Embryonal tumor with multilayered rosettes of the fourth ventricle: case report

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Embryonal tumor with multilayered rosettes (ETMR) is a recently described pathological entity. These primitive central nervous system tumors harbor amplification of the 19q13.42 locus and resultant overexpression of the LIN28A protein. Although the WHO currently recognizes 3 distinct histopathological entities—embryonal tumor with abundant neuropil and true rosettes (ETANTR), ependymoblastoma, and medulloepithelioma—recent studies indicate that these tumors have a common molecular profile and clinical course and that they are now classified as a single entity. Here the authors present a case of ETMR located in the fourth ventricle in a 12-month-old boy. The histopathology featured areas of neuropil-like stroma and highly cellular foci with characteristic multilayered rosettes. The authors discuss the clinical, radiological, and histopathological findings in this case and compare them with data in previously published cases in the literature. A review of studies assessing the molecular mechanisms underlying these tumors is also presented.

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anterior fontanelle was open, nonbulging, and 3 mm in width and his head circumference was 54 cm. Neurological exam revealed normal muscle tone in upper and lower extremities with no sign of atrophy. No gross cranial nerve deficits were apparent. Sensation was preserved bilaterally in the upper and lower extremities. Coordination examination was significant for ataxic movement in the upright position. Deep tendon reflexes were preserved in the upper and lower extremities without pathological reflexes.

Given the head CT findings, the patient was electively intubated for placement of an external ventricular drain (EVD) followed by MRI of the entire neuraxis. Magnetic resonance imaging confirmed a 5.8 × 4.7 × 4.7–cm solid mass in the fourth ventricle causing compression of the fourth ventricle and obstructive hydrocephalus. The mass was hypointense on T1-weighted imaging and hypertense on T2-weighted imaging and showed heterogeneous enhancement within the mass (Fig. 1). No metastatic deposits were visualized.

Operation
A suboccipital craniotomy for tumor resection was performed. On gross examination of the mass, the tissue appeared soft, fleshy, and pink-white in color. Gross-total excision was achieved and the aqueduct was visualized with good CSF flow. A specimen was sent for frozen section and diagnosis at this time was embryonal tumor, likely medulloblastoma as opposed to atypical teratoid/rhabdoid tumor (AT/RT).

Histopathological Examination
Histological analysis of the resected specimen showed a biphasic tumor with hypercellular areas of small blue cells with minimal cytoplasm (reminiscent of medulloblastoma/PNET) admixed with paucicellular neuropil areas (Fig. 2). Additionally numerous multilayered rosettes were found in both regions of the tumor. Immunohistochemical staining for neurofilament protein was diffusely positive, while synaptophysin highlighted the PNET and neuropil areas but was negative in the rosettes. Glial fibrillary acidic protein and NeuN were negative throughout. Immunohistochemical study for INI-1 showed retained nuclear staining in the tumor cells. Chromosome 19 expression studies were not available at our institution.

Postoperative Course
Magnetic resonance imaging performed on postoperative Day (POD) 1 revealed no evidence of residual tumor and expected postsurgical changes in the fourth ventricle (Fig. 3). His EVD was clamped and intracranial pressures were monitored. His intracranial pressure remained normal and his EVD was removed.

Chemotherapy consisting of vincristine, cisplatin, etoposide, cyclophosphamide, and methotrexate with leucovorin rescue was started on POD 18 according to the ACNS0333 protocol. Radiotherapy was not pursued since we believed that its short and long-term risks did not outweigh its benefits in a child his age. The patient’s hospital

FIG. 1. Axial (A) and sagittal (C) T1-weighted MR images showing a hypointense 5.8 × 4.7 × 4.7–cm solid mass in the fourth ventricle compressing the brainstem. Compression of the pituitary from a dilated third ventricle is visible. There is mild heterogeneous enhancement of the mass on axial (B) and sagittal (D) Gd-enhanced T1-weighted MR images.

FIG. 2. A: Paucicellular neuropil (arrow) admixed with PNET-like areas containing multilayered rosettes (asterisks). Original magnification ×40. B: Hypercellular embryonal areas of small blue cells with minimal cytoplasm. Original magnification ×100. C: Arrow indicating multilayered true rosette with distinct lumen. H & E, original magnification ×400. Figure is available in color online only.
course was complicated by bacteremia with *Enterococcus faecalis*, coagulase-negative *Staphylococcus* sp., *Candida albicans*, and *Candida glabrata* and *Clostridium difficile* in stool. He was treated with appropriate antibiotics and antifungal agents. On POD 42, the patient developed a dysconjugate gaze with bilateral cranial nerve VI palsies, and repeat MRI revealed fourth ventricle outflow obstruction. He underwent placement of a ventriculoperitoneal shunt. Chemotherapy is ongoing.

