Posterior fossa ependymoma: current insights

Yuan Y. Thompson[1,2] · Vijay Ramaswamy[1,2,3] · Phedias Diamandis[2] · Craig Daniels[1] ·
Michael D. Taylor[1,2,4]

Abstract

Background Ependymoma is the third most common malignant tumor of the posterior fossa and is a major cause of neurological morbidity and mortality in children. Current treatments, particularly surgery and external beam irradiation result in relatively poor outcomes with significant neurological and cognitive sequelae from treatment. Historical approaches have considered all ependymomas as similar entities based on their morphological appearance.

Results Recent advances in genomics and epigenetics have revealed, however, that ependymomas from different CNS locations represent distinct entities. Moreover, ependymoma of the posterior fossa, the most common location in children, is actually comprised of two distinct molecular variants. These two variants have marked differences in demographics, transcriptomes, structure, methylation patterns, and clinical outcomes. This allows for the development of new biology-based clinical risk stratification, which can both prioritize patients for de-escalation of therapy and identify those who will benefit from novel therapeutic strategies. Indeed, the identification of these two variants allows an opportunity for robust preclinical modeling for development of novel therapeutic strategies.

Conclusions Herein, we have summarized our current clinical approach to diagnosis and treatment of posterior fossa ependymoma, recent advances in understanding the biology of posterior fossa ependymoma and how these new insights can be translated into the clinic to form the basis of the next generation of clinical trials.

Keywords Ependymoma · Posterior fossa · Genomics

Yuan Y. Thompson and Vijay Ramaswamy contributed equally to this article.

✉ Michael D. Taylor
  mdtaylor@sickkids.ca

1   Developmental and Stem Cell Biology Program, Arthur and Sonia Labatt Brain Tumor Research Centre, The Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G1X8, Canada

2   Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

3   Division of Hematology/Oncology, Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G1X8, Canada

4   Division of Neurosurgery, Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G1X8, Canada

Introduction

Ependymoma is the third most common malignant pediatric brain tumor and can occur anywhere along the central nervous system (CNS) [1]. In children, the most common location is in the posterior fossa, followed by the cerebral hemispheres. Posterior fossa ependymoma has, over the past decade, been the subject of intensive investigation as it is a common cause of morbidity and mortality in children. In addition, over the past 10 years all patients with posterior fossa ependymoma have increasingly been treated in an identical manner, whereby conformal radiation is now the standard of care for all pediatric patients in North America [2]. Despite attempts to reduce the radiation field, there is a significant toxicity from radiation to the developing brain.

Recent biological insights suggest that despite having morphologically identical appearances, ependymoma in the brain comprises several different molecular variants. Even among the posterior fossa ependymomas, which are the subject of this review, there exists a degree of molecular heterogeneity not appreciable through morphological analysis alone. Based on

recent molecular genetic studies, posterior fossa ependymomas have now been designated into two main subgroups, named group A and group B [3]. In this review, we will discuss the current understanding and management of posterior fossa ependymoma in relation to the new information derived from the recent genomic and epigenomic studies.

Demographics

There is a bimodal age distribution to posterior fossa ependymoma with peaks at ages 5 and 35. Traditionally, the demographics of each location of ependymoma have not been captured separately, as the CBTRUS (Central Brain Tumor Registry of the United States) and SEER (Surveillance, Epidemiology, End Results) databases code ependymoma as one entity. As such, there is a paucity of robust demographic detail specific to each location of ependymoma and within each age group. Overall, there is a male preponderance of approximately 2:1 in children [4]. In the pediatric setting, ependymomas occur mostly intracranially with uncommon intraspinal lesions, largely confined to the filum terminale. In contrast to this, ependymomas in adults have a predilection for the spinal cord.

Clinical presentation

Children with posterior fossa ependymomas typically present with symptoms of obstructive hydrocephalus and posterior fossa compression, specifically headache, vomiting, and ataxia. Moreover, children with posterior fossa ependymoma commonly present with torticollis due to growth through the foramen of Magendie, which is distinct from other posterior fossa tumors such as medulloblastoma [5, 6]. There is rarely any family history of malignancies noted at presentation.

