Neuronal differentiation distinguishes supratentorial and infratentorial childhood ependymomas

Felipe Andreiuolo, Stéphanie Puget, Matthieu Peyre, Carmela Dantas-Barbosa, Nathalie Boddaert, Cathy Philippe, Audrey Mauguen, Jacques Grill, and Pascale Varlet

Ependymomas are glial neoplasms occurring in any location throughout the central nervous system and supposedly are derived from radial glia cells. Recent data suggest that these tumors may have different biological and clinical behaviors according to their location. Pediatric supratentorial and infratentorial ependymoma (SE and IE) were compared with respect to clinical and radiological parameters and immunohistochemistry (IHC). Neuronal markers were specifically assessed by IHC and quantitative PCR (qPCR). No single morphological or radiological characteristic was associated with location or any neuronal marker. However, there was a significant overexpression of neuronal markers in SE compared with IE: neurofilament light polypeptide 70 (NEFL)-positive tumor cells were found in 23 of 34 SE and in only 4 of 32 IE ($P < .001$). Among SE, 10 of 34 exhibited high expression of NEFL, defined as more than 5% positive cells. qPCR confirmed the upregulation of neuronal markers (NEFL, LHX2, FOXG1, TLX1, and NPTXR) in SE compared with IE. In addition, strong NEFL expression in SE was correlated with better progression-free survival ($P = .007$). Our results support the distinction of SE and IE. SEs are characterized by neuronal differentiation, which seems to be associated with better prognosis.

Keywords: child, ependymoma, location, neurofilament, supratentorial.

Ependymomas represent the third most common intracranial tumors in children and are defined as neoplasms exhibiting glial and/or epithelial morphology. Only 25%–35% of them occur in a supratentorial location. According to the WHO 2007 classification, histological variants include classic, cellular, clear cell, papillary, tanycytic, and myxopapillary ependymoma. In the supratentorial compartment, tanycytic or myxopapillary variants are exceptional. The current WHO 2007 classification distinguishes grade II from grade III, which does not accurately predict clinical outcome. The extent of surgery remains the most important prognostic indicator, although children with supratentorial ependymoma (SE) seem to have a better outcome.

Ependymomas are neoplasms thought to originate from the ependymal layer of the entire ventricular system. Therefore, SE may develop in the third or lateral ventricles, but also without direct adhesion to the ventricular system, in the white matter, and some rare cases of ependymomas have even been referred to as “cortical” in the literature. Ependymomas are morphologically similar in every CNS location but seem to display distinct chromosomal imbalances or genomic abnormalities. Interestingly, recent comparative gene expression profiles support the idea that pediatric ependymomas exhibit the patterns of gene expression recapitulating those of radial glia cells in the corresponding CNS regions. As radial glia cells are now considered neural stem cells and as they give rise to mature...
ependymal cells, it is thus possible to hypothesize that
tumor cells forming ependymomas may not only
express glial markers but may rarely exhibit differen-
tiation along neuronal lines.

The aim of the present study was to compare SE and
infratentorial ependymoma (IE) by gene expression
studies and immunohistochemistry (IHC), with empha-
sis on neuronal expression and/or differentiation.

Material and Methods

Pathology Review

We reviewed the pathological features of 43 SE and 32
IE resected from children in the years from 1994 to
2007 at the Necker Enfants Malades Hospital.
Subependymomas, myxopapillary ependymomas, and
ependymoblastomas were excluded. Slides from all par-
affin blocks were diagnosed and graded according to the
WHO 2007 classification by 2 neuropathologists (P.V.
and F.A.). The following histological characteristics
were evaluated: ependymal rosettes, perivascular pseudo-
rosettes, number of mitotic figures per 10 high-power
fields, cellular density, necrosis, and endothelial
proliferation.

After histological review, 9 cases were excluded: 1
atypical teratoid/rhabdoid tumor (reclassified on the
base of the loss of nuclear INI1 expression), 2 papillary
glioneuronal tumors, and 3 gangliogliomas with ependy-
moda as the glial component. Three ependymomas
were excluded because the residual tissue for com-
plementary IHC studies was insufficient. The remaining
34 SEs were separated into subcategories: classic (n =
27), clear cell (n = 3), papillary (n = 3), and tanycytic
(n = 1; Fig. 1A–D). These were compared with a

Fig. 1. Histological variants and IHC findings in SE: (A) classic ependymoma histology; (B) clear cell ependymoma; (C) papillary
ependymoma; (D) tanicytic ependymoma; (E) GFAP immunostaining showing a classical perivascular enhancement; and (F) EMA
staining, with positivity in dots, some cases showing an apical cell-membrane staining in true rosettes (insert). Original magnifications:
×200 (A, B, and E), ×100 (C), and ×400 (F).
group of 32 IEs from posterior fossa, separated as follows: classic (n = 30) and clear cell (n = 2).

