome associated with diverse malignancies, including brain tumors.¹⁰⁵ A key cellular function of *p53* (extensively reviewed in Bourdon, et al.²⁶) is to inhibit malignant cell transformation resulting from events such as DNA damage or ectopic oncogene expression, by induction of genes involved in cell cycle arrest and apoptosis. Most *p53* mutations in human cancers result in structural alterations with loss of wild-type *p53* growth inhibitory functions and may also generate mutant *p53* proteins with dominant negative properties or gain of new biochemical and biological functions that promote malignant cell growth.²⁴ Due to its central role in controlling cell growth, *p53* function is normally tightly regulated by a variety of cellular mechanisms that may also be targeted in tumor cells.¹⁶³ These include amplification of the *MDM2* oncogene on chromosome 12q14, a ubiquitin ligase that facilitates proteosomal degradation of *p53*. Loss of *p15ARF*, a critical upstream activator of *p53*,¹⁵¹ can also represent a common mechanism of functional *p53* loss in tumors (described in the following section).

Supratentorial PNET as well as medulloblastoma have been described in familial Li-Fraumeni syndrome,¹⁶⁶ but because PNETs of the CNS were described collectively,^{48,56,119} the specific incidence of either tumor type in Li-Fraumeni syndrome is not known. Germ line *p53* mutations described in supratentorial PNETs involve codon 213, and an unusual CAT to AAT transversion in codon 179,¹²⁹ suggesting the possibility of other atypical *p53* mutations in supratentorial PNET.

Although *p53* mutations are estimated to occur in ap-

proximately 27% of all brain tumors,¹¹⁸ these mutations occur rarely (< 1%) in sporadic malignant pediatric brain tumors.¹²⁸ The relative lack of *p53* mutations in pediatric brain tumors have been corroborated by individual studies of pediatric supratentorial PNETs. Mutational studies of a sum of 28 pediatric supratentorial PNETs in four separate studies^{30,74,98,99,133} have identified only one somatic *p53* mutation.⁹⁸ Interestingly, in a study of 14 adult and pediatric cerebral PNETs, *p53* mutations were observed in six of 11 cases in adults and none of three pediatric supratentorial PNETs.⁷⁴ These observations lead us to suggest that, similarly to pediatric high-grade gliomas,¹³⁸ childhood supratentorial PNETs may differ genetically from the corresponding tumors in adults.

Despite the rarity of *p53* mutations in sporadic supratentorial PNET, a role for this gene in supratentorial PNET pathogenesis is suggested by development of supratentorial PNET in several *p53*—/— murine brain tumor models,^{158,177} and in SV40 T antigen-driven transgenic PNET models.^{2,107} In addition, high *p53* protein expression, indicative of *p53* dysfunction, is seen in a significant proportion of supratentorial PNETs.^{31,51,87,174} The true frequency of *p53* immunopositivity in supratentorial PNETs is not known because studies have often considered medulloblastoma and supratentorial PNET collectively. Nevertheless, studies of 12 cases of supratentorial PNET in two small series (one included three medulloepitheliomas) suggested that up to 90% of supratentorial PNETs display *p53* dysfunction, as indicated by strong *p53* positivity.^{51,87} In contrast, strong *p53* immunopositivity is reported in 10 to 30% of medulloblastomas.^{51,134} Correlation of *p53* mutations was not conducted in these studies; thus the mechanisms for *p53* immunopositivity in these tumors are not known. Studies conducted in a small number of tumors indicate that *mdm2* amplification may not be a common mechanism for *p53* loss in supratentorial PNETs. Mutually exclusive mutations in *p53*, methylation of *p14ARF*, or deletion of *INK4/ARF*, have recently been demonstrated in up to 25% of medulloblastomas.⁵⁸ Thus, a greater role for *p53* and *p53* regulatory pathways in supratentorial PNET development may yet be demonstrated.

Recently, several novel members of the *p53* protein family (*p63* and *p73*) that have roles in oncogenesis have been identified.⁸² In addition, *p53* isoforms resulting from alternative splicing have also been reported.²⁵ The significance of these novel *p53* family members/isoforms in supratentorial PNET development remains to be investigated.