Initial evaluation of patients is through neuroimaging, normally by CT, followed by MRI. On CT, posterior fossa ependymomas are typically isodense, frequently with small calcifications and cyst formation. However, the differential diagnosis between ependymoma and medulloblastoma is difficult using CT alone. On MRI, posterior fossa ependymoma can commonly be distinguished from embryonal tumors such as medulloblastoma due to their location, specifically since they have invasion into the foramina of Luschka and Magendie. Moreover, posterior fossa ependymomas commonly grow into the cerebellopontine angle and also into the upper cervical canal. Ependymomas can also present with leptomeningeal dissemination, but this is rare at diagnosis and, if noted, should prompt a re-consideration of the morphological diagnosis of ependymoma.

Pathology

Definitive diagnosis is by histomorphological examination. On microscopic examination, ependymomas exist as both conventional (WHO grade II) and malignant anaplastic (WHO grade III) forms. Classic ependymoma consists of solid and well-demarcated lesions with limited infiltration of surrounding cerebellum or brainstem. Histological features include a sheet-like growth pattern interrupted by perivascular pseudo-rosettes, which consist of tumor cells surrounding blood vessels with nuclei separated from the vascular wall by radially arranged fibrillary processes (Fig. 1a, b). True rosettes are also characteristic and defined by ependymoma cells self-organizing radially around a central lumen reminiscent of ependymal canals (Fig. 1c). Grade II or classic ependymomas usually have a low mitotic index but can have nuclear atypia, foci of non-palisading necrosis, and calcification. Additional WHO grade II histological variants include clear cell, papillary and tanycytic ependymomas. Malignant WHO grade III or anaplastic ependymomas have increased cellularity, cytological nuclear anaplasia, and have elevated mitotic activity with vascular proliferation (Fig. 1d, e) [7]. Necrosis in the anaplastic variant typically shows a pseudopalisading pattern (Fig. 1f). A clear consensus on grading criteria is not well established, and thus, considerable inter-observer variability exists with the prognostic implications of conventional and anaplastic variants of ependymomas in children being unclear [8].

Radial glial cells are the candidate cell of origin for ependymoma

A major limitation of the early cytogenetic studies was the inclusion of multiple locations along the neuroaxis based on an identical histomorphological appearance. However, one fact that quickly emerged from numerous genetic studies is that despite some overlap at the structural level, different anatomical regions of the CNS have distinct cytogenetic patterns, even though the tumors appear histologically indistinguishable [9, 10]. This led to a cross-species genomics study by Taylor et al., using gene expression and array CGH profiling of 32 ependymomas, which demonstrated that tumors from different locations cluster together based on their distinct gene expression profiles and distinct cytogenetic aberrations [11]. These findings were validated using fluorescent in situ hybridization of location-specific bacterial artificial chromosomes on a non-overlapping set of 71 formalin-fixed paraffin-embedded samples.

