Perspective

Pediatric Ependymoma: Biological Perspectives

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Abstract

Pediatric ependymomas are enigmatic tumors that continue to present a clinical management challenge despite advances in neurosurgery, neuroimaging techniques, and radiation therapy. Difficulty in predicting tumor behavior from clinical and histological factors has shifted the focus to the molecular and cellular biology of ependymoma in order to identify new correlates of disease outcome and novel therapeutic targets. This article reviews our current understanding of pediatric ependymoma biology and includes a meta-analysis of all comparative genomic hybridization (CGH) studies done on primary ependymomas to date, examining more than 300 tumors. From this meta-analysis and a review of the literature, we show that ependymomas in children exhibit a different genomic profile to those in adults and reinforce the evidence that ependymomas from different locations within the central nervous system (CNS) are distinguishable at a genomic level. Potential biological markers of prognosis in pediatric ependymoma are assessed and the ependymoma cancer stem cell hypothesis is highlighted with respect to tumor resistance and recurrence. We also discuss the shifting paradigm for treatment modalities in ependymoma that target molecular alterations in tumor-initiating cell populations. (Mol Cancer Res 2009;7(6):765–86)

Introduction

Ependymomas are the third most common pediatric tumor of the CNS accounting for 6% to 12% of brain tumors in children and almost 2% of all childhood cancers (1-3). More than half of all cases occur in children under 5 years of age (4-6). Almost 90% of pediatric ependymomas are intracranial in origin, with two thirds arising in the posterior fossa (4-8). Nevertheless, pediatric ependymomas are capable of occurring anywhere within the CNS, including the parenchyma of the cerebral hemispheres and, rarely, the spinal cord (9). Intriguingly, ependymomas also share certain clinical characteristics with germ cell tumors and have been reported in the sacrococcygeal area, mediastinum, and ovary (10, 11), suggesting variable aberrant cell migration and differentiation pathways during ependymoma tumorigenesis.

The prognosis for pediatric ependymomas remains relatively poor when compared with other brain tumors in children, despite advances in neurosurgery, neuroimaging techniques, and postoperative adjuvant therapy. The 5-year survival rate ranges from 39% to 64%, with a 5-year progression-free survival (PFS) rate of 23% to 45% (12). In addition, late relapses up to 15 years after initial treatment are not uncommon (13). The ability to predict patient outcome has been hampered by the heterogeneous clinical behavior of ependymomas in children, insufficient recruitment into large prospective clinical trials, and contradictory studies of existing clinicopathological prognostic markers (14, 15).

Ependymoma is considered by some to be a surgical disease. However, although complete resection is the most consistently reported favorable clinical prognostic factor (1, 16-18), this is not a universal finding and some studies fail to show this relationship for pediatric posterior fossa tumors (19, 20). Furthermore, despite complete excision, local tumor recurrence can develop in up to 50% of cases, even following adjuvant radiotherapy (14, 15, 21).

Patient age at diagnosis and tumor location has also been suggested as prognostic factors. Historically, children below 3 years of age and infratentorial ependymomas have been associated with a poor outcome (6, 22-24). It remains unclear whether this reflects tumor biology, the surgical inaccessibility of posterior fossa tumors, which are more prevalent in younger children, or the avoidance of adjuvant radiotherapy in early life resulting from concerns about long term clinical sequelae (4, 14, 23, 25, 26).

The use of tumor grade according to the current World Health Organization (WHO) 2000 criteria remains controversial as a prognostic marker for ependymoma because of inherent subjectivity. Several studies have shown conflicting results about an association between grade and patient outcome (1, 4, 5, 13, 14, 18, 22, 27-31).

Poor outcome and the unpredictable behavior of this tumor in children have turned attention to improving our knowledge of ependymoma biology. Consequent advances

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Table 1. Abnormal Karyotypes Reported in the Literature of 58 Pediatric Primary Intracranial Ependymomas

Sex	Age (y)	Site	Hist	Karyotype*	Reference
F	2	BS		47,XX,+17	37
M	0–18	PF		50,XY,+7,+8,+9,+9	38
F	8			47,XX,+i(1)(q10)	39
F	8			45,XX,−9/46,idem,+r	39
F	3			45,X,−X,t(10;11;15)(p12;q13;p12)	56
F	6			46,X,Y,+7,10,14,+18,+20,−22,+mar	40
M	4			45,XY,der(1)t(1;3)(q24;p13),der(6)t(2;6)(p11;q22)	40
M	1			45,XY,−22	40
M	8			44,X,−Y,−22	40
M	2	PF		46,XY,+del(1)(p22),−16	41
F	16	PF		55,XX,+2,+3,+5,+7,+8,+9,+11,+19,+21	41
M	9			46,XY,+2mar	42
M	4			46,XY,inv(11)(?p11–13?q13–14)	42
M	5	PF	CL	46,fra(X)(q27.3)c LT	43
M	1	ST	CL	46,XY,?der(6)	43
F	8	PF	CL	46,XX,der(3)t(1;3)(q23;q27–29)	43
F	6	PF	AN	46,XX,trp(1)(q22q31),der(6)t(1;6)trp(1)	43
M	3	PF	CL	46,XY,der(1;6)(q10;p10)	43
F	2	ST	CL	46,XX,?der(6)	43
M	2			46,X,−Y,der(1)t(Y;1)(q12;q11)	44
F	3			49,XX,add(1)(p36),+i(1)(q10)x2,add(2)(p25),inv(2)(p25q21),?t(3;3)(q29;q25),−7,del(7)(q32),−9,−11,+add(14)(p11),+3mar/54,idem,+inv (2),+del(7),+add(14),add(16)(q23),+17,+21,+3mar	44
F	4			46,XX,der(6)t(1;6)(q11;q11)/46,XX,der(14)t(1;14)(q11;p11)	44
F	11			45,X,−X,add(19)(p13),add(22)(q?13)	44
F	0			46,XX,r(14)(p11q?23),add(16)(q11)/46,XX,der(14)t(?1;14)(?q11;p11),add(16)	44
F	7			47,XX,+i(1)(q10)	44
F	6	PF		45,XX,−22	45
M	15	PF		46,XY,add(20)(p?)	45
M	3	PF		45,XY,der(6)t(6;16)(q11;p11),−16	45
F	12	PF		45,X,t(X;18)(p11;q11),t(1;20)(q21;q13),t(2;17)(p11;p11),add(5)(p?),t(12;18) (p11;q11),13,13,t(13;14)(q11;p11),del(14)(q?),add (17),der(21)t(17;21) (p11;q1?)	45
M	10	PF		50,XY,+8,+9,+15,+19	45
M	11	PF		93,XXYY,−6,+13,+20	45
M	8	PF		48,XY,+1,t(1;2)(p33;q21),t(11;18)(q13;q21),+mar/48,X,−Y,+der(1)t(1;8) (^{31}P;q22),t(1;8),del(6)(q15),add(16)(p?),+2mar	45
M	0–18	PF		46,Y,t(X;22)(p22;q11)	46
M	0–18	PF		46,XY,add(1)(p12)	46
M	3	PF		3951,XY,del(2)(q?34),t(2;4)(q34;q35),+del(6)(q25),+12,17	47
F	3	ST		46,XX[5]/40–51,XX,del(X)(p21),der(X)del(X)(p21)del(X)(q26),−X,del(2)(q34),t(2;10)(p25;p12), del(4)(q21q25),del(4)(p14),+6,+10,+16,−17,+22,−22[cp25]	47
M	1			46,XY,t(11;17)(q13;q21)	48
M	1			50–77,XXY,del(1)(p22),dup(1)(p13p32)x2,+i(7)(q10),add(9)(p?),t(12;21),inc	48
M	3	BS		47,XY,+11	49
F	4			61–62,XXX,−1,−2,−5,−8,+9,−10,−12,−14,−21,−22/61–62,idem,tas(3;11) (q29;q25)/61–62,idem,tas(4;22)(p16;p13)/61–62,idem,tas(6;11)(q27;q25)/61–62,idem,tas(9;11)(q34;q25)/61–62,idem,tas(9;17)(q34;q25)	49
M	6			46,XY,add(17)(p13)	50
M	3	PF		45,XY,add(9)(q34),−17,add(17)(p13),add(22)(q11)	50
F	5	PF		47,X,−X,+del(1)(q21),t(1;3)(p34;q21),+add(7)(p11),+15,del(16)(q13),inv(17)(p11q21),−19/46,X,−X,t(1;3),del(4)(q25q31),add(9)(q34),del(10)(p13),der(13)t(13;17)(p12;q21),−15,−17,del(18)(p11),+mar/??,X?,t(1;2)(q21;q35),inc	50
F	1			46,XX,add(11)(q13)	57
M	13			46,XY,del(5)(q34),+7,−8/45,XY,del (5),−17/44,XY,del (5),−17,−22	51
F	9			48–49,XX,+16,+16,+20,−22,+mar	51
F	3			40–44,X,−X,8,t(11;12)(q13;q24),−15,−17,22/40–44,idem,der(6;17)(p10;q10)/40–44,idem,dic(6;17)(q13;p13)	52
F	2			46,XX,t(2;22)(p12;q13)/45,idem,−10	53
M	6			43–46,X,−Y,+7,+19,−22/44–46,X,−Y,+mar	53
F	8			46,X,−X,+7,+16,22	53
F	8			46,X,−X,+mar	53
M	9			42–47,XY,+mar	53

(Continued on the following page)

Table 1. Abnormal Karyotypes Reported in the Literature of 58 Pediatric Primary Intracranial Ependymomas (Cont'd)

Sex	Age (y)	Site	Hist	Karyotype*	Reference
M	6			46–48,XY,t(1;7)(q25;q35),+2,del(3)(q13),der (5),del(6)(q12),+7,+11,−12/45,XY,−22 46–48,XY,t(1;7)(q25;q35),+2,del(3)(q13),der (5),del(6)(q12),+7,+11,−12	53
M	2			45,XY,−22/54,XY,+5,+7,+11,+13,+14,+15,+19,+21 54,XY,+5,+7,+11,+13,+14,+15,+19,+21	53
M	2	PF		46,XY[16]	55
M	13	PF		46,XY[12]/46,XY,del(5)(q34),+7,−8[5]/45,XY, del(5)(q34),−17[7]/44,XY,del(5)(q34),−17,22[8]	55
F	18	ST		46,XY,der(5)t(5;?)(p;?),der(8)t(8;17)(q24;q23), del(17)(q23)[8]/46,XY,der(5)t(5;?)(p;?),−8, der(8)t(8;17)(q24;q23),−11,del(17) (q23),del(22)(q13)[4]	55
M	6			47,XY,+8,+13,−22	54

Abbreviations: M, male; F, female; Hist, histology; PF, posterior fossa; ST, supratentorial; CL, classic; AN, anaplastic.
*Normal cell lines not shown.

have been made, including the identification of Nucleolin expression as a potential biological prognostic marker for children with intracranial ependymoma (32). Nevertheless, an enhanced understanding of the biology of ependymoma remains crucial if we are to identify additional prognostic markers, discover molecular targets for novel or existing therapeutic agents in the clinic and allow adjuvant therapy to be tailored according to tumor-specific molecular characteristics. Progress in these areas could minimize the long term adverse effects of therapy and improve patient survival.