The *INK4/ARF* Pathway. The *cdk/INK4/pRB* pathway is a key mediator of mitogenic and antimitogenic signals necessary for normal cellular proliferation. Not surprisingly, this pathway is deregulated in more than 80% of human tumors by genetic and epigenetic alterations of different components, as reviewed in various studies (Fig. 3).^{120,144,151}

The *INK4a/ARF* locus on chromosome 9p21 encodes two distinct tumor suppressor proteins (*p16INK4a* and *p15ARF*), which normally act in parallel to regulate the function of the *pRB* and *p53* tumor suppressors, respectively. In normal cells, wild-type *p16INK4a* maintains the *pRB* tumor suppressing function by inhibition of *cdk4* and *cdk6* activity, resulting in *pRB* hypophosphorylation, E2F repression, and induction of *G₁* arrest. In tumor cells, deletions or mutations of *p16INK4a* renders it ineffective for regulation of *cdk4/6* activity, resulting in *pRB* hyperphos-

phorylation, loss of E2F repression, and activation of genes permissive for G₁-S phase transitions and unregulated cellular proliferation. Under normal cellular growth conditions, p15ARF acts to stabilize p53 by binding to MDM2. Loss of P15ARF function through genetic and epigenetic mechanisms in tumor cells results in functional loss of p53, as reviewed in Roussel¹⁴⁴ and Sherr.¹⁵¹

The INK4 proteins (a-d) are highly conserved and have partly redundant functions that remain incompletely elucidated. Like INK4a and b, the INK4c and d proteins regulate pRB function by specifically inhibiting cdk4 and cdk6 activity.⁶⁸ Unlike P15INK4b and P16INK4a, however, the P18INK4c and/or P19INK4d show temporal and spatial expression in the developing and mature brain, leading to the suggestion that these proteins may have context-specific tumor suppressor functions. Loss of Ink4c/d or p53⁴⁹ alone do not predispose mice to brain tumors. Nevertheless, Zindy, et al.,¹⁷⁷ described a spectrum of tumors, including brain tumors in p53 null mice with homozygous loss of Ink4c or Ink4d. Mice with homozygous or heterozygous loss of Ink4c developed cerebellar tumors resembling medulloblastoma. Interestingly, mice with only Ink4d loss developed cerebral tumors resembling human supratentorial PNET. In addition to cerebellar tumors, mice heterozygous for Ink4d and Ink4c developed supratentorial PNET-like tumors originating from periventricular zones and thalamic locations. It is noteworthy that Ink4d is normally expressed in the progenitor cells of the rat telencephalon and neonatal subventricular zone,⁴² and that it has been implicated in inhibition of postnatal neuronal differentiation.¹⁷⁶ These observations indicate cooperative tumorigenic effects of p53 and the INK4c and INK4d proteins, and perhaps a more specific requirement for INK4d inactivation in supratentorial PNET development.

As described in the preceding section, there is evidence of p53 dysfunction in a substantial proportion of supraten-

torial PNETs studied to date. The status of the INK4/ARF and cdk4/6 loci has not been specifically investigated in supratentorial PNET. It is significant that although only rare p16 deletions in medulloblastoma had been identified in previous studies,^{10,124,144,147} more recent reports demonstrate that up to 25% of the large cell aggressive variant of medulloblastoma show evidence of genetic or epigenetic lesions in the INK4/ARF/p53 pathway.⁵⁸ It is interesting that in the study by Zindy, et al.,¹⁷⁷ mice heterozygous for both Ink4c and Ink4d developed both medulloblastoma and midline supratentorial PNET-like tumors, thus raising the possibility that a spectrum of medulloblastoma, such as the anaplastic variant, may share etiological pathways with supratentorial PNETs.