Clinical Characteristics of the Population

Relevant clinical and follow-up data were obtained from patients’ records or eventually by contacting patients’ practitioners. Extent of surgical resection was assessed from the surgeon’s report and immediate postoperative contrast-enhanced CT scan or on magnetic resonance imaging (MRI) performed before adjuvant therapy. A recurrence was defined as a new lesion that appeared in situ, after gross total resection. Tumor progression was defined as an enlargement of a residual tumor. Progression-free survival (PFS) was defined as time from first surgery to recurrence or progression. Overall survival (OS) was calculated from the time of first surgery to recurrence or progression. Dead includes in supratentorial and infratentorial control groups are shown in Table 1.

These patients were treated according to age groups. After surgery, children younger than 5 years received chemotherapy. In the case of relapse, they were reoperated and received focal radiotherapy (50–55 Gy). Children older than 5 years received postoperative radiotherapy. At relapse, these patients received chemotherapy after a reoperation when feasible. Adjuvant therapy was used for all patients who had an incomplete resection.

IHC Analysis

Four-micrometer sections were deparaffinized and subjected to microwave antigen retrieval for 30 minutes at 98 °C. After blocking of nonspecific endogenous peroxidase by H2O2 and nonspecific antibody-binding sites, sections were incubated with one of the following primary antibodies: MIB-1 (1/10, Zymed), antineuronal nuclei (NeuN) (1/500, clone VMA377, Abcys), antineurofilament light polypeptide 70 (NEFL) (1/50, clone 2F11, Dako), antichromogranin A (1/75, clone LK2H10, Immunotech), antisympathophysin (1/50, clone SY38, Progen), antigial fibrillary acidic protein (GFAP) (1/200, clone 6F2, Dako), antionigoloidendrocyte transcription factor 2 (Olig2) (1/25, polyclonal goat anti-human, R&D), antiepithelial membrane antigen (EMA) (clone E29, 1/1, Dako), and anti-INI1 (1/50, clone BAF47, BD Biosciences) for 1 hour at room temperature. The reaction was revealed using the diaminobenzidine chromogen (kit DAB K3468, Dako).

To evaluate neuronal differentiation, we examined the expression of a panel of 4 immunomarkers: NEFL, chromogranin A, synaptophysin, and NeuN, besides the proliferation index MIB-1 and the oligodendrocyte lineage marker Olig2. Immunostains for GFAP (Fig. 1E), EMA (Fig. 1F), and INI1 were performed in some cases to confirm the diagnosis of ependymoma. For NEFL immunostaining, a semiquantitative analysis was used with a staining score scale: score 0, negative in all blocks containing a viable tumor, including sonic aspiration specimens; score 1, positive in <5% tumor cells; and score 2, positive in >5% of tumor cells. The MIB-1 proliferation index was scored as a percentage of positive cells (as of most positive areas, total 200 cells counted per area).

Quantitative PCR

For quantitative PCR (qPCR), the following genes involved in neurogenesis/neuronal differentiation were selected based on the literature review: NEFL, T-cell leukemia homeobox 1 (TXL1), LIM homeobox protein 2 (LHX2), forkhead box G1B (FOXG1), neuronal pentraxin receptor (NPTXR), reelin (RLN), tenasin C