Here we review current knowledge of pediatric ependymoma biology, including a discussion of putative immunohistochemical and genomic prognostic markers. Genomic profiles pertinent to tumor location and patient age are highlighted through a meta-analysis of all CGH studies done on primary ependymomas, implying underlying tumor heterogeneity. There is moreover increasing awareness that brain tumors, similar to leukemia and many solid tumors, are organized as a developmental hierarchy that is maintained by a small fraction of cells endowed with many shared properties of tissue stem cells. Recent advances reveal radial glia as candidate neural precursors upon which neoplastic transformation occurs (33, 34). The paradigm presented by our current understanding of tumor hierarchy and tumor initiation provides a new conceptual and technical framework upon which to develop testable hypotheses about ependymoma biology. The controversies surrounding the ependymoma cancer stem cell and its therapeutic implications will be discussed.

Molecular Distinctions between Pediatric and Adult Ependymomas

Even accounting for differences in clinical factors and therapy, pediatric ependymomas have a worse prognosis than those in adults (8, 14, 35, 36). One interpretation of this is that pediatric

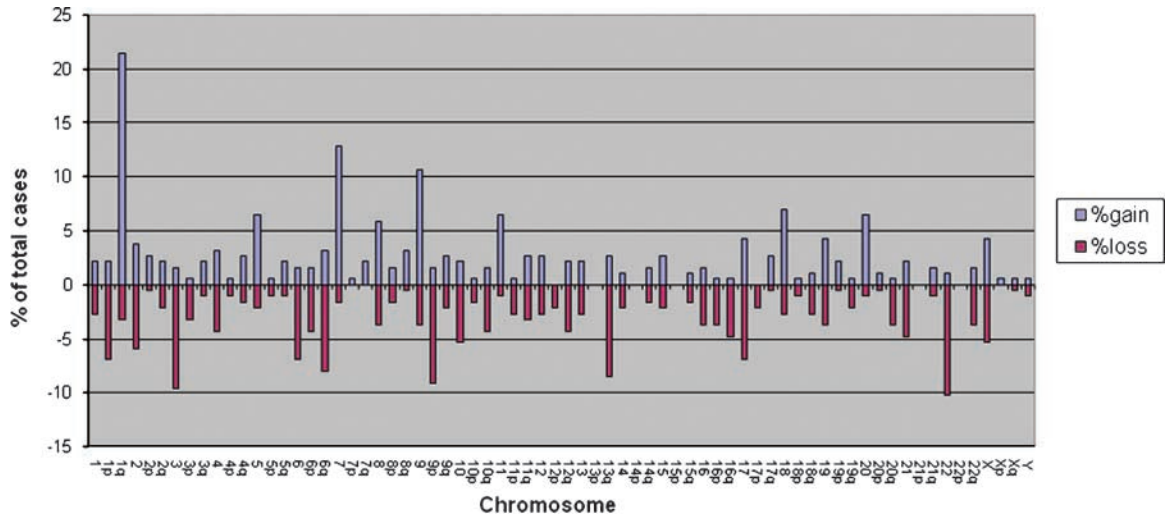

FIGURE 1. Genomic anomalies in 187 primary pediatric ependymomas detected by CGH analysis. The most frequent genomic gains involve chromosome 1q (40/187), 7 (24/187), and 9 (20/187). The most frequent genomic losses involve chromosome 22 (19/187), 3 (18/187), 9p (17/187), 13q (16/187), 6q (15/187), 1p (13/187), 17 (13/187), and 6 (13/187).

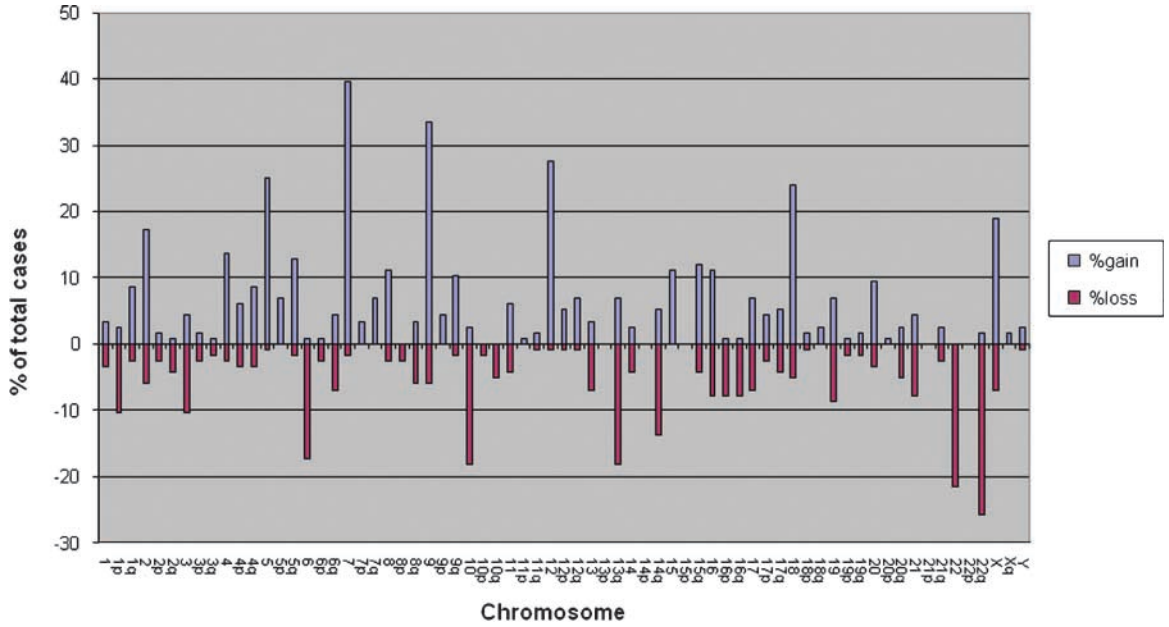

FIGURE 2. Genomic anomalies in 116 primary adult ependymomas detected by CGH analysis. The most frequent genomic gains involve chromosome 7 (46/116), 9 (39/116), 12 (32/116), 5 (29/116), 18 (28/116), X (22/116), and 2 (20/116). The most frequent genomic losses involve chromosome 22q (29/116), 22 (25/116), 10 (21/116), 13q (21/116) 6 (20/116), and 14q (16/116).

and adult ependymomas are biologically distinct, with growing evidence to support this at a molecular level.

Before the advent of detailed genomic analysis, karyotypic studies had found that pediatric ependymomas showed a spectrum of complexity ranging from single rearrangements to structural and numerical aberrations. Our review of data from 21 karyotypic studies of 65 primary pediatric intracranial ependymomas revealed abnormal karyotypes in 58 (89.2%) of cases, which are presented in Table 1 (14, 37-57). However, whereas abnormalities involving chromosomes 22 and 1q were present in almost 30% and 20% of cases, respectively, no cytogenetic abnormality was characteristic of pediatric ependymoma and the frequency of specific aberrations seemed similar between children and adults (14). This implied that a higher resolution analysis of the ependymoma genome was required to identify age-specific anomalies.

The most frequently used technique for high resolution genomic analysis of ependymomas to date has been CGH. This procedure involves the simultaneous hybridization of genomic tumor and constitutional DNA, each labeled with different fluorescent markers, to normal target metaphase chromosomes. Differences in fluorescent intensities between the two markers along the length of each chromosome subsequently identify regions of genetic gain and loss within the tumor genome. A review of literature on CGH analyses of ependymoma since the year 2000 revealed 13 studies, collectively evaluating 303 primary tumors with genomic abnormalities (58-70). We have undertaken a comparative meta-analysis of this cohort. All tumors demonstrating genomic anomalies were included in the analysis, whereas samples without detectable aberrations were excluded. Abnormalities involved either a chromosome arm or a whole chromosome. The cohort was subsequently analyzed according to the clinical subgroups of age and tumor location, with the number of anomalies within each subgroup calculated as a percentage. A pediatric age was defined as equal to or below 16 years. Statistical significance between clinical subgroups was ascertained using Fisher's exact test.

The meta-analysis revealed a clear distinction between pediatric and adult cases in the chromosomal regions most commonly affected by genomic imbalance, which contrasts karyotypic findings (Figs. 1 and 2). All pediatric ependymomas most frequently show gain of chromosomes 1q, 7, and 9 and loss of chromosomes 22, 3, 9p, 13q, 6q, 1p, 17, and 6, whereas the most common genomic aberrations in adult ependymomas are gain of chromosomes 7, 9, 12, 5, 18, X, and 2 and loss of 22/22q, 10, 13q, 6, and 14q.

One of the most striking differences between the two age groups analyzed by CGH is the genomic gain of 1q seen in more than 20% of pediatric ependymomas, but in only 8% of their adult counterparts ($P < 0.004$). In contrast, gains of chromosomes 7, 9, and 12 seem more prevalent in adults ($P < 0.001$). Location specific genomic anomalies of ependymomas may account for this, reflecting the contrasting tendency of pediatric ependymomas to arise in an intracranial location with the spinal predilection of adult tumors (Fig. 3A–F). Gain of 1q, together with loss of chromosomes 6q and 22, is also a common finding in recurrent pediatric intracranial ependymal tumors (Fig. 4A–C), which suggests that these anomalies may be potential markers of aggressive disease.

In addition to the chromosomal location of genomic imbalances, the other important distinction between pediatric and adult ependymomas relates to the number and complexity of genomic anomalies. By comparing the meta-analysis data from all primary ependymoma CGH studies, we observe that adult tumors display more chromosomal aberrations than pediatric tumors with mean genomic anomalies of 7.5 and 3.8 per tumor,

respectively (Table 2). This is reinforced by the CGH finding that a balanced genomic profile, without chromosomal gain or loss, can be seen in 36% to 58% of pediatric ependymomas and is significantly associated with children under 3 years of age. By contrast, a balanced genome is found in less than 10% of adult cases (58, 59, 62, 69, 71).