This same study went on to compare the gene expression profiles of ependymoma from distinct anatomical locations to the corresponding neural progenitor cells from the developing murine nervous system. The results showed that embryonic
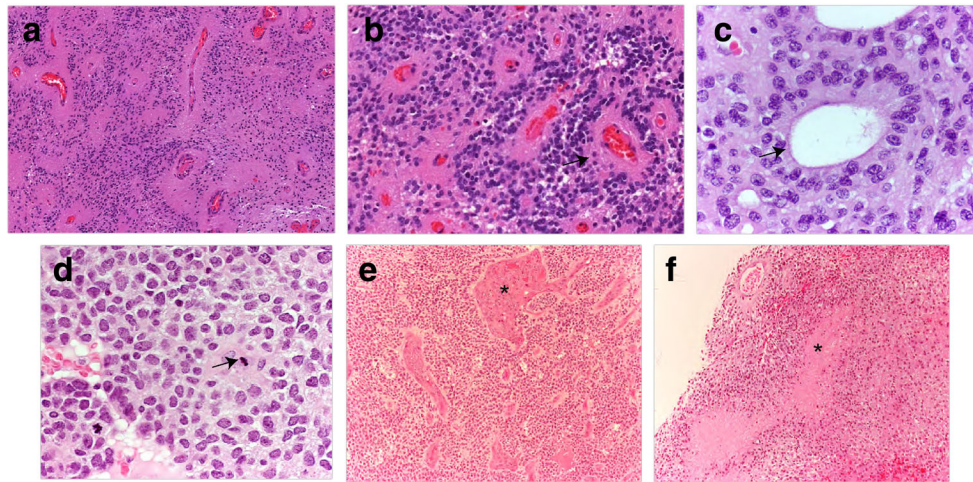
Fig. 1 Histomorphological features of classic and anaplastic ependymomas. Hematoxylin and eosin stain of a WHO grade II ependymoma showing sheets of neoplastic cells forming numerous perivascular pseudo-rosettes (**a**, **b**) consisting of tumor cells surrounding blood vessels (*black arrow*) and true ependymal rosettes (**c**) comprised of neoplastic cells lining a central lumen reminiscent of ependymal canals (*black arrow*). Examples of anaplastic WHO grade III ependymomas showing increased nuclear pleomorphism and mitotic activity (**d**, *black arrow*), endothelial proliferation (**e**, *asterisk*), and areas of pseudopalisading necrosis (**f**, *asterisk*)

radial glial cells (RGCs) from different anatomical locations have distinct gene expression profiles, and that ependymoma cancer stem cells are a rare self-renewing population, which resemble radial glial cells isolated from the corresponding anatomical location. This finding, that the gene expression patterns of ependymoma recapitulate the radial glial cells of their corresponding anatomical location, suggests that ependymoma tumors either maintain or reiterate the developmental expression profiles of anatomically restricted progenitor cells. A subsequent cross species study of 83 ependymomas again confirmed that the gene expression profiles of ependymoma from distinct locations preserve the gene expression profile of candidate embryonic neural progenitor cells from the corresponding anatomical location [12].

Early publications had suggested, based on morphology that ependymomas arose from ependymal cells. This is very unlikely as ependymal cells are post-mitotic and are expected to have little capacity for neoplastic transformation. There is significant data supporting the cross-species genomic findings that radial glial cells are the candidate cells of origin for ependymoma. Radial glial cells are key ubiquitous progenitor cells that originate in the subventricular zone and one of their primary purposes is to facilitate neuroblast migration. They act as guide cells for neuroblast migration and are essential for patterning and region-specific differentiation during the development of the CNS of all vertebrates [13, 14]. Studies have shown that ependymal cells are born from radial glial cells during embryogenesis and reach maturation within the early postnatal period [15]. Interestingly, there is evidence that radial glial-like cells are present, not only during early development, but persist into adulthood specifically in the subventricular zone (SVZ) as adult neural stem cells (NSCs) that generate new neurons continuously throughout adulthood

[16]. These adult radial glial cells may serve as the cells of origin for ependymomas that occur beyond infancy and early childhood [17].

To date, no preclinical model of posterior fossa ependymoma has been established. Blbp expression characterizes several neuronal progenitors including radial glial cells during CNS development. Using the available knowledge for this marker, preclinical murine models have now been generated where E14.5 Blbp-positive supratentorial cells can be transformed into murine tumors which morphologically and genetically resemble ependymoma [12]. Supratentorial ependymomas frequently exhibit deletion of the *CDKN2A* locus as well as upregulation of the EphB-Ephrin signaling pathway; this can be modeled by overexpressing the *EPHB2* receptor on the *Ink4a/Arf* (*Cdkn2a*) null background. Using this, Johnson et al. were able to induce oncogenic transformation of radial glial cells taken from the cerebrum of mouse embryos into forming supratentorial ependymoma-like tumors in immunocompromised mice [12]. Recently, an oncogenic fusion between *RELA*, the principal effector of canonical NF-κB signaling, and an uncharacterized gene *C11orf95* has been found in a large fraction of supratentorial ependymomas [18]. Introduction of the *C11orf95-RELA* fusion into embryonic cerebral RGCs, the cells of origin for supratentorial ependymoma, was once again able to induce ependymoma formation as allograft in mice [18].