The PMS2 Pathway. Both medulloblastoma and supratentorial PNET have been linked to Turcot syndrome, which is characterized by concurrence of primary brain tumor and multiple colorectal adenomas. Two types of syndromes are recognized: Turcot Type 2 occurs in the setting of familial adenomatous polyposis and involves germ line mutations of the APC gene on chromosome 5q21.⁷¹ The predominant brain tumor in Turcot Type 2 is medulloblastoma, in which syndrome-associated mutations in APC or beta-catenin mutations have been demonstrated.^{78,156,178}

Turcot Type 1 comprises a second syndrome with clinical findings of nonfamilial adenomatous polyposis-associated colorectal cancer (hereditary nonpolyposis colorectal cancer), skin lesions (including café-au-lait spots-), and glioblastoma. It is a genetically heterogeneous disease resulting from germ-line defects in several DNA mismatch repair genes, including *hMLH1* (3p21 locus), *hMSH2* (2p16), *hMSH3* (5q11-q13), *hMSH6* (2p16), *hPMS1* (2q31), and *hPMS2* (7p22). Defects in these loci lead to genetic instability in which short repeated sequences (microsatellites) are characteristically altered due to accumulation of unrepaired somatic mutations.⁷¹ The PMS2 and hMLH1 gene products form heterodimers and provide one of the major DNA mismatch repair activities in mammalian cells. Although MLH1 mutations are commonly seen in hereditary nonpolyposis colorectal cancer, germ-line mutations of PMS2 have been described only recently.^{46,114,148} DeVos, et al., reported on two siblings with supratentorial PNETs in a heavily consanguineous family with cosegregation of learning difficulties, cutaneous café-au-lait spots, and early-onset brain tumors inherited in a recessive fashion. A homozygous missense mutation in exon 14 of *PMS2* leading to protein truncation was demonstrated in all affected individuals.

In addition to the detection of germ line PMS2 mutations in supratentorial PNETs, several other observations indicate that signaling pathways involved in DNA damage repair are important in supratentorial PNET development. These include, as described earlier, high-level expression of p53 protein in a large proportion of supratentorial PNETs and development of supratentorial PNET radiation-induced secondary malignancies as well as rare supratentorial PNETs in the setting of multiple sequential malignancies.¹³⁵ In addition, we have observed unusual karyotypes indicative of an underlying DNA breakage syndrome in a case of sporadic supratentorial PNET (Fig. 4; L Lafay-Cousin and A Huang, unpublished data). Furthermore, supratentorial PNETs have been reported in two different medulloblastoma mouse models with disruptions of genes that have im-

Fig. 3. Chart showing regulation of pRB and p53 by the INK4a/ARF tumor suppressor pathway. A simplified schema of the INK4a/pRB/p53 pathway is shown. The INK4a locus encodes p16INK4a and ARF through the use of alternate reading frames. The p16INK4a and related INK4 proteins (INK4b-d) inhibit cdk4/6 activity with consequent hypophosphorylation of pRB. Hypophosphorylated pRB acts with E2F proteins to repress transcription of genes necessary for the G₁-S phase transition. The P14^{ARF} regulates p53 activity by inhibiting MDM2-mediated degradation of p53, allowing p53 to induce cell cycle arrest.

portant functions in DNA repair, such as *p53*¹⁷⁷ or *PARP-1* (a DNA damage response gene).¹⁵⁸

The status of the other DNA mismatch repair genes and of *PARP-1* in sporadic supratentorial PNET remains to be investigated. It is interesting to note that overlapping losses centering around chromosome 3p21 have been reported in CGH studies of supratentorial PNETs,¹¹⁵ thus suggesting potential for hMLH1 loss in a proportion of supratentorial PNETs.

The prevalence of PMS2 mutations in hereditary and sporadic supratentorial PNET is also not known, and may prove challenging to determine because multiple PMS2 pseudogenes are located on chromosome 7. Recent studies^{46,114} demonstrate that these pseudogenes on chromosomes 7q13-q12, 7q11, and 7q22 can obscure detection and lead to underdiagnosis of PMS2 mutations. Use of a recently developed PMS2 antibody may help identify patients with putative PMS2 mutations for comprehensive mapping studies in supratentorial PNET.