Our meta-analysis also revealed that the genomic imbalances characteristic of adult and spinal ependymomas regularly involve whole chromosomal rearrangements, unlike the partial and complex imbalances frequently seen in pediatric cases and several aggressive adult cancers (Figs. 1, 2, and 3A and D; refs. 72, 73). CGH and DNA cytometry studies have suggested that a high number of genomic anomalies in ependymoma are associated with tumors of a lower histological grade and a favorable patient outcome (8, 58, 59, 67, 74). This finding could reflect intermediate ploidy, a phenomenon associated with a favorable outcome in acute lymphoblastic leukemia, in which imbalances involve whole chromosomes (75). This explanation is supported by the CGH analysis of 53 pediatric ependymomas by Dyer and colleagues that identified three genetically distinct subgroups within their cohort: the "balanced" genomic group already discussed that was significantly associated with an infant age, a "structural" group demonstrating infrequent and often partial genomic imbalances, and a third "numerical" group showing frequent anomalies similar to those often seen in adult ependymomas. The subdivisions were significantly associated with prognosis, with the numerical and structural groups demonstrating the best and worst patient outcome, respectively. Consistent with this observation, almost all recurrent ependymomas analyzed exhibited a structural profile (59).

The advent of array CGH (aCGH) has enabled the identification of genomic imbalances at a higher resolution than conventional, metaphase CGH. Despite this, similar findings were shown by one aCGH study of 24 intracranial ependymomas that revealed that tumors from younger patients had significantly

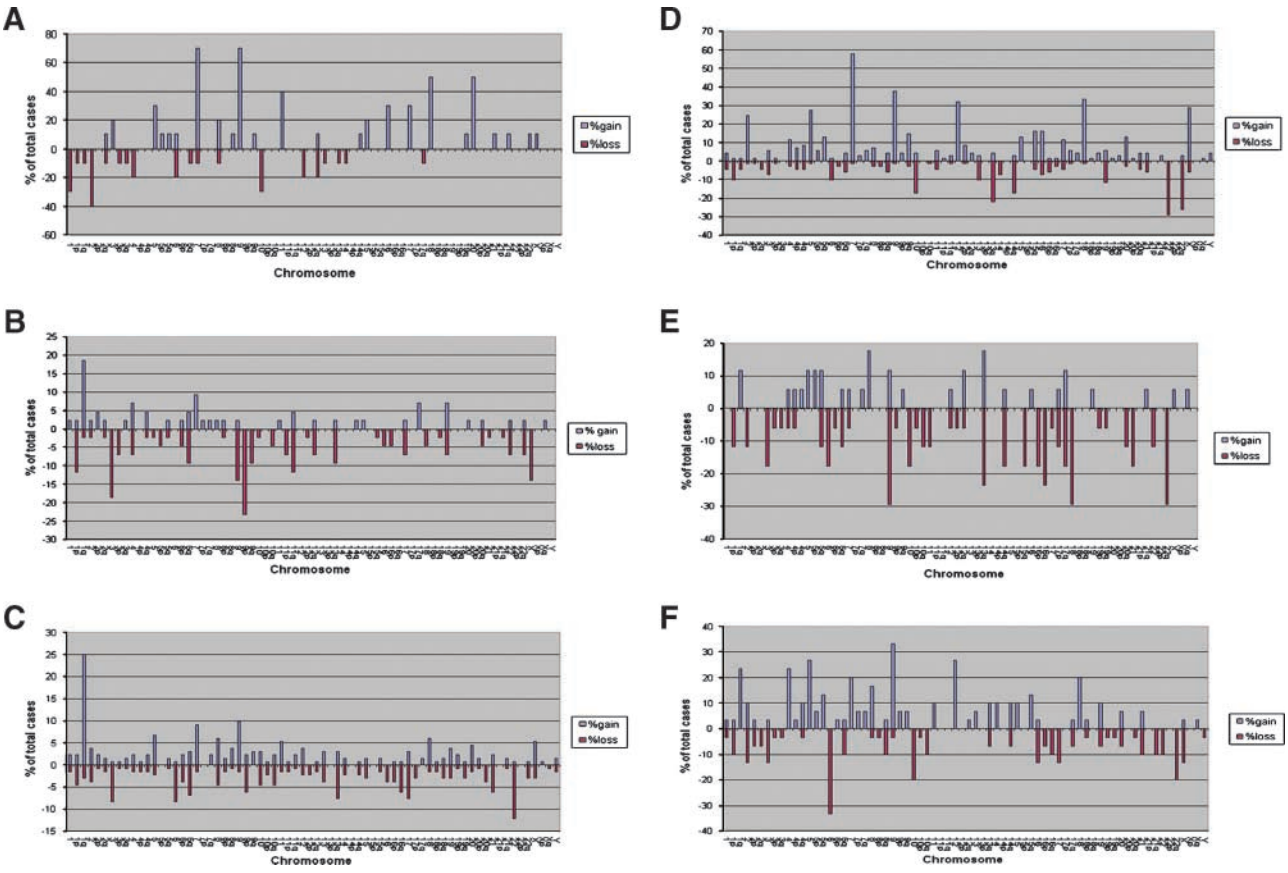

FIGURE 3. Genomic anomalies in 301 primary pediatric ependymomas, grouped according to tumor location within the CNS and age, as detected by CGH. The pediatric tumor group, (**A–C**), consisted of 10 spinal tumors, 43 supratentorial tumors, and 132 infratentorial tumors. The adult tumor group (**D–F**) consisted of 69 spinal tumors, 17 supratentorial tumors, and 30 infratentorial tumors. The most frequent genomic anomalies in 10 pediatric spinal ependymomas (**A**) include whole chromosome gains of chromosomes 7 (7/10), 9 (7/10), 18 (5/10), and 20 (5/10) and loss of chromosome 2 (4/10), 1 (3/10), and 10 (3/10). The most frequent genomic imbalances in 43 supratentorial tumors (**B**) are gain of chromosome 1q (8/43) and loss of chromosome 9p (10/43), 3 (8/43), and X (6/43). In contrast, the most common genomic anomalies in 132 infratentorial ependymomas (**C**) are gain of chromosome 1q (33/132), yet loss of chromosomes 22 (16/132), 3 (11/132), 6 (11/132), 13q (10/132), and 17(10/132). The most frequent genomic anomalies in 69 adult spinal ependymomas (**D**) include whole chromosome gains of chromosomes 7 (40/69), 9 (20/69), 18 (23/69), 12 (22/69), X (20/69), 5 (19/69), and 2 (17/69) and loss of chromosome 22 (20/69), 22q (18/69), 13q (15/69), 14q (12/69), 10 (12/69), and 19 (8/69). The most frequent genomic imbalances in 17 supratentorial tumors (**E**) are gain of chromosome 13q (3/17) and 8 (3/17) and loss of chromosome 9 (5/17), 18 (5/17), 22q (5/17), 13q (4/17), and 16q (4/17). In contrast, the most common genomic anomalies in 30 infratentorial ependymomas (**F**) are gain of chromosomes 9 (10/30), 5 (8/30), 12 (8/30), 1q (7/30), 4 (7/30), 7 (6/30), and 18 (6/30) yet losses of chromosomes 6 (10/30), 22q (6/30), and 10 (6/30).

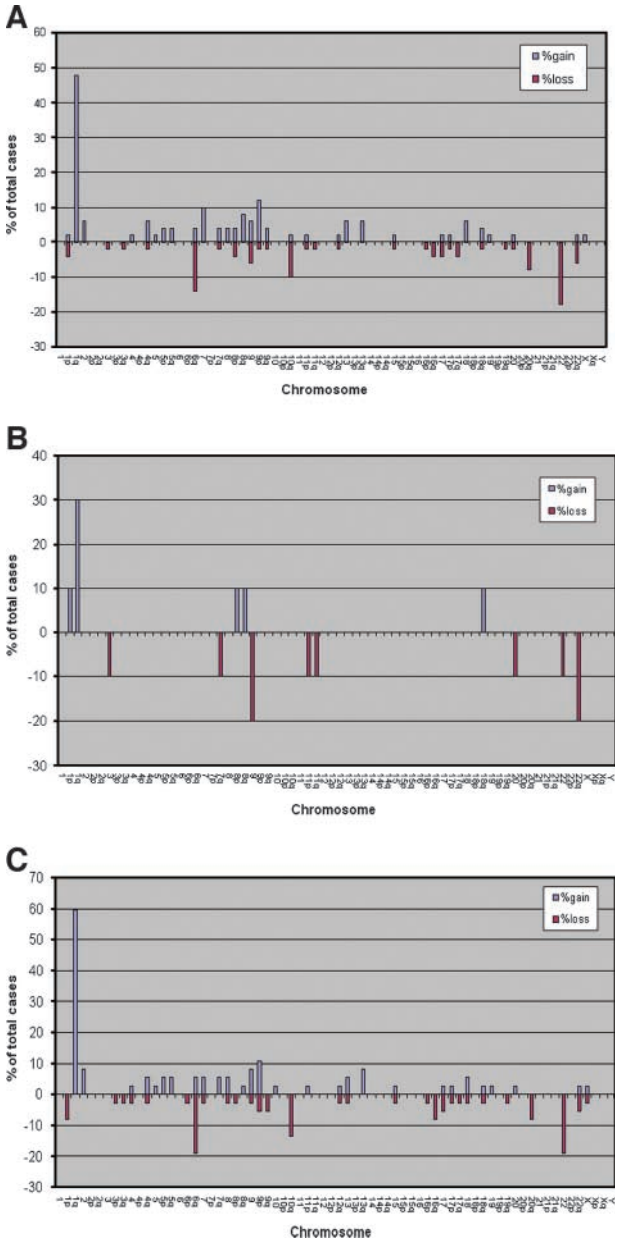

FIGURE 4. Genomic anomalies in 50 intracranial recurrent pediatric ependymomas detected by CGH analysis. Analysis was conducted collectively (**A**), then subgrouped according to supratentorial location (**B**), 10 tumors or infratentorial location (**C**) 37 tumors. The location of three of 50 tumors was not specified in the literature. Overall (**A**), the most frequent genomic gain involves 1q (24 of 50), and the most frequent genomic losses involve chromosomes 22 (9 of 50) and 6q (7 of 50). In addition, location specific anomalies include the loss of chromosome 9 in 2 off10 supratentorial tumors (**B**) and the loss of chromosomes 6q (7 of 37) and 10q (5 of 37), which are exclusive to posterior fossa recurrences (**C**).

smaller, partial regions of genomic imbalances than older patients (76).

Other studies have highlighted genetic and epigenetic characteristics of pediatric ependymomas that differentiate them from the molecular profile of adult tumors. These include the overexpression of genes *LDHB* and *STAM* (77), the downregulation and deletion of members of the Protein 4.1 superfamily (78), and the reduced methylation of *CDKN2A, CDKN2B*, and *p14ARF* in posterior fossa ependymomas (79).