Posterior fossa ependymoma is comprised of two distinct molecular variants

A large study of 83 frozen ependymomas, using combined gene expression profiling and high resolution copy number

profiling, revealed that there are three distinct molecular variants of ependymoma and that a subset of posterior fossa tumors occur in older children and cluster with spinal ependymomas [12]. This finding was extended in an integrated genomic study of two large independent cohorts (177 ependymomas in total). The analysis consistently revealed three transcriptionally defined subgroups of ependymoma that correspond to the supratentorial, posterior fossa, and the spinal cord tumor locations, again, with a subset of posterior fossa ependymomas being more similar to spinal tumors [3]. These findings highlight the importance of recognizing the genetic heterogeneity present in ependymomas arising from different anatomical locations and suggest that supratentorial, posterior fossa, and spinal ependymomas are molecularly distinct despite their histological similarities. Ependymomas from different parts of the nervous system should therefore be considered as distinct entities with their own respective demographics, transcriptomics, genetics, epigenetics, clinical course, and prognosis.

In addition to confirming that there are three principal subgroups of ependymoma largely defined by their site within the CNS, Witt and Mack et al. further identified and characterized two transcriptionally, genetically, demographically, and clinically distinct subtypes of posterior fossa (PF) ependymoma, termed PFA and PFB. PFA tumors cluster together into a purely PF group whereas PFB tumors preferentially cluster with spinal ependymomas. Pathway analysis has identified distinct biological processes and signaling pathways between PFA and PFB ependymomas. PFB tumors are defined by gene sets involved in ciliogenesis, microtubule assembly, and mitochondria/oxidative metabolism. PFA tumors, on the other hand, were characterized by many pathways and gene networks well known in promoting and sustaining oncogenesis, including angiogenesis (HIF-1a signaling, VEGF signaling, cell migration), PDGF signaling, MAPK signaling, EGFR signaling, TGF-b signaling, integrin signaling, extracellular matrix assembly, tyrosine-receptor kinase signaling, and RAS/small GTPase signaling. These clear differences in the biological pathways affected in the tumors further supports the distinct nature of PFA and PFB ependymomas.

PFA tumors arise in younger patients (median age 2.5 years) with a 70 % male gender bias, whereas PFB tumors occur predominantly in older patients (median age 20 years). Anatomically, two thirds of PFA tumors occur laterally with one third exhibiting cerebellar invasion, whereas PFB ependymomas are most likely to be found in the midline without invasive growth into the cerebellum.

The genetic difference between PFA and PFB ependymomas is striking. PFA tumors exhibit largely balanced genomic profiles, with 1q gain being the most frequently observed genomic aberration, found in about 15 % of cases [3]. Indeed the structural alterations identified across PFA and

PFB are in line with previously published cytogenetic characterizations of ependymoma. PFA ependymomas fall predominantly into high risk groups 2 and 3 devised by Korshunov et al. [10]. In contrast, PFB tumors show high levels of genetic instability with numerous cytogenetic abnormalities involving whole chromosomes or chromosomal arms [9]. PFB ependymomas fit into the low-risk group 3 or the "numerical" group based on the Korshunov et al. classification system.

An independent study by Wani et al. [19] using unsupervised clustering of gene expression microarray data from 67 posterior fossa ependymomas confirmed the existence of two distinct subgroups of posterior fossa ependymoma. They revealed two major posterior fossa ependymoma subgroups with remarkable correspondence to the PFA and PFB tumors reported by Witt and Mack et al. [3]. Their group 1 tumors (similar to PFA) overexpressed genes associated with mesenchyme (i.e., response to wound healing, inflammation, migration, and cell adhesion) resembling the mesenchymal signature observed in glioblastoma multiforme (GBM). Like PFA tumors, group 1 tumors arose in patients of younger age and showed worse progression-free and overall survival than group 2 tumors (similar to PFB).