The *hSNF5/INI1* Pathway. Central nervous system atypical rhabdoid teratoid tumors are aggressive lesions that have overlapping clinical, histological, and radiographic features with medulloblastoma or PNET.¹⁴¹ In particular, the clinical diagnosis and differentiation of supratentorial PNETs from atypical rhabdoid teratoid tumors in a young child have posed significant challenges in the past because both tumors can show predominantly undifferentiated histological features with focal areas of divergent differentiation.⁹⁹ A tumor suppressor (*hSNF5/INI1*) mapping to chromosome 22 has now been identified for atypical rhabdoid teratoid tumors. The *hSNF5/INI1* gene product is a component of the SW1/SNF chromatin remodeling complex.⁸⁰ Nonrandom association of monosomy on chromosome 22 that typically characterizes atypical rhabdoid teratoid tumors has also been reported for histologically confirmed

medulloblastoma and, less frequently, in supratentorial PNET. Studies of *INI1* in medulloblastoma and supratentorial PNET have revealed only very few *INI1* mutations,^{18,99} indicating that a distinct chromosome 22 tumor suppressor gene is involved in medulloblastoma and/or supratentorial PNET development. Nonsense mutations resulting in a truncated *INI1* gene product were reported in two of 17 supratentorial PNETs in an investigation of *hSNF5/INI1* alterations in 229 tumors of various origins.^{149,150} Germ-line *INI1* mutations have been reported in rare families with choroid plexus carcinomas, central PNETs, or medulloblastomas presenting in very young children. In addition, de novo constitutional *INI1* mutation associated with a supratentorial PNET and a renal rhabdoid tumor has been described.^{149,150}

Molecular Correlates of Disease Phenotypes in Supratentorial PNET

Prognostic roles for *erbB2*, *myc*, *p53*, and *Trk* receptor expression have been demonstrated in medulloblastoma. The status of *erbB2* in supratentorial PNET is not known. Apart from *p53*, the other markers have only been examined in a handful of supratentorial PNETs^{67,116} collectively with medulloblastoma. Because only very few tumors have been studied with individual markers, their correlation, if any, with clinical phenotypes in supratentorial PNETs cannot be determined based on the current data. It is interesting to note, however, that in studies in which supratentorial PNETs and medulloblastomas have been collectively analyzed for individual markers such as *p53* immunopositivity,⁵¹ Notch 2,⁵⁴ or *hTERT*⁵⁵ gene activation, the supratentorial PNETs tend to segregate with more aggressive medulloblastoma variants. It will clearly be of interest to determine whether aggressive variants of medulloblastoma share additional overlapping molecular features with supratentorial PNETs.

PINEOBLASTOMAS

Pineoblastomas make up 45 to 50% of pineal parenchymal tumors, and are classified as WHO Grade IV tumors with typical variable reactivity to neuronal and glial markers.¹¹⁰ Like cerebral supratentorial PNET, pineoblastoma is mainly a childhood disease, with cases only occasionally described in adults.^{91,111} Due to their poorly differentiated histopathological features, they have generally been studied together with other undifferentiated embryonal intracranial PNETs. They have been collectively analyzed with cerebral PNETs in most therapeutic and molecular studies, comprising 20 to 50% of reported supratentorial PNETs in such series.^{40,47,108,123,135,157} Nevertheless, there is some suggestion that in very young children (< 18 months), pineal tumors may occur at a higher frequency than nonpineal lesions.⁶³ Indeed, tabulation of published cytogenetic data indicates that 40 to 50% of pineoblastomas occur in children less than 1 year old (Table 2).

It is noteworthy that pineoblastomas may exhibit histopathological features indicative of photosensory differentiation ranging from focal expression of retinal S-antigen to characteristic Flexner–Wintersteiner rosettes and fleurettes reminiscent of retinoblastic differentiation (Fig. 5).^{9,110} Embryologically, the pineal gland develops from primitive

Fig. 4. Unusual karyotype of a sporadic primary parietal supratentorial PNET in a child without the syndrome is shown. Radial figures that are normally characteristic and indicative of DNA breakage syndromes such as Bloom syndrome are indicated by arrows. (Image courtesy of Dr. I. Teshima, Hospital for Sick Children, Toronto, Ontario, Canada.)

neuroepithelial cells, which line the diencephalic roof¹⁴⁵ and have features of photoreceptor organs, with photosensory and neuroendocrine differentiation. These cells are believed to be ontogenetically linked to the photoreceptor cells of the retina. The histological features of pineal and suprasellar PNETs arising in the context of trilateral retinoblastoma (described later), can bear exceptionally close resemblance to retinoblastoma, and this tumor type has been labeled by some as “primary ectopic intracranial retinoblastoma.”^{9,92} Thus, at least a proportion of pineoblastomas may share ontogenic aspects with precursor cells targeted for transformation during retinoblastoma development.