Taken together, these findings suggest that ependymomas in children are genetically distinct from their adult counterparts. Furthermore, genetic subdivisions within the pediatric age range may exist. Ependymomas with few and often partial chromosomal imbalances may confer a worse prognosis and are more likely to occur in younger children. At present, the explanations for this remain unclear. It has been hypothesized that the association between partial chromosomal rearrangements and poor prognosis in pediatric ependymomas could be anticipated because the acquisition of numerous, partial genomic imbalances may have biological effects that exceed those preserving a broad genomic balance across individual chromosomes. A difference in the number of genomic aberrations between ependymomas from both age groups (Table 2) seems consistent with a canonical multistep cancer initiation/progression model for adult ependymoma. It also suggests that fewer defects in cell-regulating processes are needed to initiate ependymoma in children, because the behavior of cells from immature normal tissue is similar to that of cancer cells with respect to differentiation, survival, and self-renewal (80, 81). Pediatric ependymomas demonstrating balanced genomic profiles or subtle imbalances substantiate this theory. This would imply that the genes affected by mutation in the younger age group are potent oncogenes or tumor suppressor genes, or are genes responsible for regulating cell differentiation and self-renewal that only exert an oncogenic effect within a particular developmental environment or temporal window. Alternatively, epigenetic phenomena, such as methylation and acetylation signatures, may be affecting the expression profiles of an unknown number of genes in pediatric ependymomas by altering their transcription and expression without a corresponding detectable genomic imbalance. Further studies are required to validate these theories, such as analyzing the gene expression profiles of "balanced" ependymomas, examining tumor epigenetics, and using techniques such as the single nucleotide polymorphism (SNP) array to analyze the ependymoma genome at a higher resolution for as yet undetected subtle genomic imbalances.

Ependymoma Heterogeneity and Tumor Location

Despite histological similarities, ependymomas arising from the spinal canal, infratentorial, and supratentorial compartments of the CNS show diverse clinical behavior (1). Molecular genetic studies, including our meta-analysis of all CGH studies done exclusively on primary pediatric and adult ependymomas (Fig. 3A–F), show location-specific differences that suggest biological tumor heterogeneity.

Pediatric spinal ependymomas (Fig. 4A) frequently show whole chromosomal imbalances such as gain of chromosomes 7, 9, 11, 18, and 20 or loss of chromosomes 1, 2, and 10, whereas intracranial ependymomas are characterized by gain of chromosome 1q and often show loss of chromosomes 22, 3, 9p, and 13q (58-64, 66-71). This again reinforces a possible association between intermediate ploidy and improved patient outcome, which is often seen with cases of intramedullary

ependymoma (82). Spinal ependymal tumors also seem to have a gene expression profile that is distinct from their intracranial counterparts. Whereas the latter group seems to upregulate the oncogene homolog *RAF-1*, spinal ependymomas overexpress genes responsible for peptide production and activity including *PLA2GS* and *ITIH2*, and members of the homeobox (*HOX*) family involved in normal anteroposterior development of the spinal canal (34, 77, 83). Mutation analysis of the Protein 4.1 superfamily member *NF2* on chromosome 22q12 has revealed that, in association with loss of heterozygosity (LOH) for chromosome 22, somatic *NF2* mutations exist in a proportion of ependymomas. However these findings are restricted exclusively to spinal cases in adults (82, 84). In addition, *NF2* mutations are seen in patients with Neurofibromatosis type 2, a cancer predisposition syndrome regularly associated with the occurrence of spinal ependymomas. Disparate genetic alterations in other Protein 4.1 members have also been reported between spinal and intracranial ependymomas, such as *4.1B* deletion and *4.1R* loss of expression (78, 82, 84, 85).

Further genetic and epigenetic diversity seems to exist between pediatric ependymomas from different intracranial locations. Gene expression profiling of supratentorial ependymomas has shown the distinct overexpression of several components of the EPHB-EPHRIN and Notch cell signaling pathways responsible for regulating cell proliferation and differentiation (34). In addition, the genomic loss of 9p occurs preferentially in pediatric supratentorial ependymomas (Fig. 4B). This is supported by studies of *CDKN2A* (p14ARF), a tumor suppressor gene located at 9p21.3 that regulates neural stem cell proliferation and whose deletion has been shown to rapidly expand progenitor cell numbers in developing neural tissue (33, 34). Whereas gene expression analysis has revealed that *CDKN2A* is upregulated in spinal tumors (77, 86), it is downregulated by a much stronger magnitude in intracranial tumors (86). Furthermore, while epigenetic analysis has shown a significant difference in the methylation status of neighboring *CDKN2B* between spinal and intracranial tumors (79), fluorescent *in situ* hybridization (FISH) has shown that *CDKN2A* deletion is virtually exclusive to supratentorial ependymomas (34). Other genes on 9p that seem to be downregulated exclusively in supratentorial ependymal tumors include *FREM1, C9orf24*, and *KIAA116* (76).

In contrast, posterior fossa ependymomas in children often show loss of chromosomes 22, 6, and 17 (Fig. 4C), and a relatively high degree of epigenetic silencing of the 17p tumor suppressor gene *HIC-1* by promoter DNA methylation (87). At recurrence, the loss of chromosome 6q seems exclusive to posterior fossa tumors, as determined by CGH (Fig. 3C). Additional subdivision of posterior fossa ependymomas based on their gene expression profiles has also been suggested by one study of 103 ependymomas that identified three infratentorial subgroups including one characterized by numerous DNA amplifications and one demonstrating recurrent genomic gains of chromosome 1q (34). Collectively, these data support the current consensus that tumor hierarchical organization underlies genetic and molecular distinctions in pediatric ependymoma.

Ependymoma-Initiating Cells

The general impetus for cancer researchers has been to interrogate genetic anomalies that occur in highly malignant cells from late stages of tumor progression. More recently, the focus has shifted to dissecting the biology of the first transformed tumor cell, viewing the tumor as the consequence of aberrant developmental processes. There is accumulating evidence that ependymoma genesis is a phenotypic outcome of dysregulated neurogenesis, with tumors viewed conceptually as abnormally differentiated neural tissue.

From the initial discovery in hematopoietic tumors, researchers of most solid cancers have defined restricted subsets of tumor cells that share characteristics of the corresponding normal tissue stem cells (88-92). Peter Dirks and colleagues have previously isolated a minority population of pediatric brain tumor cells, based on the expression of the cell surface antigen CD133, known to be highly expressed in normal neural stem cells. These putative brain cancer stem cells were isolated from medulloblastoma, pilocytic astrocytoma, glioblastoma multiforme, and anaplastic ependymoma and possessed a marked capacity for self-renewal (evidenced by the formation of floating aggregates termed tumorspheres), proliferation, and differentiation (93). Crucially, only the CD133+ fraction was capable of tumor initiation (medulloblastoma and glioblastoma multiforme) upon xenograft transplantation in nonobese diabetic, severe combined immunodeficient (NOD-SCID) mouse brain (94). Not only was the CD133+ fraction able to recapitulate the original tumor hierarchy, but it also produced a tumor that could be serially transplanted into secondary recipients, providing evidence of self-renewal capacity *in vivo* (94).

More recently, a study using oligonucleotide gene expression arrays and aCGH showed first that supratentorial, posterior fossa, and spinal ependymomas have distinguishable gene expression signatures and second, that signature genes of supratentorial and spinal ependymomas are similarly expressed by murine embryonic radial glial cells in the corresponding region of the CNS (33, 34). Embryonic and postnatal ependymal cells have previously been shown to be derived from a subset of radial glia, suggesting that these cells are neural stem or precursor cells (95). The ability to prospectively identify such radial glial-like subsets from pediatric ependymoma is necessary to refute the tumor-initiating attribute of these

Table 2. Comparison of the Number of Genomic Imbalances in 116 Adult Primary Ependymomas and 187 Pediatric Primary Ependymomas by CGH

Age Group	Number of Primary Ependymomas	Total Number of Gains	Total Number of Losses	Total Number of Genomic Anomalies	Mean Number of Anomalies per Tumor
Adult	116	507	362	869	7.5
Pediatric	187	331	376	707	3.8

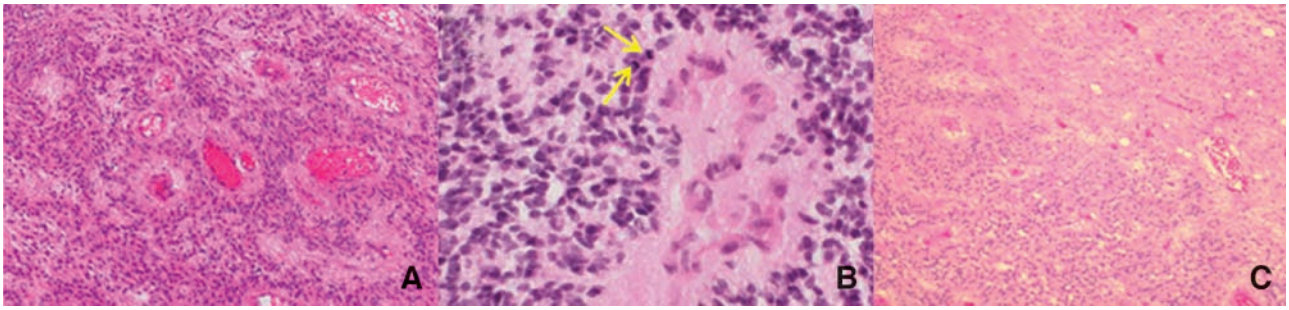

FIGURE 5. Histological appearance of pediatric ependymoma. **A.** Classical (grade II) ependymoma with perivascular pseudorosettes. **B.** Anaplastic (grade III) ependymoma with high mitotic activity (arrows) and vascular proliferation. **C.** Ependymoma displaying tumor heterogeneity with a grade II region (top right) and a grade III region (bottom left) in the same section. Magnifications ×10, ×40, and ×10, respectively.

cells. Taylor and colleagues used protein markers representing early stages of differentiation in the subventricular zone. Neuroepithelial cells are the immediate precursors of radial glia and express CD133 and Nestin (96, 97), whereas brain lipid-binding protein (BLBP) is expressed exclusively on glial cells (98). All ependymoma-derived tumorspheres displayed a CD133+/Nestin+/RC2+/BLBP+ immunophenotype, which permitted the sorting and characterization of this subset. The capability of these radial glial-like cells to self-renew and exhibit multilineage differentiation in vitro and form orthotopic tumors *in vivo* renders them a convincing candidate for the ependymoma-initiating cell, the cell within which the initial transformation event occurs. An average of ~1% of cells within CD133+ sorted populations of ependymoma displayed a radial glial-like immunophenotype. In contrast, ependymoma subsets that lacked expression of the CD133 antigen did not give rise to tumors upon xenotransplantation (34), even when large number of cells were injected, consistent with reports from other pediatric brain tumors (94).