CpG island methylator phenotype defines PFA ependymomas

The fact that PFA ependymoma exhibits a mostly balanced genomic profile was an enigma that perplexed early cytogenetic researchers. Indeed, even using next-generation sequencing, no recurrent single nucleotide variant was identified across a cohort of 47 posterior fossa ependymomas [18, 20]. In fact, looking across various cancers, posterior fossa ependymoma has the lowest rate of somatic mutations of any malignancy sequenced to date.

In order to determine if epigenetic changes might be driving the tumorigenesis of posterior fossa ependymoma, Mack and Witt et al. proceeded to perform genome-wide methylation arrays on 79 ependymomas [20]. Initial, unsupervised clustering of these epigenomic profiles divided ependymomas into the same supratentorial, posterior fossa, and spinal groups previously described based on gene expression profiles; this reinforces the biological basis for studying and treating ependymomas from different anatomical regions as distinct diseases. Methylation profiling revealed dramatic differences between PFA and PFB, whereby PFA tumors had a relative increase in CpG methylation, and these areas of CpG hypermethylation converged on targets of the polycomb repressor complex 2 (PRC) [20]. The polycomb repressor complex 2 (PRC2) represses stem cell differentiation and maintains pluripotency through trimethylation of H3K27, suggesting that this may be a possible driver of the PFA ependymoma [21, 22]. More significantly, treatment of patient-derived PFA

ependymoma cell cultures with DNA-demethylating agents or drugs that target the PRC2 complex and/or H3K27 trimethylation has been shown to decrease PFA ependymoma tumor cell survival and growth both in vitro and in vivo [20]. This not only provides functional evidence that CpG hypermethylation and/or hyperactivity of PRC2 is a key mechanism for PFA ependymoma tumorigenesis but also advocates effective therapeutics specifically targeting the biological basis of this disease. More broadly this suggests that epigenetic modifiers (i.e., DNA methylation inhibitors either alone or in combination with EZH2 inhibitors) represent a novel treatment strategy for PFA ependymomas of infancy.

Prognosis and treatment

In terms of clinical outcome, PFA ependymomas have a significantly increased 5-year incidence of tumor recurrence and mortality compared to PFB tumors, with 5-year progression free and overall survival rates being at 44 and 65 % for PFA tumors compared to 75 and 95 % for PFB tumors [3]. The mainstay of therapy for ependymoma is maximal safe surgical resection. Several studies have suggested that an incomplete resection is the strongest predictor of poor outcome in posterior fossa ependymoma [4, 23, 24]. However, posterior fossa ependymoma commonly invades the cranial nerves, particularly the lower cranial nerves, and also the floor of the fourth ventricle, thus precluding a complete resection without devastating neurological morbidity. The use of intraoperative monitoring can be invaluable, particularly for those cases invading the, floor of the fourth ventricle, auditory canal, and cervical spinal cord. Somatosensory-evoked potentials, motor-evoked potentials, brainstem auditory-evoked potentials, and electromyography have all been used in the intraoperative setting to help reduce morbidity associated with aggressive surgical resections. However, cases with lower brainstem invasion or extensive involvement of the lower cranial nerves are notoriously difficult to resect without the need for tracheostomies and gastrostomy tubes.

As will be discussed in more detail below, recent biological data suggests that the "extent of resection" may not be as critical, when accounting for biological risk stratification but this issue is currently unclear [3]. Both chemotherapy and external beam irradiation have been investigated in treatment of posterior fossa ependymoma. Historically, children under 3 have been treated with chemotherapy only; however, the results of these chemotherapy approaches have been very disappointing. More recently, however, conformal 3D external beam irradiation is becoming the standard of care in North America for all children with posterior fossa ependymoma, regardless of age.