**Discussion**

Embryonal tumors typically present in young children and adolescents and are often associated with an aggressive course. According to the 2007 WHO classification system, these tumors are classified as medulloblastomas (the most common type), AT/RTs, or CNS PNETs. In CNS PNETs, there are 3 recognized histological variants: ETANTR, medulloepithelioma, and ependymoblastoma. Recent molecular studies have shown that these 3 tumor types harbor a common molecular phenotype and may in fact represent a histological spectrum that is now unified under the term “ETMR.” We maintain the term “ETANTR” for the purposes of reviewing the literature since it was the term used in most previous studies.

ETANTR was first described in 2000 by Eberhart et al. In this seminal article these authors reported 7 cases of CNS PNETs, characterized histologically by undifferentiated neuroepithelial cells, areas of well-differentiated neuropil, and ependymoblastic rosettes arising from paucicellular regions of the neuropil. In 2009, a follow-up of this original report was published, which included 22 additional cases and follow-up on the original 7 cases. This updated report demonstrated that the majority of tumors were found in the supratentorial region and were predominately seen in females with a mean age of 2 years. The majority of patients showed little or no response to chemotherapy and radiation, with over 75% of patients dying within 30 months of initial presentation and the longest survival being only 42 months.

Since the original report in 2000, fewer than 100 additional cases of ETANTR have been reported in the literature. In the majority of cases, including those described in the original report, the tumor was found in the supratentorial region (65 total), whereas only 35 were found in the infratentorial region—16 in the brainstem and 1 in the spinal cord. There was a slight female predominance (52%), with an average age of 28 months at initial presentation. Of the supratentorial tumors, most involved the parietal or frontal lobe. Two of the 65 supratentorial tumors invaded the dura mater, and 1 showed spinal metastasis at initial presentation.

Of the 35 infratentorial tumors reported in the literature, only 1 has been described as occurring in the fourth ventricle, as in the patient in the present case. Similar to the patient we report here, this other child with a fourth ventricle tumor underwent resection and was started on a treatment regimen that included 5 cycles of chemotherapy with vincristine, cisplatin, etoposide, cyclophosphamide, and methotrexate with leucovorin and stem cell rescue.

Sixteen tumors documented in the literature appeared in the brainstem, with 2 presenting with spinal metastasis at initial diagnosis. One tumor was exclusively found in the spinal cord—this patient did not undergo resection and died 6 months after presentation.

The prognosis associated with ETANTRs is poor with a 69% mortality rate overall and more than 75% of these deaths occurring within a year of initial presentation. Of all reported cases, brainstem tumors are associated with the worst prognosis with only 7% of patients disease-free at follow-up, although supratentorial and cerebellar tumor patients have a slightly better prognosis with 11% and 9% disease-free at follow-up, respectively. The worse prognosis is probably attributable to the fact that brainstem tumors are often unresectable because of their location, as compared with lesions in the cortex or cerebellum. Only 38% of tumors documented in the brainstem were resected (either completely or partially), compared with 72% of cerebellar tumors and 69% of supratentorial tumors. Notably, the longest reported survival for a patient with ETANTR without residual disease is 7 years. However, it is not clear whether other long-term survivors exist, as there is very limited long-term follow-up data on ETANTR patients in the literature, which has been attributed to the fact that most reported series are in pathology journals, and thus clinical follow-up is not discussed in detail.
Recent studies have attempted to elucidate the genetic and molecular profile of these tumors, with the goal of identifying tumor-specific markers that can be used for diagnostic, prognostic, and therapeutic purposes. The first cytogenetic studies of ETANTRs included a case with isochromosome 17, a finding typically seen only in medulloblastoma, and another case with tumor cells exhibiting polysomy of chromosomes 2, 8, 17, and 22 on fluorescence in situ hybridization (FISH) analysis. In 2009, using array comparative genomic hybridization, Pfister et al. reported a tumor that, in addition to exhibiting polysomy of chromosomes 2 and 19, showed amplification of a chromosome band at 19q13.42, which encompasses a cluster of microRNAs (miRNAs) denoted “C19MC.” Interestingly, 2 of the miRNAs in this cluster, mir-372 and mir-373, have been implicated as oncogenes in the p53 regulatory pathway.