Heritable Pineoblastoma

Tumors presumed to be heritable nonsyndromic pineoblastomas have unknown molecular sources and have been described only rarely. These include cases of pineoblastoma in a mother and a daughter,¹⁰¹ sequential development of pineoblastoma and pilocytic astrocytoma in one individual,²⁹ and identical twins with discordant presentation of PNET; one with pineoblastoma and the other with a medulloblastoma.¹⁶⁵ A rare association of pineoblastoma with familial adenomatous polyposis has also been reported.⁷⁹

Significantly, pineoblastoma can present in the setting of heritable and sporadic retinoblastoma. Trilateral retinoblastoma, a condition first described in 1971,⁸⁸ is characterized by an intracranial neuroblastic tumor that typically occurs in the setting of germ-line *RB1* gene mutations and heritable disease that manifests as bi- or multifocal disease or family history. Less frequently, trilateral retinoblastoma can manifest with unilateral ocular retinoblastoma.¹⁷⁰ Rare germ-line mutations of *RB* manifest as “forme fruste” Rb, with only intracranial PNET, and no ocular tumors.¹²² Up to 6% of patients with bilateral retinoblastoma and 10% of patients with a family history of these lesions have been reported to harbor an intracranial PNET in one study.²² Trilateral retinoblastoma develops within a median duration of 21 to 23 months from the retinoblastoma diagnosis, has a dismal prognosis, and a 6 month median survival duration following diagnosis of the intracranial tumor.^{92,122} The bleak outlook warrants measures for reductive therapy and early screening in hereditary retinoblastoma.

Trilateral retinoblastomas with intracranial disease that

precede the development of retinoblastoma have been exceptional.^{9,57} More than 80% of PNETs associated with retinoblastomas are pineal in location, whereas supra- or parasellar tumors comprise less than 20% of intracranial tumors in trilateral retinoblastoma. Rare cases of this disease with cerebellar/fourth ventricular PNET/medulloblastoma have been reported.^{53,57} Nevertheless, we are aware of only one case of cerebral PNET reported in association with retinoblastoma. Dorfmueller, et al.,⁵⁰ described a child in whom a temporal-parietal supratentorial PNET developed 5 years after radiation therapy for a unilateral retinoblastoma. Nevertheless, the atypical location and exceptionally long lag time to development of the intracranial lesions suggests a therapy-related secondary tumor rather than a manifestation of trilateral retinoblastoma.

The observed differences in histological features and location of trilateral retinoblastoma-associated PNET suggest that pineal/suprasellar PNETs and nonpineal cerebral supratentorial PNETs may arise from distinct precursor cell populations. In this respect, it may be relevant that pineal PNETs presenting in very young children are reported to have worse outcomes than cerebral supratentorial PNETs.^{63,157} Curiously, several therapeutic studies have reported opposite trends in older children, with superior survival rates and lower incidences of metastatic disease in pineal compared with nonpineal supratentorial PNETs.^{40,47,86} Whether these observed differences reflect true age-related differences in tumor biology or result from earlier clinical manifestations and hence detection of pineal region tumors is not known.

The *RB1* Locus

The gene of origin for retinoblastoma on chromosome 13q14 (*RB1*) was one of the first tumor suppressors identified through studies of heritable cancer syndromes.^{59,100} The pRB pathway has been expertly discussed in several reviews,^{72,154,167} and only its salient features are detailed here and illustrated in Fig. 6.