Chemotherapy has usually been reserved for young children in a radiation-sparing approach; however, this has yielded relatively poor 5-year survivals of around 30 %, even when high-dose chemotherapy with autologous stem cell support is used [25–27]. Trials in both North America and Europe using chemotherapy alone for the treatment of ependymoma have yielded relatively poor outcomes, although a report from the Pediatric Oncology Group (POG9233) and the UKCCSG/SIOP suggests that up to 40 % of infant ependymomas can be treated with prolonged post-operative chemotherapy without radiation therapy [28, 29]. However, these two studies, which suggest a role for a chemotherapy-only approach appear to be outliers that need to be evaluated with extreme caution, as they do not provide any data suggesting an objective response to chemotherapy nor do they clearly account for site differences between ependymomas of the supratentorial region and posterior fossa [30]. In the light of the poor responses to chemotherapy, conformal radiation has emerged as a possible upfront treatment for posterior fossa ependymoma in children and infants, in whom RT had usually been avoided. A prospective study at St. Jude's Children's Research Hospital, Memphis suggested that conformal radiation can be safely administered to children as young as 1 year, and results in 7-year event-free survival rates of over 60 % [23]. This approach is currently being validated in a multicenter study from the Children's Oncology Group, where they are specifically asking whether there is a role for combined chemotherapy and conformal radiation following incomplete resection (ACNS0121 and ACNS0831).

Upon PFA tumor recurrence, treatment consists primarily of re-resection and re-irradiation, which appear to increase survival post-recurrence; no conventional chemotherapy has shown any activity in this setting [2, 31, 32]. Although the site of first recurrence is in the tumor bed, metastatic recurrences are not infrequent (particularly the second recurrence), and as such at the Hospital for Sick Children in Toronto, the standard of care is now craniospinal irradiation for all recurrent ependymoma [32–34]. Indeed, we have shown previously that re-irradiation can be safely administered in this setting; however, longer observation of our cohort is still required. As re-irradiation is likely a temporizing measure, new and unique approaches are urgently required for both the treatment and risk stratification of posterior fossa ependymoma.

Conclusion and future directions

Current treatment for posterior fossa ependymoma heavily relies on surgical resection, followed by adjuvant radiotherapy and occasionally chemotherapy without proven efficacy. Stratification of ependymoma based on histology alone has not been informative. Thanks to recent research findings focusing on the molecular biology of this disease, there is now almost unanimous appreciation and recognition that ependymomas should be categorized into subgroups based

Table 1 Characteristics distinguishing PFA vs. PFB ependymomas

	PFA	PFB
Demographics	Median age 2.5 years 70 % male	Median age 20 years No sex bias
Anatomical site	Lateral PF location with cerebellar invasion	Medial PF location without cerebellar invasion/ spinal cord
Cytogenetics	Balanced genome 1q gain	Numerous chromosome gains and losses
Epigenetics	CIMP +ve	CIMP −ve
Clinical outcome	High rate of recurrence Low rate of survival	Low rate of recurrence High rate of survival

on their CNS location and that posterior fossa ependymomas can be further subdivided into two distinct subgroups with significantly different characteristics as summarized in Table 1.

This enhanced molecular understanding of posterior fossa ependymomas provides a greater prognostic power to clinicians, which in turn translates into improved personalized patient treatment based on risk stratification.

Furthermore, the identification of CpG hypermethylation through activation of PRC2 as a key mechanism in PFA ependymoma tumorigenesis offers important insights necessary for modeling this disease in animals as well as for establishing novel targeted therapies for patients [35–40]. Based on the epigenetic-driven nature of PFA ependymoma, drugs that target DNA CpG island methylation, PRC2, trimethylation of H3K27, and/or histone deacetylase inhibitors are very promising therapeutics against PFA ependymoma as they provide a rational approach in targeting the underlying cause of this disease and should be tested in clinical trials. The paucity of somatic mutations in posterior fossa ependymomas suggests that typical routes to rational therapy will not be possible and supports the use of more innovative approaches. Relating PF ependymoma biology to that of the normal development of the brainstem and cerebellum may allow improved modeling of the disease, and perhaps the development of rational targeted therapies. Further efforts are currently focused on how to translate these findings to the bedside through refined preclinical modeling and development of rational therapeutic strategies.

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