To an extent, this realization represents a paradigm shift in our understanding of tumorigenicity. The vast majority of cells within the ependymoma hierarchy are therefore nontumorigenic, with only a minority capable of tumor initiation. At present, this does not exclude the possibility of more committed progenitors or even mature cells reverting to a stem-like phenotype to provide a pool from which cancer stem cells may mature. It could also be argued that the mouse may not represent a physiologically relevant microenvironment for engraftment and growth of human tumors, thus potentially resulting in an underestimation of cell types capable of tumor initiation. Indeed, recent data have emerged showing that modifications in xenotransplantation assays can dramatically increase the proportion on melanoma-initiating cells by several orders of magnitude (99). An additional unresolved question is whether the cell type displaying the CD133+/Nestin+/RC2+/BLBP+ immunophenotype and capacity for self-renewal *in vitro* is the same cell type that is tumor-initiating *in vivo*. Although likely, definitive experiments have not yet been established. Current data do not exclude the possibility that the tumor cell of origin varies depending on the particular tumor, or intriguingly, that it varies depending on the age of the patient at which the initial malignant transformation event occurs. Definitive evidence using gene expression comparisons between human radial glia and ependymoma will be required to verify this primitive neural cell as the ependymoma cell of origin. At present, it is unknown whether normal ependymal cells can transform directly into a neoplastic cell, a question hampered by the inability to culture ependymal cells for a sufficient period of time.

The cancer stem cell field is at present contentious, largely due to semantic misinterpretations (100). The term cancer stem cell is often applied to cells identified by varying methods and criteria that do not share many characteristics. The danger here is the application of the term to cells with no evidence of cancer-initiating capacity. It is important to clarify what ependymoma stem cell refers to in the present context: these are cells that have cancer-initiating ability upon orthotopic transplantation; show the ability to self-renew *ex vivo* and *in vivo*; harbor ependymoma specific karyotypic and or genetic alterations; and show aberrant differentiation and a capacity to generate a phenocopy of the original tumor, consisting of both tumorigenic and nontumorigenic cells.

Biological Markers of Prognosis in Pediatric Ependymoma

Currently the only accepted clinical prognostic marker available for children with ependymoma is the degree of tumor excision at surgery. However, the need to identify new correlates of outcome in pediatric ependymoma has intensified because tumor recurrence can occur in up to 50% of children, despite complete surgical resection and adjuvant radiotherapy, with only approximately 15% of patients subsequently surviving beyond 5 years (2, 14, 15, 21).

Histology, Tumor Morphology, and Prognosis

Ependymomas are neuroepithelial tumors of variable morphological appearance. The histology of this tumor group remains an extremely controversial correlate of prognosis. Although several classification systems have been proposed, the most frequently used is the WHO 2007 grading system (101). This identifies four major types of ependymoma: subependymoma and myxopapillary ependymoma (grade 1), classic or low grade (grade II), and anaplastic (grade III; Fig. 5). The latter two grades are the principal variants found in children. Classic ependymomas often show dense cellularity and perivascular pseudorosettes (20), whereas a diagnosis of

Table 3. Statistically Significant Studies of Putative Prognostic Markers (Immunohistochemical and Genomic) for Pediatric Ependymoma

Immunohistochemical Marker	Source	Prognostic Feature	Patient Outcome (P value)	Use as a Prognostic Marker in Pediatric Ependymoma	
				Strength	Weakness
Glial fibrillary acidic protein (GFAP)	108	Increased expression in >30% tumor cells	Improved OS ($P < 0.05$)	Pediatric cohort analyzed exclusively.	Small cohort size ($n = 16$)
		GFAP to vimentin ratio <1	Worse OS ($P < 0.05$)	Statistical significance in univariate analysis	Other pediatric study refutes prognostic association (e.g., ref. 127). No prospective validation
Bromodeoxyuridine (BrdU) labeling index	104	Labeling index >1%	Early recurrence rate ($P < 0.05$)	Statistical significance in univariate analysis	Mixed age cohort
		Correlation between BrdU index and time to recurrence	Inverse correlation ($P < 0.05$)		Small cohort size ($n = 32$) No prospective validation
Proliferating cell nuclear antigen (PCNA)	122	High expression in supratentorial cases	Worse OS ($P < 0.05$)	Statistical significance in univariate analysis	Both studies: Mixed age cohort
	126	PCNA >5%	Worse OS in univariate analysis ($P = 0.005$)	Statistical significance in univariate analysis	No statistically significant association in multivariate analysis. No prospective validation
Metallothionine	114	Increased expression	Lower recurrence risk ($P = 0.005$)	Statistical significance in multivariate analysis	Korshunov study: Mixed age cohort
				Relatively large cohort size ($n = 76$)	Cohort size remains inadequate for reliable multivariate analysis.
Metalloproteinase expression	124	Increased MMP2 and MMP14 expression in tumors with CR	Lower OS ($P < 0.05$)	Pediatric cohort analyzed.	Snuderl study: Small cohort size ($n = 28$)
				Statistical significance in multivariate analysis	Both studies: No prospective validation
P-glycoprotein	114	Increased expression	Lower recurrence risk ($P = 0.02$)	Statistical significance in multivariate analysis	Mixed age cohort
				Relatively large cohort size ($n = 76$)	No prospective validation Cohort size remains inadequate for reliable multivariate analysis.
p53	35, cohort expansion of 113, 112	Immunohistochemical positivity (LI 14–32%)	Worse PFS for entire cohort ($P = 0.0001$) and high grade tumors ($P = 0.0001$)	Large cohort size ($n = 112$)	All studies: No prospective validation P53 antibody detects mutant *and* wild type p53.
			Higher recurrence risk ($P = 0.01$)	Statistical significance in multivariate analysis	Mutations of p53 seem rare in ependymoma.
	126	Increased expression >1%	Worse OS in univariate analysis ($P = 0.02$) and multivariate analysis ($P = 0.013$)	Relatively large cohort size ($n = 51$). Statistical significance in multivariate analysis	Other studies refute prognostic association (e.g. ref. 123). Verstegen and Korshunov studies: Mixed age cohorts
	127	Increased expression	Worse PFS ($P < 0.001$)	Pediatric cohort analyzed Statistical significance in univariate analysis	Zamecnik study: Small cohort ($n = 31$) and no statistical significance in multivariate analysis

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Table 3. Statistically Significant Studies of Putative Prognostic Markers (Immunohistochemical and Genomic) for Pediatric Ependymoma (Cont'd)

Immunohistochemical Marker	Source	Prognostic Feature	Patient Outcome (*P* value)	Use as a Prognostic Marker in Pediatric Ependymoma	
				Strength	Weakness
Apoptotic index (AI, using ISEL)	35, cohort expansion of 113, 112	Apoptotic index <1%	Worse PFS for entire cohort ($P = 0.002$) and high grade tumors ($P = 0.0001$)	Large cohort size ($n = 112$) Statistical significance in univariate analysis	No statistically significant association in multivariate analysis. Mixed age cohort. No prospective validation
p14 ARF labeling index	35, cohort expansion of 113	Labeling index <10%	Worse PFS for entire cohort ($P = 0.0001$) and high grade tumors ($P = 0.0001$)	Large cohort size ($n = 112$)	Mixed age cohort
			Higher recurrence risk ($P = 0.001$)	Statistical significance in multivariate analysis	No prospective validation
				CDKN2A recognized TSG.	Other studies refute prognostic association of CDKN2A (e.g., ref. 120).
bcl-2	127	Increased expression	Worse PFS and OS ($P < 0.001$)	Pediatric cohort analyzed exclusively.	No statistically significant association in multivariate analysis
				Statistical significance in univariate analysis	Small cohort size ($n = 31$)
				No prospective validation	
				Other studies refute prognostic association (e.g., ref. 126).	
Cyclin D1 labeling index	127	Labeling Index >5%	Worse PFS ($P = 0.049$)	Pediatric cohort analyzed exclusively. Statistical significance in univariate analysis No prospective validation	No statistically significant association for OS in univariate analysis or OS and PFS in multivariate analysis Small cohort size ($n = 31$)
				Other studies refute association with prognosis (e.g. ref. 117).	
Topoisomerase-II alpha (Ki-S1) labeling Index	35, cohort expansion of 113, 112	Labeling index >5%	Worse PFS for entire cohort ($P = 0.001$) and low grade tumors (0.0001)	Large cohort size ($n = 112$) Statistical significance in univariate analysis	All studies: No statistically significant association in multivariate analysis No prospective validation
	127	Labeling index >12%	Worse PFS and OS ($P < 0.001$)	Pediatric cohort analyzed exclusively. Statistical significance in univariate analysis	Variable LI cut offs between studies Korshunov study: Mixed age cohort Zamecnik study: Small cohort ($n = 31$)

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Table 3. Statistically Significant Studies of Putative Prognostic Markers (Immunohistochemical and Genomic) for Pediatric Ependymoma (Cont'd)

Immunohistochemical Marker	Source	Prognostic Feature	Patient Outcome (*P* value)	Use as a Prognostic Marker in Pediatric Ependymoma	
				Strength	Weakness
Ki-67/MIB-1 labeling index	121	High MIB-1 labeling index (13% versus 2%)	Tumor tendency for CSF dissemination ($P = 0.02$)	Statistical significance in univariate analysis; large cohort size ($n = 140$)	All studies: No prospective validation
	36	MIB-1LI >20%	Worse OS ($P = 0.0013$)	Statistical significance in CART analysis	Other pediatric studies refute association with prognosis (e.g., refs. 32, 123). Variable LI cutoffs to define prognostic groups
	110	MIB-1LI >9%	Worse OS and PFS ($P < 0.001$)	Relatively large cohort ($n = 81$)	Rezai, Ritter, Ho, Preusser, Verstegen studies: Mixed age cohorts
				Significance in univariate analysis	Zamecnik, Ritter studies: Small cohort sizes ($n = 36$ and 34)
	127	MIB-1 LI >7%	Worse OS and PFS ($P = 0.002$)	Pediatric cohort analyzed exclusively. Statistical significance in multivariate analysis	Verstegen study: No statistically significant association in multivariate analysis
	119, cohort expansion of 118	MIB-1 LI >20.4%	Worse OS in univariate analysis ($P = 0.00001$) and multivariate analysis ($P = 0.010$)	Statistical significance multivariate analysis Large cohort ($n = 100$)	
	126	MiB-1 LI > 1%	Worse OS in univariate analysis ($P = 0.02$)	Statistical significance multivariate analysis Relatively large cohort ($n = 51$)	
	106	Elevated Ki-67 LI in infratentorial cases (>25%)	Worse OS ($P < 0.002$)	Pediatric cohorts analyzed exclusively; relatively large cohort ($n = 74$); Statistical significance in multivariate analysis	All studies: No prospective validation Variable LI cutoffs to define prognostic groups
	109	Ki-67 LI >25%	Worse OS ($P < 0.009$)	Pediatric cohorts analyzed exclusively; relatively large cohort ($n = 89$).	Cohort sizes remain inadequate for reliable multivariate analysis.
	107	Ki-67LI >1%	Worse PFS ($P < 0.006$)	Pediatric cohorts analyzed exclusively.	Other studies refute association with prognosis (e.g., refs. 32, 117). Gilbertson and Figarella-Branger studies: No statistically significant association in multivariate analysis Figarella-Branger study: Small cohort size ($n = 37$)
p27/Kip 1 labeling index	35, cohort expansion of 112	Labeling Index <20%	Reduced PFS in entire cohort ($P = 0.01$) and high grade tumors ($P = 0.00003$)	Large cohort size ($n = 112$)	No statistically significant association in multivariate analysis. Mixed age cohort.
				Statistical significance in univariate analysis	No prospective validation
Nucleolin	32	Increased expression	Reduced EFS and OS ($P = 0.007$)	Relatively large cohort size ($n = 80$) Pediatric cohort exclusively analyzed. Statistical significance in multivariate analysis	No prospective validation