The *RB1* gene product is a nuclear protein that is expressed in many cell types. In addition to a critical role in cell cycle regulation, pRB is also implicated in cellular differentiation, senescence, apoptosis, and embryonic development. One of the best-characterized and critical functions

TABLE 2
Literature review and summary of published karyotypes for pineoblastomas*

Authors & Year	Tumor No.	Patient Age, Sex	Comment	Karyotype
Griffin, et al., 1988	3	3 yrs, F	trilat retinoblastoma	42;46;XX,del(1)(p13-21) der(1)t(1;?)(p36)
	4	10 mos, M	trilat retinoblastoma	45-47;XY,der(1)t(1;?)(q44)
Sreekantaiah, et al., 1989	8	2.5 mos, M	primary	46, del(11)(q13.1q13.5)
Kees, et al., 1994	9	8 mos, NR	cell line PER-452	iso 17q†
Agamanolis & Malone, 1995	2	10 yrs, F	primary	46,XX
Bigner, et al., 1997	1	1.5 yrs, F	primary	46,XX,+14,-22/46, idem; -20, +mar ³
Kees, et al., 1998	10	11 mos, NR	cell line PER-480	46,XY,der(10)t(10;17)(q21;q22-23), der(16)t(1;16)(q12;q11.2)‡
Roberts, et al., 2001	5	NR	primary	46,XX,-22
	6	NR	primary	63-82,XXX;+2,-3,+4,+5,+7,+9,+11,+12,-14,+15,+16,+17,+18,+20,+21,+22(cp6)
Batanian, et al., 2003	7	8 mos, F		50,XX,YYY,+9; +13, +1q; +19p: 17; complex t(1;11;18)

* NR = not reported.

† N-myc protein overexpression reported.

‡ c-myc protein overexpression reported.

Fig. 5. Photomicrographs showing characteristic histopathological features of a pineoblastoma. A: A lower-power (original magnification $\times 100$) image showing high tumor cellularity. B: Higher-power (original magnification $\times 600$) image showing characteristic Homer-Wright and Flexner-Wintersteiner rosettes. H & E.

of the pRB protein is that of a transcriptional repressor, a function that is mediated through interactions with different E2F (E2F1–5) protein family members. The E2F proteins normally activate gene transcription by binding to consensus positive regulatory DNA elements in gene promoters. The E2F:RB interactions are tightly controlled by the oncogenic cell cycle-specific proteins; cyclins D1 through D3, cyclin E, and their associated cdks (cdk2/4/6).¹⁵⁴ In quiescent cells, hypophosphorylated pRb exists in a complex with E2F4 and other proteins, including p130 and DP1, and acts to recruit chromatin-remodeling proteins such as histone deacetylase^{27,103,104} to effect “closed chromatin conformation” and consequent gene repression. When normal cells are stimulated with mitogens, hyperphosphorylated pRB generated by increased activity of cyclin:cdk4/6 complexes dissociates from E2F proteins, and enables “switching on” of gene transcription mediated through E2F-binding DNA elements.¹⁶⁸ The silencing of genes needed for cells to enter the G₁-S phase of the cell cycle is believed to be one of the critical cellular functions of pRB, which is abrogated in cells with genetic or functional loss of this protein.

Structurally, pRB belongs to a family of so-called pocket proteins (other members are the p107 and p130 proteins), characterized by a small “pocket domain” critical for interactions with a variety of cellular proteins, including D-cyclins, associated cdk6, and HDAC1 (a histone deacetylase molecule involved in transcriptional repression by pRB). These proteins share a short conserved LXCXE peptide motif that binds the small pocket domain in pRB and other pRB-related proteins.¹⁵² Significantly, similar motifs are found in oncogenic DNA tumor viruses such as E1A, SV40 large T, and the HPV E7 antigens,^{77,125} which are known to target pRB through the pocket domain during cellular transformation. These observations underscore the importance of pRB transcriptional repressor functions in maintaining normal cellular phenotypes.

Pineoblastoma in Rb Knock-Out Mice

The RB1 gene product has a central function in cell growth regulation, and is ubiquitously expressed in many

cell types and tissues. The reason for the narrow spectrum of human cancers seen with germ-line RB1 mutations remains unknown, but is proposed to be related to the intrinsic death resistance phenotypes of retinoblastoma precursor cells.^{28,36} Surprisingly, several Rb+/- mouse models generated by homologous recombination do not develop retinoblastoma even after prolonged observations, perhaps suggesting differences in human and mouse Rb precursor cells.

