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Embryonal Tumors With Abundant Neuropil and True Rosettes:

A Distinctive CNS Primitive Neuroectodermal Tumor

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Abstract

Embryonal neoplasms of the central nervous system (CNS) generally arise in the early years of life and behave in a clinically aggressive manner, but vary somewhat in their microscopic appearance. Several groups have reported examples of an embryonal tumor with combined histologic features of ependyoblastoma and neuroblastoma, a lesion referred to as "embryonal tumor with abundant neuropil and true rosettes" (ETANTR). Herein, we present 22 new cases, and additional clinical follow-up on our 7 initially reported cases, to better define the histologic features and clinical behavior of this distinctive neoplasm. It affects infants and arises most often in cerebral cortex, the cerebellum and brainstem being less frequent sites. Unlike other embryonal tumors of the CNS, girls are more commonly affected than boys. On neuroimaging, the tumors appear as large, demarcated, solid masses featuring patchy or no contrast enhancement. Five of our cases (18%) were at least partly cystic. Distinctive microscopic features include a prominent background of mature neuropil punctuated by true rosettes formed of pseudo-stratified embryonal cells circumferentially disposed about a central lumen (true rosettes). Of the 25 cases with available follow-up, 19 patients have died, their median survival being 9 months. Performed on 2 cases, cytogenetic analysis revealed extra copies of chromosome 2 in both. We believe that the ETANTR represents a histologically distinctive form of CNS embryonal tumor.

Keywords

pediatric brain tumors; embryonal tumor; divergent differentiation; neuropil; ependymoblastic rosettes

Embryonal neoplasms of the central nervous system (CNS) usually occur in children and are clinically aggressive. Most are formed in large part of undifferentiated cells having the capacity to differentiate along glial, neuronal, and occasionally mesenchymal lines. According to the 2007 World Health Organization (WHO) classification, this group is divided into 3 broad categories: medulloblastoma,⁵ CNS primitive neuroectodermal tumor (PNET),¹⁰ and atypical teratoid rhabdoid tumor (AT/RT).⁸ The most common entity, medulloblastoma, has been extensively investigated with respect to its molecular profile and histogenesis.^{2,5} The AT/RT also represents a rather well-defined entity in terms of its microscopic appearance, immunophenotype, and genetic profile.^{7,8} The remaining group of CNS PNETs contains several accepted entities defined on the basis of their histopathologic features, including medulloepithelioma, ependymoblastoma, CNS neuroblastoma, and CNS ganglioneuroblastoma.¹⁰

Medulloepithelioma is a rare, nonsmall cell neoplasm resembling primitive medullary epithelium.¹⁰ Ependymoblastoma is a rare, embryonal neoplasm seemingly committed to differentiation along ependymal lines, defined by the presence of true rosettes and ependymal canals in association with small cells.¹⁰ The 2007 WHO classification designates tumors composed of, in part, such undifferentiated or poorly differentiated neuroepithelial cells, but lacking specific features such as those seen in medulloepithelioma and ependymoblastoma, as CNS PNET. This term represents an extension of the supratentorial PNET category of the prior WHO classification, and underscores the fact that PNET can be identified in the brain stem and spinal cord.

Herein, we describe 29 cases of CNS PNET with distinctive histology and a particularly poor clinical outcome. These include 7 cases previously reported under the designation “pediatric neuroblastic tumor with abundant neuropil and true rosettes.”³ Fourteen additional examples were subsequently reported by 4 other groups.^{1,3,4,9} Some of these reports applied the term “embryonal” rather than “neuroblastic,” and employed the acronym embryonal tumors with abundant neuropil and true rosettes (ETANTR). Histologically, ETANTR is characterized by the presence of undifferentiated neuroepithelial cells resembling those of classic PNET, broad zones of well-differentiated neuropil, and ependymoblastic rosettes arising abruptly from paucicellular regions of neoplastic neuropil. Homer Wright rosettes may also be present. The 22 new cases of ETANTR we present herein are accompanied by additional follow-up on the 7 we originally reported. We seek to better define the histopathology and clinical behavior of this uncommon tumor which, to us, represents a defined clinicopathologic entity distinct from the general group of CNS PNET and more common than some embryonal tumors, such as medulloepithelioma and ependymoblastoma.

MATERIALS AND METHODS

Sixteen surgically obtained specimens (cases 1 to 16 and case 29) were received in consultation at the Johns Hopkins Hospital. An additional case (case 18) came from the Mayo Clinic, 5 from the consultation files of Marc K. Rosenblum at Memorial Sloan-Kettering Cancer Center (cases 23 to 27), and 2 (cases 15 and 28) from The Sick Children Hospital, Toronto. The others were contributed by the Department of Pathology, Catholic University, Italy (cases 19 to 21), the University of Padua (cases 22), and 1 from the Department of Experimental Medicine and Pathology, University of Rome “La Sapienza” (case 17). The first 7 cases enumerated were previously reported, but survival data were updated for the purpose of this study.³ The specimens examined included 11 biopsies and 18 resections. All had been formalin-fixed, routinely processed, and paraffin-embedded. Five-micron sections were stained with hematoxylin and eosin. A Gomori reticulin stain was performed in 1 instance (case 22).