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Table 3. Statistically Significant Studies of Putative Prognostic Markers (Immunohistochemical and Genomic) for Pediatric Ependymoma (Cont'd)

Immunohistochemical Marker	Source	Prognostic Feature	Patient Outcome (P value)	Use as a Prognostic Marker in Pediatric Ependymoma	
				Strength	Weakness
hTERT	125	Increased expression	Reduced PFS and OS ($P = 0.002$)	Relatively large cohort size ($n = 87$) Pediatric cohort exclusively analyzed. Statistical significance in multivariate analysis	All studies: No prospective validation Re-evaluation of antibody used to detect hTERT verified the actual target to be Nucleolin.
	86	Increased expression	Worse OS ($P = 0.01$)	Large cohort size ($n = 170$) IHC validated genomic gains at hTERT locus detected by aCGH	Mendrzyk study: Mixed age cohort No statistically significant association in multivariate analysis
Vitronectin	128, cohort expansion of 127	Immunohistochemical detection at tumor invasion front	Worse PFS ($P = 0.005$)	Pediatric cohort analyzed exclusively. Statistical significance in univariate analysis	Small cohort size ($n = 36$) No statistically significant association in multivariate analysis No prospective validation
Tenascin	35, cohort expansion of 112	Immunohistochemical positivity	Worse PFS for entire cohort ($P = 0.0001$) and low grade tumors ($P = 0.0001$) Higher recurrence risk ($P = 0.001$)	Large cohort size ($n = 112$) Statistical significance in multivariate analysis	Korshunov study: Mixed age cohort No prospective validation Zamecnik study: Small cohort size ($n = 36$) No prospective validation
	128, cohort expansion of 127	Immunohistochemical detection in intercellular spaces and blood vessel walls	Worse PFS ($P = 0.012$)	Pediatric cohort analyzed exclusively Statistical significance in multivariate analysis	
VEGF	35, cohort expansion of 112	Immunohistochemical positivity	Worse PFS for entire cohort ($P = 0.003$) and low grade tumors ($P = 0.001$)	Large cohort size ($n = 112$) Statistical significance in univariate analysis	No statistically significant association in multivariate analysis Mixed age cohort No prospective validation Other studies refute prognostic association (e.g., ref. 116).
Hypoxia-related tissue factors	119	High hypoxia score (increased expression of 2 or 3 of the markers VEGF, carbonic anhydrase 9 (CA9) and hypoxia-inducible factor 1 alpha (HIF-1α)	Worse OS ($P = 0.0402$)	Large cohort size ($n = 100$) Statistical significance in univariate analysis	No statistically significant association for individual markers in univariate analysis No statistically significant association for hypoxia score in multivariate analysis Mixed age cohort No prospective validation
Survivin	118	Increased expression	Worse OS ($P = 0.0032$)	Statistical significance on univariate analysis Relatively large cohort size ($n = 63$)	No statistically significant association in multivariate analysis Cohort size remains inadequate for reliable multivariate analysis Mixed age cohort Other pediatric studies refute prognostic association (e.g., ref. 32) or show an association between low expression and aggressive disease (e.g., ref. 103).
Mos protein	105	Labeling index >10%	Worse PFS ($P = 0.05$)	Statistical significance in univariate analysis	No statistically significant association for OS No multivariate analysis Mixed age cohort Small cohort size ($n = 34$) No prospective validation

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Table 3. Statistically Significant Studies of Putative Prognostic Markers (Immunohistochemical and Genomic) for Pediatric Ependymoma (Cont'd)

Immunohistochemical Marker	Source	Prognostic Feature	Patient Outcome (P value)	Use as a Prognostic Marker in Pediatric Ependymoma	
				Strength	Weakness
RTK1 family (EGFR, EEBB2–4)	35, cohort expansion of 112	Immunohistochemical positivity	Worse PFS for entire cohort ($P = 0.005$) and low grade tumors ($P = 0.002$)	Large cohort size ($n = 112$)	All studies: No statistically significant association in multivariate analysis No prospective validation
	109	Combination of incomplete tumor resection and ERBB2/ERBB4 coexpression or Ki-67 LI >25%	Worse OS ($P < 0.0001$)	Relatively large cohort size ($n = 59$) Pediatric cohort exclusively analyzed	Other pediatric studies refute prognostic association of all RTK1 family (e.g., ref. 32). Gilbertson study: ERBB2/ERBB4 coexpression alone not associated with poor outcome
	86	Immunohistochemical positivity	Worse OS ($P = 0.002$)	Large cohort size ($n = 170$) IHC validated genomic gains and amplifications at EGFR locus detected by aCGH	Korshunov and Mendrzyk studies: Mixed age cohorts
Genomic/Genetic Marker	**Source**	**Prognostic Feature**	**Patient Outcome (P value)**	**Use as a Prognostic Marker in Pediatric Ependymoma**	
				Strength	**Weakness**
Genomic gain of 1q	58	Gain of 1q (CGH)	Worse OS for intracranial tumors ($P < 0.05$)	Statistical significance in univariate analysis	All studies: Mixed age cohort
	86	Gain of 1q25 (aCGH/FISH)	Worse PFS ($P < 0.001$) and OS ($P = 0.003$) in intracranial tumors	Statistical significance in multivariate analysis Large cohort	No prospective validation Carter study:
		Gain of 1q21.1–32.1 (aCGH/FISH)	Higher recurrence rate in intracranial tumors ($P < 0.001$)	FISH validated aCGH findings	Small cohort size ($n = 31$) No statistically significant association in multivariate analysis
Number and complexity of genomic aberrations	59	Structural group of tumors with few and partial chromosomal imbalances (CGH)	Worse OS than numerical tumors ($P = 0.05$) or balanced tumors ($P = 0.02$)	Statistically significant in multivariate analysis Pediatric cohort exclusively analyzed	Small cohort size ($n = 42$) No prospective validation
Protein 4.1 family	78	Deletion of *4.1G* locus – 6q23 (FISH)	Worse EFS ($P = 0.009$)	Statistically significant on Fisher's exact test Large cohort ($n = 84$)	Mixed age cohort No multivariate analysis No prospective validation
NFκB2 gene	21	Underexpression (expression microarray)	Prediction of recurrence ($P = 2.05 \times 10^{-5}$)	Statistically significant on PAM analysis Pediatric cohort exclusively analyzed	Very small cohort size ($n = 13$) No prospective validation Not identified in unsupervised or supervised hierarchical clustering of cohort gene expression
PLEK gene	21	Underexpression (expression microarray)	Prediction of recurrence ($P = 6.29 \times 10^{-5}$	Statistically significant on PAM analysis Pediatric cohort exclusively analyzed	Very small cohort size ($n = 13$) No prospective validation Not identified in unsupervised or supervised hierarchical clustering of cohort gene expression
LOC374491 gene	21	Overexpression (expression microarray)	Prediction of recurrence ($P = 8.08 \times 10^{-5}$)	Statistically significant on PAM analysis Pediatric cohort exclusively analyzed.	Very small cohort size ($n = 13$) No prospective validation Not identified in unsupervised or supervised hierarchical clustering of cohort gene expression

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Table 3. Statistically Significant Studies of Putative Prognostic Markers (Immunohistochemical and Genomic) for Pediatric Ependymoma (Cont'd)

Immunohistochemical Marker	Source	Prognostic Feature	Patient Outcome (*P* value)	Use as a Prognostic Marker in Pediatric Ependymoma	
				Strength	Weakness
Imbalance of aCGH BAC clones on chromosome 19	76	Loss of BAC clones on chromosome 19 (aCGH)	Increased incidence of recurrence ($P < 0.0001$)	Statistically significant on Fisher's exact test Pediatric cohort exclusively analyzed	Small cohort size ($n = 24$) No multivariate analysis No prospective validation
6q25 deletion	115	Presence of 6q25 deletion in anaplastic intracranial tumors (microsatellite markers)	Improved OS ($P = 0.013$)	Statistically significant in multivariate analysis Pediatric cohort exclusively analyzed.	Very small cohort size ($n = 15$) Mixed age cohort No prospective validation 6q loss feature of recurrent pediatric disease on CGH meta-analysis
Loss of *RAC2* (22q13) and amplification of *TPR* (1q25)	111	Loss of *RAC2* and amplification of *TPR* in pediatric intracranial tumors (qPCR)	Shorter OS ($P = 0.0492$ and $P < 0.0001$, respectively)	Statistically significant in multivariate analysis Pediatric cohort exclusively analyzed.	Small cohort size ($n = 47$) No prospective validation

NOTE: For studies analyzing the same markers in sequentially larger cohorts, the study analyzing the largest patient number is shown with reference to the smaller related series.

Abbreviations: OS, overall survival; EFS, event-free survival; BAC, bacterial artificial chromosomes; IHC, immunohistochemistry; ISEL, in situ end labeling; CART, classification and regression tree analysis; PAM, prediction analysis of microarrays. Mixed age, adult and pediatric (<16 y).

anaplasia requires the presence of necrosis, calcification, increased microvascular proliferation, mitotic activity, and cell density (1).

Although specific grades have been recognized, the reality of assigning such classifications to an ependymoma remains subjective (2). Benign, classic, and anaplastic morphology probably represent different points along a continuous pathological spectrum, whereas tumor heterogeneity may result in islands of anaplasia occurring within classic histological regions (Fig. 5C), causing uncertainty about the degree of focal anaplasia that represents true anaplasia. This is highlighted by the proportion of anaplastic ependymomas in reported series ranging from 7% to 89% (1) and a study of 34 ependymomas in which tumor grade was revised in almost a quarter of cases (16). Furthermore, the current criteria defining anaplasia has met with criticism from some neuropathologists who do not believe endothelial proliferation or necrosis reflect this tumor grade (102).