In the majority of human trilateral retinoblastomas, the ocular tumors precede pineoblastoma. These observations suggest possible differences in the cell of origin or transformation events required for development of RB and pineoblastoma. The Rb+/- mice developed a high incidence of pituitary neoplasms after a prolonged latency, and RB-deficient embryos showed defective development of the hindbrain due to increased cell death.⁸⁴ When Rb+/- mice are crossed with p53+/-, a variety of tumors develop, including pineoblastoma. Remarkably, in Rb+/-, p53+/- mice the incidence of pineoblastomas increased to 40%, contrasted with 1% in the Rb+/-, p53+/- mice.¹⁷¹ These observations indicate that functional interactions and cooperative tumorigenic effects of pRb and p53 are important in the development of pineoblastoma. A requirement for concurrent inactivation of p53 and pRb may explain the relatively frequent development of pineal and midbrain PNETs in transgenic mice generated from viral oncogenes (described later) that are known to have pRb and p53 binding activity. Whether both p53 and pRb inactivation is required for development of human pineoblastomas is not known.

Trilateral Rb in Transgenic Mice

Tumors resembling pineoblastomas as well as midbrain PNETs are reported in several retinoblastoma transgenic mouse models driven by different viral oncogenes. In one model in which SV40 large T expression was driven by a Moloney murine sarcoma virus enhancer, pineal hyperplasia developed in adult mice culminating in highly infiltrative midbrain PNET-like tumors with accompanying

Fig. 6. Diagram showing pRB transcriptional regulatory activity. The simplified schema illustrates regulation of pRB transcriptional activity by the INK4/CDK/cyclin proteins. In quiescent cells, INK4 proteins act to inhibit the cdk/cyclin complexes and consequent pRB phosphorylation. Hypophosphorylated pRB acts with DP1 and E2F proteins to enhance transcriptional repression by recruiting histone acetylase (HDAC) to target gene promoters. In proliferating cells, cdk/cyclin complexes are activated and effect pRB phosphorylation. Hyperphosphorylation of pRB provides a permissive chromatin state for E2F protein complexes to activate target gene transcription.

destruction of the pineal gland. The resulting tumors had poorly differentiated histological features with minimal expression of neuron-specific enolase and NF-H, but expressed S antigen, a marker characteristic for pinealocytes and rhodopsin, thus indicating a potential pineal origin for these midbrain tumors.⁹⁶ Interestingly, there were no retinal tumors but instead the mice displayed heritable defects in lens fiber differentiation, retinal dysplasia, and pancreatic tumors.⁶⁶ A second transgenic model more closely resembling human trilateral retinoblastoma with early-onset pineal PNET associated with retinoblastoma has been described in transgenic mice with SV40 expression targeted to photoreceptor cells by a murine interstitial retinol-binding protein promoter. Mice exhibited early pineal hyperplasia and both retinoblastoma-like and pineal tumors developed by the time they reached 2 weeks of age.⁷⁶

Transgenic mice with PNET-like tumors of the diencephalon and/or midbrain regions in association with retinal tumors have also been generated using viral transgenes, and have been postulated to resemble the less frequent association of suprasellar and parasellar tumors in human trilateral retinoblastoma. Transgenic mice with targeted retinal expression of the SV40 large T antigen through a luteinizing hormone β subunit promoter developed multifocal, bilateral retinoblastoma, and produced offspring with retinoblastoma and a high incidence (27%) of midbrain tumors.¹⁰⁷ The resulting primitive intracranial tumors were found to originate not from the pineal gland but from subependymal cells of the cerebral aqueduct.⁹³ Similarly, al-Ubaidi, et al.,² found early development of retinal and brain tumors in a large number of transgenic mice in the embryonic and early postnatal stages, with targeted expression of SV40 large T antigen to rod and cone photoreceptors observed using the human interstitial retinol-binding protein promoter. Nevertheless, in contrast to pineal PNET

in the murine interstitial retinol-binding protein-directed transgenic mice, primitive brain tumors with neuron-specific enolase and S antigen expression developed in the diencephalon, adjacent to the third ventricle.