### Table 1. Clinical and IHC characteristics of childhood ependymomas

<table>
<thead>
<tr>
<th></th>
<th>Supratentorial tumors (34)</th>
<th>Infratentorial tumors (32)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at diagnosis (yrs)</td>
<td>6.35 (0.2–14.9)</td>
<td>4.6 (0.6–12.6)</td>
<td>.08</td>
</tr>
<tr>
<td>Sex</td>
<td>16 M, 18 F</td>
<td>13 M, 18 F</td>
<td>NS</td>
</tr>
<tr>
<td>Gross total resection</td>
<td>26 (76%)</td>
<td>22 (69%)</td>
<td>NS</td>
</tr>
<tr>
<td>Subtotal resection</td>
<td>8 (23%)</td>
<td>10 (31%)</td>
<td>NS</td>
</tr>
<tr>
<td>Radiotherapy/chemotherapy</td>
<td>8 (23%)/12 (35%)</td>
<td>13 (40%)/19 (60%)</td>
<td>NS</td>
</tr>
<tr>
<td>Histological grade</td>
<td>4 II, 30 III</td>
<td>5 II, 27 III</td>
<td>NS</td>
</tr>
<tr>
<td>NEFL expression</td>
<td>23 (67.6%)</td>
<td>4 (12.5%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>NeuN expression</td>
<td>14 (41.2%)</td>
<td>8 (25%)</td>
<td>NS</td>
</tr>
<tr>
<td>Synaptophysin expression</td>
<td>6 (18%)</td>
<td>3 (9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Chromogranin expression</td>
<td>10 (32%)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Olig2 expression</td>
<td>22 (64.7%)</td>
<td>29 (90.6%)</td>
<td>.018</td>
</tr>
<tr>
<td>Recurrence/progression</td>
<td>19 (56%)</td>
<td>21 (70%)</td>
<td>NS</td>
</tr>
<tr>
<td>Progression-free survival (2/5 yrs)</td>
<td>55% /46.5%</td>
<td>45.3% /27.9%</td>
<td>NS</td>
</tr>
<tr>
<td>Overall survival (5/10 yrs)</td>
<td>68.3% /54.7%</td>
<td>58% /33.7%</td>
<td>NS</td>
</tr>
<tr>
<td>Dead</td>
<td>10 (28.6%)</td>
<td>16 (50%)</td>
<td>NS</td>
</tr>
<tr>
<td>Follow-up (yrs)</td>
<td>4 (1–14)</td>
<td>4.2 (0.8–14)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: M, male; F, female; NS, not significant; NEFL, neurofilament light polypeptide 70; NeuN, neuronal nuclei; Olig2, oligodendrocyte transcription factor 2.
(TNC), and NOTCH1. RNA was extracted from 10 SE and 11 IE snap-frozen fresh samples using the QIAGEN Microkit (Qiagen). Approximately 1 μg of total RNA was used to synthesize cDNA using random hexamers and the SuperScript Vilo kit (Invitrogen). qPCR was carried out using Taqman Gene Expression Assays on Demand (Applera) and ABI Prism 7700 Sequence Detector (Applied Biosystems). Expression profile in each specimen was assessed by using the comparative threshold cycle \(2^{-\Delta\Delta CT}\) method. The TBBP gene was used as an endogenous control and normal whole brain RNA (Stratagene) as a calibrator, as shown previously.9

**Imaging Analysis**

Radiological features were assessed by a pediatric neuroradiologist (N.B.) and 2 neurosurgeons (S.P. and M.P.) who were blinded to the histopathological data and outcome. Preoperative MRI scans were available for all patients.

The following image features were analyzed: location, edema, gadolinium enhancement, and ventricular contact on MRI sequences.

**Statistical Analysis**

The chi-square test was performed for binomial procedures concerning location, histological features, and radiological presentation. The nonparametric Mann–Whitney rank-sum test was also performed to test the differences between the groups, and the Kaplan–Meier analyses were performed for survival data using the log-rank test. The level of significance was \(P < .05\). Analyses were performed using SPSS 16.0 for windows.

**Results**

**Radiological Features**

Tumors were divided into 3 groups according to their imaging features on MRI. The most important radiological group included 18 giant tumors with multiple solid and cystic components extending to more than 1 cerebral lobe. The second group consisted of 11 smaller lesions with a deep cyst and a superficial solid component; 6 were located in the frontal lobe and 5 in the parietal lobe. Seven had no contact with the ventricles. The remaining 5 tumors were located in the midline.

Contrast enhancement was present in all solid components of the tumors regardless of the tumor radiological group. In the second group, thin and often weak contrast enhancement of the margins of the cyst was present in all cases. Peritumoral edema was present in 9 tumors, mostly in the giant tumor group (8 of 9) but also around the cystic component of 1 tumor of the second group. Calcifications were present in 5 of 11 tumors for which CT scans were available. Particular radiological subtypes were not associated with OS and the expression of neuronal markers.