The inherent ambiguity present in the current histomorphological classification of ependymoma undoubtedly contributes to the contradictory results of studies investigating a prognostic role for tumor grading. Several studies have linked the morphological features of anaplasia to a poor outcome or suggested ependymoma grade affects survival postradiotherapy (1, 5, 13, 14, 27-31, 51). However other studies refuted this or failed to establish an association between anaplasia and prognosis, including those adopting a WHO classification system (reviewed in refs. 1, 4, 14, 22, 31). Moreover, studies analyzing the prognostic role of specific histological features such as necrosis, mitotic activity, cellularity, pleomorphism, and vascular proliferation, have yielded conflicting results (14, 31).

Recent attempts to reduce the prognostic ambiguity of ependymoma histology have included the meta-analysis of increased numbers of ependymomas using cooperative neuropathology reviews to reduce subjective interpretation bias. Tihan and colleagues found that WHO tumor grading was an independent prognostic factor for event-free survival, but not overall survival, in 96 pediatric posterior fossa ependymomas in which histology had been reviewed by three neuropathologists (18). In contrast, a panel of five European neuropathologists devised a novel histological grading scheme following consensus review of 233 intracranial ependymomas from children enrolled in four pediatric clinical trials (SFOP infant, CNS9204 infant, CNS9904, and AEIOP; ref. 103). Using the new scheme, tumors were divided into two grades: II and III. Cell density, nodularity, mitotic activity, and angiogenesis were considered important histological criteria for reclassification; however necrosis and cytological atypia were not. The group concluded that concordance on grading among the five pathologists improved significantly after devising this new classification scheme. Consensus about grade and the histopathologic variables analyzed was significantly associated with patient survival, but only in older children from one of the four clinical trials (AEIOP). Therefore the appropriate clinical setting for this scheme seems not to be ependymoma in children aged less than 3 years, but could be ependymoma in older children. An explanation for discrepant data between children in the AIEOP and CNS9904 trials was not given, although there was a significant difference between the proportions of tumors with a gross total resection in the two studies.

These recent studies suggest that undertaking large-scale histological reviews should be encouraged, feasible through national and international collaborations. Until consensus is reached on uniform classification criteria, potentially for different age categories within the pediatric population, the use of tumor grade as a prognostic marker in pediatric ependymoma will remain contentious.

Immunohistochemical and Genomic Markers

Several putative biological prognostic markers for ependymoma have been suggested using immunohistochemistry and genomic analysis on retrospective cohorts (Table 3; refs. 21,

32, 35, 36, 58, 59, 76, 78, 86, 104-129). However, only a few of these candidates have been analyzed in sufficient numbers of childhood ependymoma to allow consideration as prognostic markers in this age group. These include human telomerase reverse transcriptase (hTERT) and its nuclear chaperone Nucleolin (15, 32), members of the receptor tyrosine kinase 1 (RTK1) family and the Ki-67 labeling index (107, 110).

Telomerase functions as a homodimer and contains a catalytic component with reverse transcriptase activity (hTERT), which plausibly contributes to pediatric tumor development through failure of hTERT to be transcriptionally repressed (130). Characteristic features of neoplastic cells include the capacity for unlimited proliferation and the ability to avoid senescence, both of which require maintenance of telomere length (15). Telomeres are protective nucleoprotein structures that cap human chromosomal termini and contribute to genomic stability (131, 132). In normal somatic cells successive mitotic divisions result in telomere erosion, culminating in either replicative senescence or apoptosis (133, 134). In contrast telomere maintenance is present in almost all types of malignant cells, mediated by telomerase (135). The gene encoding hTERT is located on chromosome 5p13.3, a region found to be amplified in a proportion of intracranial ependymoma cases analyzed by aCGH (86).

An immunohistochemical analysis of 65 pediatric ependymomas seemed to implicate hTERT in prognosis, demonstrating positive staining for hTERT in 58% of cases and revealing hTERT expression as the strongest predictor of patient survival in multivariate analysis (126). However, re-evaluation of the antibody (NCL-hTERT) used to detect hTERT in this study has verified the actual target to be Nucleolin, a phosphoprotein that acts as a chaperone for hTERT during its transport from cytoplasm to nucleolus (136).

We have previously undertaken a retrospective immunohistochemical approach, independently validating several candidate biological prognostic markers, including Nucleolin, Ki-67 LI, RTK1 members, and Survivin in 80 pediatric intracranial ependymomas (32). We found that the only marker of prognostic significance was Nucleolin expression, as detected by NCL-hTERT and an independent monoclonal antibody (Ab13541 Abcam). Ependymomas exhibiting high Nucleolin expression were associated with a poor outcome when compared with tumors with low Nucleolin expression, with a 5-year event-free survival for patients of 31% versus 74%, respectively.

Through the analysis of 21 primary and recurrent pediatric ependymal tumors, we have further shown telomerase-induced telomere maintenance in more than 70% of cases, with telomere lengthening apparent in more than half of the relapsed tumors. However, neither telomerase activity, nor telomere length correlated with patient outcome, possibly owing to the small cohort size (32). This does not, however, exclude a prognostic role for hTERT expression in childhood ependymoma, because telomerase-induced telomere maintenance is present in most malignant cells (15), while telomere maintenance is a valuable prognostic marker in other CNS tumors (137, 138). The current technical issue of hTERT detection at an immunohistochemical level remains elusive.

The RTK1 family of proteins includes ERBB2, ERBB3, ERBB4, and epidermal growth factor receptor (EGFR) and is responsible for a variety of cellular processes such as division, motility, and survival. More recent data suggest ERBB2 overexpression may potentiate radial glia proliferation (33). Members of the RTK1 family have been proposed as potential prognostic markers in pediatric ependymoma. An immunohistochemical assay of 59 ependymal tumors revealed that ERBB2/ERBB4 coexpression, together with a raised Ki-67 tumor proliferation index, was associated with reduced pediatric patient survival. Interestingly, ERBB2/ERBB4 coexpression alone did not correlate with outcome (110). Support for EGFR as a prognostic marker comes from two studies analyzing mixed age cohorts. An immunohistochemical analysis of 46 ependymomas proposed EGFR as a relapse marker for low grade tumors, whereas frequent genomic gains and high-level amplifications covering the *EGFR* locus at 7p11.2 were noted by an aCGH study of 68 ependymomas and correlated with a poor prognosis (35, 86). In contrast, our extensive immunohistochemical analysis of pediatric ependymomas did not confirm the prognostic value of RTK1 markers for children. Low EGFR, ERBB2, and ERBB4 expression levels was evident in our cohort and did not correlate with patient survival (32). These findings suggest a possible difference in the mechanism of EGFR, ERBB2, and ERBB4 expression between pediatric and adult ependymomas, reinforcing the notion that these two subgroups are distinct genetic entities and implying that anti-RTK1 therapy may not prove efficacious at a young age. The current Pediatric Brain Tumor Consortium trial of Lapatinib (GW572016), a dual inhibitor of EGFR and ERBB2 receptor signaling for children with CNS tumors, may help to resolve the latter concern.

Ki-67 protein is expressed in proliferating cells but is absent in nondividing cells (139). The Ki-67 labeling index (Ki-67 LI) has been identified as a putative prognostic marker in two large studies of pediatric ependymoma (107, 110). The threshold used in these studies to define prognostic groups was above 25%. This could explain why other large pediatric studies, using lower Ki-67 LI prognostic cutoffs, found no association between this marker and patient survival (32, 127). Nevertheless Ki-67 has been associated with survival in small pediatric series using very low LI thresholds of 1% and 7% (108, 128, 129), making definitive conclusions on its use as a pediatric prognostic marker difficult. Moreover Ki-67 has been shown to correlate with tumor grade, supporting the view that increased proliferation seems to be a feature of anaplasia (32, 140-142).

Our CGH meta-analysis has revealed 1q gain as the most common genomic imbalance in both primary and recurrent pediatric ependymomas (Figs. 1 and 3), justifying its further assessment as a prognostic marker in children. The gain of chromosome 1q is associated with an unfavorable outcome in Wilms' tumors, neuroblastomas, and Ewing's sarcomas, which suggests a generic role in pediatric tumor progression and recurrence (143-145). Conventional cytogenetics suggests that 1q gain results from a variety of unbalanced rearrangements with material from numerous partner chromosomes rather than a single, recurrent translocation (43, 44). Therefore, the accumulation of genomic material on 1q could be more important for initiating and propagating tumor growth than the corresponding loss from translocated partner chromosomes.

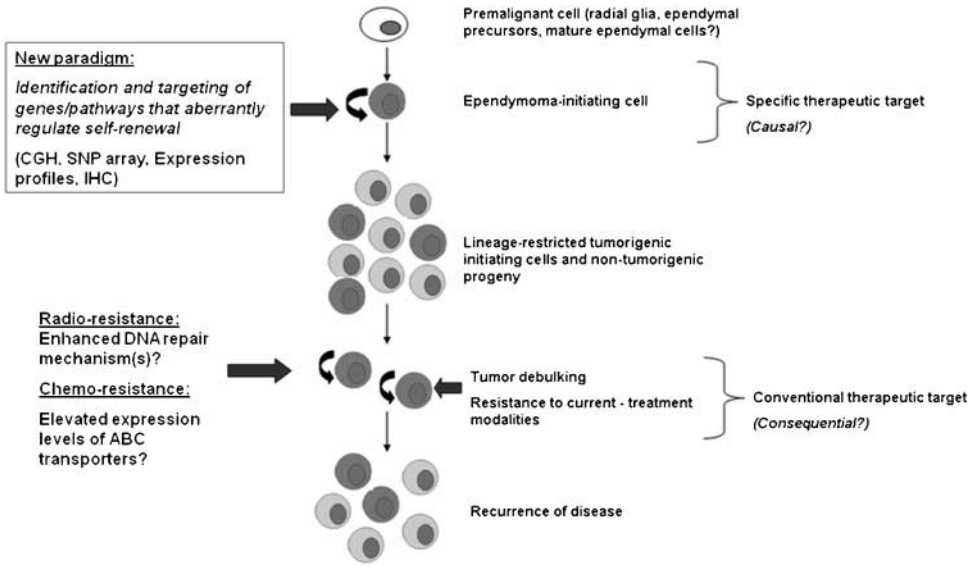

FIGURE 6. Tumor resistance and targeted therapeutics in ependymoma. Contemporary evidence shows that ependymoma cells share gene expression signatures that closely resemble radial glia, making the latter strong candidates for the ependymoma premalignant cell upon which the initial transformation event at tumor birth occurs. It is unknown at present whether more committed progenitors or mature ependymal cells can initiate tumors directly *in vivo* or whether they can revert to an immature stem-like precursor with tumor-initiating capacity. Conventional cancer therapeutics, generally focused on eradicating rapidly proliferating cells, achieve tumor debulking but do not prevent recurrence of disease in most cases. The ependymoma stem cell hypothesis postulates that the tumor-initiating subpopulation will have increased defense and repair mechanisms to counteract genotoxic and cellular stress. In this regard, a new generation of ependymoma treatment modalities must aim to identify and target molecular pathways aberrant exclusively in tumor-initiating cells.