The fidelity of viral oncogene-driven transgenic mouse models to human diseases is often limited by lack of promoter specificity. Nonetheless, these studies suggest that loss of pRB can lead to transformation of pineal precursor cells and progenitors in the subventricular germinal matrix. It is likely that loss of pRB and/or p53 occurs in sporadic human pineoblastoma or midbrain PNET; however, the extent of involvement and mechanisms of inactivation remain to be determined.

Molecular Genetic Studies of Pineoblastoma

It is not known whether direct mutations of *RB1* or alternate epigenetic mechanisms of *RB1* gene inactivation or functional pRB loss occur in sporadic pineoblastoma. Recent observations, which show significantly worse outcomes in patients with pineoblastoma associated with constitutional *RB1* defects compared with sporadic pineoblastoma,¹²⁶ suggest possible biological differences between these diseases. A number of explanations may underlie these observations, including possible differences in *RB1* genotype-phenotype correlations and involvement of distinct loci of origin in these diseases. Alternatively, the more aggressive disease course in pRB-associated pineoblastoma may reflect the greater genomic instability reported to occur with constitutional pRB loss.⁶ It is not known whether changes in chromosomes 1q31 and 6p22, which have been reported in 50% of human retinoblastoma,^{35,37} occur in PNETs associated or not associated with retinoblastoma.

To date only very limited cytogenetic and molecular data are available for this rare tumor. Informative karyotypic studies have been reported for seven cases of primary pineoblastoma; two of these were associated with trilateral retinoblastoma, and two were cell lines (Table 2). As with the cerebral supratentorial PNET, patients with cytogenetically normal karyotypes and isolated monosomy 22 (one case each) were identified. No consistent cytogenetic change can be inferred from these small numbers. Curiously, although pineoblastomas have been linked to hereditary retinoblastoma as part of the trilateral retinoblastoma complex, none of the sporadic cases of pineoblastoma exhibited a visible change in the region of the *RB1* locus at chromosome 13q14.

The CGH profiles for pineoblastoma have so far only been reported collectively with cerebral supratentorial PNETs, and therefore pineoblastoma-specific information cannot be separately derived from the publications. Recently, in a CGH study of pineocytomas, intermediate (WHO Grade III) and high-grade (WHO Grade IV [pineoblastoma]) pineal parenchymal tumors,¹³⁷ it was reported that pineoblastomas exhibited an average of 5.6 chromosomal changes, in contrast with minimal to no changes in pineocytomas. High-level gains of chromosomes 1q, 5p and q, 6p, and 14q were observed in pineoblastomas, which also frequently demonstrated monosomy 22 (two of three cases). In a recent microarray study, investigators attempted to identify genes that are differentially expressed between pineoblastoma and fetal pineal gland; however, the small number of tumors analyzed (two) significantly limits the interpretation of this study.³³

Single-gene investigations of pineoblastoma have included five pineal tumors with negative results for p53¹⁶⁰ and Waf1/p21¹⁶¹ gene aberrations. Biegel, et al.,¹⁸ reported a missense INI1 mutation in a 7-month-old infant with a histologically confirmed pineoblastoma without rhabdoid features, and with monosomy 22 as the only abnormality. It is not known whether INI1 mutations are more likely to occur in pineoblastomas presenting in very young children with particularly aggressive disease. The recent development of INI1-specific antibodies⁸⁹ should facilitate a broader scope of investigations into the genetic and functional role of the INI1 locus in the development of pineoblastoma.

CONCLUSIONS

Molecular genetic studies of supratentorial PNET and pineoblastoma are limited by the rare incidences of these tumors and the ongoing challenges in their histopathological diagnosis. Nonetheless, our review of existing clinical, molecular genetic, and relevant mouse studies implicates a role for the pRB/Ink4/p53 and DNA repair pathways in the development of supratentorial PNETs. Clearly, future concerted collaborative efforts, through dedicated and coordinated clinical, histopathological, and molecular studies are needed to identify the underlying molecular genetic defects involved in supratentorial PNET development. Such studies will be crucial for the development of appropriate disease models and more effective treatment regimens for these tumors.

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