**Outcome**

Results of clinical outcome for patients are shown in Table 1. As in previous reports, the only significant clinical variable for survival in SE was the extent of surgical resection with better OS \(P = .026\) and PFS \(P < .0001;\) Fig. 2). Tumor grade, location, and patients' age and sex were not significant prognostic factors in SE.

**Histopathological and IHC Findings**

After review, the majority of both SE (30 of 34) and IE (27 of 32) were classified as grade III (Table 1). The
immunoexpression of NEFL, NeuN, Olig2, chromogranin A, and synaptophysin is reported in Table 1. The expression rates of NEFL and chromogranin A were statistically associated with ST location ($P < .0001$ for both; chi-square test), whereas the expression of Olig2 was associated with the location in posterior fossa ($P = .018$, chi-square test). The NEFL score in the supratentorial group was established as low or negative for 24 tumors (score 0: $n = 12$ and score 1: $n = 12$) and as high (score 2) for 10 tumors (Fig. 3, Table 2). Immunoexpression of NEFL was associated with a better PFS at 5 years, 57.8% and 30% for the groups expressing and not expressing NEFL, respectively, but it did not reach statistical significance. Among children with tumors that expressed NEFL, survival correlated with the scoring. Indeed, an NEFL score of 2 was associated with a better OS ($P = .10$, log-rank test) and was a significant predictor of good PFS ($P = .009$, log-rank test; Table 2, Fig. 4). There was no association between NEFL expression and the quality of surgical resection, radiological features, or age. We found an association between the positive expression of NEFL and Olig2, chromogranin A, and synaptophysin (chi-square test, $P = .038$, .008, and .04, respectively). The median MIB-1 index was 10% among IE and 23% among SE ($P = .003$).

Quantitative PCR

The genes $NEFL$, $LHX2$, $FOXG1$, $TLX1$, and $NPTXR$ were markedly overexpressed in SE compared with IE, whereas $TNC$ and $RELN$ were overexpressed in IE. $NOTCH1$ was expressed equally in IE and SE (Fig. 5).
Discussion

Our study shows that childhood SEs often exhibit neuronal differentiation in the form of immunexpression of neuronal markers such as NEFL and chromogranin. Except for 1 recent study, which reported 6 SE and IE in children with immunophenotypic neuronal differentiation,16 little is known about neuronal differentiation in ependymomas, and to the best of our knowledge no previous data from a large pediatric cohort are actually available. Neuronal differentiation within typical ependymoma implies that such tumors may histogenetically originate from a glioneuronal progenitor rather than from a committed glial progenitor.12,17 Interestingly, tumors showing definite morphological features of neuronal differentiation have been reported and support the existence of a neuronal differentiation spectrum in ependymal tumors.16,18,19 We excluded from this series 3 published cases of gangliogliomas with ependymal differentiation, which we consider to be a different entity. They exhibit benign behavior and display important perivascular inflammation, granular bodies, and often binucleated ganglion cells.13 However, it could be hypothesized that these may represent terminal differentiation of neuronal precursors in ependymomas, which is an established phenomenon in medulloblastomas and other primitive neuroectodermal tumors.20,21

Expression of NEFL and chromogranin was strongly correlated with supratentorial location, which supports the suggestion that SE and IE are different entities, in view that there are molecular differences between ependymomas according to the location.8,9,22 Although this hypothesis is based on a limited number of SE, our studies support this idea CGH array showing a gain of 9q33-34 is significantly more frequent in IE than in SE.10

Strong expression of NEFL in SE was significantly associated with a better PFS. Classically, the infratentorial location is associated with a worse outcome in most,6,23,24 but not all, studies.25 The predominance of neuronal markers in SE, particularly NEFL, may be related in some as yet undefined manner to the different behavior of SE and IE, as it has been shown that a neuronal molecular signature is associated with better prognosis in high-grade gliomas.26,27

In our series, RELN was significantly overexpressed in IE compared with SE, confirming earlier studies.8 RELN is implicated in cell-fate decision as it can induce a radial glial cell phenotype in neural stem cell progenitors via the activation of NOTCH.28 Similar to RELN, TNC is an extracellular matrix protein also