Several ependymoma genomic studies associate gain of region 1q21–32 with an anaplastic histology and an adverse prognosis (58, 59, 86). Specifically, an aCGH study of 68 ependymomas showed that gain of the 1q25 locus as detected by FISH represented an independent prognostic marker for both recurrence free and overall survival, although the cohort analyzed included both adults and children (86). The same analysis also identified a particular area of recurrent gain at 1q23.3, a locus that contains the gene *DUSP12*. This gene is thought to have a role in cell proliferation and therefore has been proposed as a potential candidate oncogene. Gene expression analyses have shown the upregulation of additional genes located within 1q21–32, including *laminin* (1q31), *PRELP* (1q32), *HSPA6* (1q23), *GAC1* (1q32), members of the S100 family (1q21.3), and *CHI3L1* (1q32.1; refs. 114, 146-148). *GAC1* amplification has already been implicated in the pathogenesis of malignant gliomas (148). *CHI3L1* upregulation may be involved in pediatric intracranial ependymoma recurrence, whereas its corresponding protein expression has been correlated with tumor necrosis (147). These genes and the locus 1q25 merit further investigation with high resolution genomic techniques and gene expression studies that analyze larger numbers of pediatric ependymomas as an independent cohort. These methods could also establish the prognostic role of genes such as *NRXN2, TPR, SEMA5, NRCAM, CDK4*, and *ADRM1* that have already been associated with outcome in small studies of pediatric patients with intracranial ependymoma (83, 112), or examine other regions of genomic imbalance potentially harboring markers of disease progression and prognosis in children including the loss of chromosomes 22q and 6q.

Implicated genes on chromosome 22q other than *NF2* are probable because its deletion seems to have no role in the pathogenesis of intracranial ependymoma that predominates in children. Studies of non-NF2 familial ependymomas support this, identifying regions of loss distinct from the *NF2* locus such as 22q11.2 (149, 150), a region already identified in other ependymoma genomic studies (151, 152). The tumor suppressor gene *hSNF5/INI1* is located here (153, 154). However, despite analyzing more than 200 ependymomas, no mutation of this gene has been found nor does it seem to be silenced by methylation (155), making it an unlikely candidate.

The region 22q13.3 has also been highlighted as a frequent region of genomic loss in ependymoma (76, 156, 157). Gene expression analysis within this locus has revealed downregulation of *SULT4A*, a gene widely expressed in several compartments of the human brain (76, 77, 148). Reduced expression of other genes within 22q13.1–13.33 has been observed, including *CBX7, G22P1*, and *MCM5*, which may be involved in gene silencing, DNA repair, and cell proliferation, respectively (77, 148). Real-time quantitative PCR of 47 pediatric intracranial ependymomas revealed frequent loss of *C22orf2* and *RAC2*, the latter being associated with reduced overall survival in the cohort (112).

Deletion of chromosome 6q23 has been associated with a worse event-free survival in a mixed age ependymoma cohort, warranting further analysis of this locus for prognostic gene markers (78). 6q24–26 also seems a region of frequent genomic loss in ependymomas (63, 156). Expression analysis of genes within this latter region has revealed underexpression of *SASH1*, a gene whose deletion has been reported in breast cancer, and *TCP1*, which is involved in a variety of cellular processes via the production of the protein tubulin. The underexpression of genes mapping to 6q21 has also been observed

including *ADM1* and *CDK11*, two genes involved in cell proliferation (77, 148).

Unfortunately, most biological studies of ependymoma prognostic markers to date either involve small retrospective series or analyze children and adults collectively, often using nonstandardized immunohistochemical criteria to define prognostic subgroups (Table 3). For instance, Korshunov and colleagues reported on the immunohistochemical expression of various biological markers in 112 patients with intracranial ependymomas (35). The results reinforced conclusions drawn from two preceding prognostic studies assessing these markers in smaller series (113, 114). They found that for low grade tumors, a reduced PFS time was associated with increased expression of topoisomerase II-α, tenascin, VEGF, and EGFR. For high grade tumors, the PFS was shortened for increased p53 expression and reduced labeling indices for apoptosis, p27, and p14ARF. The cohort analyzed in this study comprised 60 adults and 52 children. However, as the results were not reported separately for each age group, it is not possible to determine the role of tenascin, topoisomerase II-α, p53, and p14ARF as biological correlates of outcome for childhood ependymoma. Furthermore, FISH analysis of 84 ependymomas found that neither deletion of p14ARF's encoding gene *CDKN2A*, nor the *RB* tumor suppressor gene on chromosome 13q correlated with patient survival (121). Nevertheless, these markers should not be disregarded. Tenascin is a glycoprotein responsible for producing gliogenic precursors and has been a target for radioimmunotherapy in brain tumors (65, 158). The tenascin c (TNC) gene on chromosome 9 seems upregulated in infant ependymomas (76), and its protein expression seems relatively high in pediatric cases (35), whereas the presence of intercellular tenascin has been identified as an adverse prognostic marker in an albeit small immunohistochemical study of pediatric ependymomas (129). The authors also found that unfavorable outcome was associated with increased expression of topoisomerase II-α, bcl2, and cyclin D1 and p53 (128). Analysis of a further mixed age cohort provides support for aberrant p53 expression as a prognostic marker in ependymoma (127), although *p53* gene mutations in pediatric ependymomas are rare (159-162), whereas its regulator MDM2 has failed to establish a prognostic role in this tumor group (114).

The identification of biological correlates of clinical outcome for pediatric ependymoma from a retrospective population is an important finding. However, verification within an independent cohort treated in a homogenous manner is required, ideally within the setting of a clinical trial. Moreover, the detection method for any identified marker must be rapid, robust, standardized, and easy to interpret to allow diagnostic laboratories to identify patients suitable for a particular therapeutic proposal. At present, published prospective validation is lacking for pediatric ependymoma. This must be encouraged by large national and international collaborative studies to allow multivariate analysis of recognized and novel putative prognostic markers and consequently to develop new therapeutic strategies.

Therapeutic Implications for Research

The cancer stem cell model permits a conceivable explanation of tumor recurrence in ependymoma. If only a minority of cells within the tumor is tumorigenic, conventional approaches would be expected to remove bulk (nontumorigenic) tumor mass, but crucially fail to eradicate the tumor-initiating population(s) that may be inherently resistant to current therapies. There is evidence to substantiate this, hinting at enhanced defensive attributes of the tumor stem cell (Fig. 6). Pediatric glioblastoma stem cells display resistance to radiation because of preferential activation of the DNA damage checkpoint (163, 164), whereas xenograft models have attributed varying radiation resistance between ependymomas to possible inherent differences in p53 mediated growth arrest (160). Overexpression of the DNA repair protein and chemosensitivity marker MGMT in a small series of recurrent pediatric ependymomas suggests a possible implication in anaplastic ependymoma chemoresistance (165). Additionally, distinct populations of forebrain neural stem and progenitor cells can be isolated on the basis of their ability to efflux the fluorescent dye Hoechst 33342, indicative of high expression of ATP-binding cassette (ABC) drug transporters (166, 167). This finding supports the hypothesis that brain tumor stem cells have the potential to express high levels of ABC transporters and that this may represent a mode of action for chemoresistance via the efflux of cytotoxic agents (Fig. 6). Indeed, a recent report has shown increased resistance of CD133+ glioblastoma cells in response to treatment with chemotherapeutic agents such as temozolomide, carboplatin, paclitaxel (Taxol), and etoposide (VP16; ref. 168), suggesting this may also be a method of resistance worth analyzing in ependymoma. The next generation of chemotherapeutic drugs may include a cocktail of chemosensitizers and cytotoxic agents that alter ABC-transporter activity. Further studies will also be required to elucidate whether the (tumor-initiating) cell conferring chemoresistance is the same (tumor-initiating) cell that confers radioresistance.

In this regard, conventional therapeutic protocols have been targeting the result of dysregulated expansion of the tumor-initiating cell rather than the causal cell itself (Fig. 6). Future ependymoma therapeutic targets will require a better understanding of ependymoma stem cell biology and of signaling pathways that control self-renewal. The refinement of methods to prospectively and exclusively identify and isolate ependymoma-initiating cells, coupled with advances in genome-wide association methodology and RNA expression profiling, may herald a new research focus that targets tumorigenic, therapy-resistant cells. Identification of DNA alterations and gene expression levels that differ not only from normal tissue stem cell counterparts (such as radial glia), but nontumorigenic ependymoma cells, will provide evidence about what cancer mutations occur upstream of canonical oncogene and tumor suppressor activation and inactivation, respectively. These are likely to be mutations that cause deregulation of self-renewal mechanisms in ependymoma, the likely candidates of which are the Wnt, Notch, and Sonic Hedgehog pathways. A modest number of patients with Turcot syndrome Type 2 (TS2) develop intracranial ependymomas and are known to have disruptions to the Wnt/Wingless pathway, including mutations in *APC* and *CTNNB1*. However a study of 77 sporadic ependymomas, including 23 pediatric tumors, failed to find mutations in *APC* and *CTNNB1*, which suggests other pathway components are

implicated (169). Maintenance of self-renewal and multipotentiality in adult neural stem cells has been shown through upregulation of Jagged 1, the ligand of Notch (170), whereas *Notch 1* deletion depletes this stem cell fraction (171). In addition, activation of *Notch 1* in the subventricular zone of the forebrain of embryonic mice promotes the maintenance of radial glial identity (172). Inhibitors of γ secretase, an enzyme that mediates the activation of the Notch pathway, could soon be introduced as a trial drug in children with supratentorial ependymomas, whereas the contribution of aberrant Notch activation and deletion of *CDKN2A* (p14ARF) to transformation of radial glia into ependymal tumor stem cells, as recognized by Taylor and colleagues, warrants further investigation (34). Mouse model work has also shown that small molecule inhibitors of the Sonic Hedgehog Pathway can restrict the growth of medulloblastomas (173). This finding may prove fruitful for use in pediatric ependymomas as a proportion of tumors exhibit upregulation of this pathway, although initial assessment using ependymoma cell lines is necessary. Novel targets arising from basic research of this sort may result in adjuvant therapies that involve agents targeting upstream alterations in tumor-initiating cells, in conjunction with agents that have consistently been shown to eradicate bulk nontumorigenic progeny.

Our knowledge of the biology of ependymoma and its role in tumor development and progression in children has advanced significantly in the past decade, allowing some insight into the heterogeneous behavior of this tumor. This work demands continued progress if we are to achieve our essential goal of improving pediatric patient survival on the basis of this understanding of the underlying biology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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