Following this initial report of a copy number variation at this miRNA locus, Li et al. described amplification of this cluster in 11 additional cases of supratentorial PNETs and hypothesized that this amplification might be associated with ependymoblastlastic differentiation. To test this hypothesis, Korshunov et al. performed FISH analysis on 41 tumor samples histologically diagnosed as ependymoblastomas and ETANTRs and found that 97% of the samples showed amplification at the 19q13.42 locus, suggesting that ETANTRs and ependymoblastomas may not be separate entities. Subsequently, multiple other reports have demonstrated amplification of this region in tumors with ependymoblastic rosettes. In 2010, Paulus and Kleihues coined the term “embryonal tumor with multilayered rosettes.”

After the original report by Pfister et al. showing chromosome 19 amplification in ETMRs, other studies documented molecular abnormalities in these tumors. In 2012, Korshunov et al. proposed LIN28A overexpression as a potential biomarker for ETMRFs after finding by immunohistochemistry that 100% of the ETMRs they analyzed were LIN28A positive compared with only 12% (6/50) of the AT/RTs and none of the 41 CNS PNETs (non-ETMR variants), 334 medulloblastomas, 223 anaplastic ependymomas, and 131 pediatric glioblastomas. LIN28A and its homolog LIN28B encode proteins that bind and repress the let-7 family of miRNAs. They are thought to act as oncogenes when overexpressed via let-7 repression and subsequent upregulation of let-7 targets such as MYC and RAS. Similarly, a study by Spence et al., which included 128 medulloblastomas, 45 AT/RTs, 105 ependymomas, 50 high-grade gliomas (HGGs), 20 choroid plexus carcinomas, and 103 CNS PNETs, revealed that amplification at 19q13.42 was observed only in CNS PNETs, whereas LIN28A immunoreactivity was observed in 19.5% of HGGs and 24.4% of AT/RTs in addition to a subset of CNS PNETs. Together these studies suggest that LIN28A positivity may be sensitive to, but not specific for, ETMR. Conversely, 19q13.42 amplification appears to be specific for ETMRs.

In a similar analysis, Korshunov et al. evaluated 97 tumors from patients diagnosed with ETANTR, ependymoblastoma, or medulloepithelioma and demonstrated that these tumors could not be distinguished from each other at a molecular level based on 19q13.42 amplification and LIN28A positivity. Thus, they corroborated previous suggestions that these tumors should be considered a single diagnostic entity. Additionally, they noted that these tumors share a similar clinical course, typically occurring in children younger than 3 years of age, usually supratentorial in origin, and associated with a poor prognosis.

Most recently, Kleinman et al. looked at the methylation patterns of ETMRs, hypothesizing that the miRNA cluster amplification seen in these tumors may be affecting global methylation patterns. Interestingly, they reported that DNMT3B, a methyltransferase involved in DNA methylation and thought to be important in embryonic differentiation, was overexpressed in the 12 ETMRs they studied as compared with other tumors in The Cancer Genome Atlas, making overexpression of this protein another potential diagnostic biomarker. Moreover, exon 1B of DNMT3B is predominately expressed during Week 8 of fetal development and was only found to be expressed in ETMRs, suggesting that aberrant splicing during neurogenesis may result in tumor formation. Future work should attempt to define the normal expression of this miRNA cluster and its methylation pattern over early human neurodevelopment, in a manner similar to the Kleinman et al. study of DNMT3B, as perhaps dysregulation of expression of this miRNA cluster early in development drives subsequent molecular and histopathological changes seen in these tumors.

Conclusions

ETANTRs, now belonging to the classification ETMRs, are a group of rare tumors usually found in the supratentorial region in young children. Treatment of this tumor includes resection, chemotherapy, and radiotherapy in most cases, although even with appropriate treatment the prognosis is poor. Molecular studies have recently identified amplification of the C19MC miRNA cluster and overexpression of LIN28A in these tumors, suggesting a potential molecular mechanism for the pathogenesis of this cancer. Furthermore, such findings may prove useful as diagnostic biomarkers for this tumor and potential targetable therapies. Future work concentrating on understanding the normal function of the C19MC miRNA cluster in human brain development may lead to a better understanding of the molecular pathogenesis of ETMRs and identify additional molecular biomarkers for targetable therapies and prognostic utility in these aggressive tumors.

References

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Disclosure
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