### Table 2. Clinical and IHC characteristics of childhood SEs according to NEFL expression

<table>
<thead>
<tr>
<th></th>
<th>NEFL High (10)</th>
<th>NEFL Low/negative (24)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at diagnosis (mos)</td>
<td>79.15</td>
<td>61.27</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>6 M, 4 F</td>
<td>10 M, 14 F</td>
<td>NS</td>
</tr>
<tr>
<td>Gross total resection</td>
<td>9 (90%)</td>
<td>17 (71%)</td>
<td>NS</td>
</tr>
<tr>
<td>Subtotal resection</td>
<td>1 (10%)</td>
<td>7 (29%)</td>
<td>NS</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>4 (40%)</td>
<td>4 (17%)</td>
<td>NS</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>2 (20%)</td>
<td>10 (42%)</td>
<td>NS</td>
</tr>
<tr>
<td>Progression-free survival (3 yrs)</td>
<td>90%</td>
<td>28%</td>
<td>.007</td>
</tr>
<tr>
<td>Overall survival (5 yrs)</td>
<td>90%</td>
<td>60%</td>
<td>.14</td>
</tr>
<tr>
<td>NeuN expression</td>
<td>6 (60%)</td>
<td>12 (50%)</td>
<td>NS</td>
</tr>
<tr>
<td>Synaptophysin expression</td>
<td>3 (30%)</td>
<td>3 (12%)</td>
<td>.041</td>
</tr>
<tr>
<td>Chromogranin expression</td>
<td>6 (60%)</td>
<td>4 (17%)</td>
<td>.008</td>
</tr>
<tr>
<td>Olig2 expression</td>
<td>9 (90%)</td>
<td>14 (58%)</td>
<td>.038</td>
</tr>
<tr>
<td>MIB-1a index (&lt;30%; &gt;30%)</td>
<td>6 (66.7%)/3 (33.3%)</td>
<td>16 (80%)/4 (20%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: NEFL, neurofilament light polypeptide 70; M, male; F, female; NS, not significant; NeuN, neuronal nuclei; Olig2, oligodendrocyte transcription factor 2.

*The MIB-1 proliferation index was performed for 29 of 34 patients.*

![Fig. 4. PFS in months for SE, according to neurofilament light polypeptide (NEFL) immunostaining—strong vs weak/no staining.](image-url)

**NEFL-score**

- 0 + 1
- 2
- 0 + 1-censored
- 2-censored

Cum survival

PFS (mos)

MIB-1a index (<30%; >30%)

6 (66.7%)/3 (33.3%)

16 (80%)/4 (20%)

P=0.009

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implicated in radial glial cell phenotype and which has recently been described as a target gene for NOTCH in gliomas.²⁹ Moreover, TNC is located on the same chromosomal region as NOTCH1.¹⁰ The TNC mRNA levels were higher in IE compared with SE, and both were higher than those in normal brain. However, NOTCH1 was not differentially expressed by SE and IE, according to the previous reports from our group.¹⁰ This suggests that NOTCH1 expression is driven differently according to the location of the ependymoma.

TLX1 was overexpressed in SE compared with IE. TLX1 participates in early differentiation of the mammalian nervous system and sensory ganglia¹⁰ and is a neural oncogene; some alternative transcripts of this gene have been implicated in the genesis of pediatric neural tumors such as neuroblastoma and primitive neuroectodermal tumor of the CNS.³¹ The nuclear receptor TLX has also been implicated in neural stem cell self-renewal.³² LHX2 was significantly overexpressed in SE compared with IE, and as previously suggested might be related to tumor location.³³ LHX2 is a transcriptional factor involved in brain development/neural stem cell differentiation and patterning of early telencephalon, which is overexpressed in both SE and supratentorial pilocytic astrocytoma. FOXG1 is involved in neurogenesis in the retina and the maintenance of neural stem cell phenotype, patterns early telencephalon,³⁴ and was also significantly overexpressed in SE when compared with IE. NPTXR is another gene of neuronal differentiation we found to be overexpressed in SE, which codes for an integral membrane protein, a neuronal synaptic receptor with higher expression in Purkinje and granule neurons of the cerebellum, also present in the hippocampus and cerebral cortex.³⁵

Taken together our data and the literature show that SE and IE have different genomic, gene expression, and IHC signatures. Different oncogenic pathways may be involved depending on the location, driving to a neuronal phenotype in SE. The better prognosis of children with SEs may be partly related to their neuronal differentiation.
Acknowledgments

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Conflict of interest statement. None declared.

References


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