Immunohistochemistry (IHC) employed the avidinbiotin peroxidase complex method, diaminobenzadine serving as the chromogen. In Figure 3, IHC was performed using antibodies for vimentin (DAKO, Glostrup, Denmark), desmin (Ylem, Avezzano, Italy), synaptophysin (Ylem), smooth muscle actin (DAKO), glial fibrillary acidic protein (GFAP) (DAKO), neurofilament protein (Ylem), BAF47/INI1 (BD Biosciences, Palo Alto, CA), and NeuN (Neomarkers, Fremont, CA). Proliferation was evaluated using the anti-MIB-1 antibody (DAKO), and a MIB-1 index derived by counting positive and negative tumor cells in multiple fields from the most highly proliferative areas.

Cytogenetic analysis was performed and obtained in cases 15 and 24 using standard techniques. In brief, fresh tumor material was exposed to collagenase for approximately 1 to 4 hours, then washed and cultured in RPMI medium supplemented with 20% fetal calf serum, L-glutamine, penicillin/streptomycin. Cells were monitored for growth and harvested at 1 to 7 days in culture. Metaphases were collected after colcemide and hypotonic exposure, then slides were made and G-banded karyo-types prepared.

RESULTS

Clinical Findings

Clinical and radiographic data regarding the ETANTR in this series are summarized in Table 1. The tumors occurred in both infants and young children (range, 9 to 48 months; mean, 24 months; female:male ratio = 2:1). The lesions showed a preference for the supratentorial region, most arising in the frontal lobes (13 cases), either in isolation or with extension to adjacent lobes, or in the parietal lobe (2 cases) or thalamus (1 case). An only somewhat smaller subset was infratentorial, affecting brainstem (5 cases), cerebellum (5 cases), or the pineal/tectal plate (1 case). One tumor presented as multiple lesions in the spinal cord. Lastly, the location was unknown in case 8. Headaches, nausea, vomiting, and visual disturbances were the most common presenting symptoms, but movement disorders were present in some infratentorial cases.

The tumors were generally large, ranging from 2 to 8 cm in diameter, and solid, although 5 included a cystic component. In neuroradiologic terms, they were well demarcated, and exhibited variable, sometimes extensive mass effect and also surrounding edema. Minute calcifications were noted in rare cases. Three supratentorial tumors were dura-based. Spinal lesions interpreted as “drop metastases” were identified in 1 patient with a cerebellar tumor, and in 1 with a brainstem lesion, suggesting that like other CNS embryonal tumors ETANTR can disseminate through the cerebrospinal fluid.

In general, magnetic resonance imaging scans revealed hypodensity on T1-weighted (Fig. 1D) and hyperintensity on T2-weighted studies (Figs. 1A, E). Proton nuclear magnetic resonance spectroscopy, performed in cases 19 and 20, revealed a choline peak with a high choline/N-acetyl aspartate ratio suggesting high cellularity (Figs. 1B, C). Patchy postgadolinium contrast enhancement was observed in at least 7 cases, whereas in 6 cases they lacked contrast enhancement (Fig. 1F). Only 1 tumor (case 1) showed diffuse enhancement. The spinal metastases in case 13 were detected using MRI, but the enhancement status of the tumor and other details of the scan are not available.

Clinical follow-up data were available for 25 cases (89%). Nineteen of these patients (76%) died from 1 to 30 months after initial presentation (median survival, 9 months). Most patients showed little or no response to chemotherapy and/or radiation therapy, although the widespread leptomeningeal metastases in 1 patient (case 13) resolved transiently after intrathecal therapy. Only a single long-term survivor (case 12) was alive without evidence of disease at last follow-up (42 months after initial surgery), having received both chemotherapy and radiation therapy (Table 1). The microscopic appearance of this tumor did not differ from that of other tumors in the series. One additional patient (case 26) is alive without recurrent disease 18 months after initial diagnosis.

Histopathologic Findings

Collectively, the tumors were variably cellular, but featured in general a mixture of hypercellular regions and many paucicellular fields composed largely of a fibrillar, neuropil-like matrix occasionally containing rare ganglion cells or scattered microcalcifications (Figs. 2A, B). In some cases, the neuropil had a streaming quality similar to that seen in medulloblastoma with extensive nodularity. The distinctive feature of the ETANTR was the presence of ependymoblastic rosettes. These were sometimes observed in hypercellular areas, but were most distinctive when arising abruptly in the almost anuclear neuropil. Sometimes numerous, they were formed of pseudo-stratified elongate cells circumferentially arranged around a central, circular, or slitlike lumen (Figs. 2C, D). Nuclei were peripherally disposed and fibrillar cell processes extending toward the lumen. In some rosettes, the lumen was filled with granular debris, whereas in others they were clear (Fig 2D). Scattered mitotic figures were noted in the ependymoblastic rosettes. Hypercellular areas were composed of small hyperchromic cells with round to oval nuclei and indistinct cell borders (Figs. 2A, B). Homer Wright rosettes and perivascular pseudo-rosettes were sometimes observed in this tissue component. Mitoses and apoptotic bodies were frequent. Large foci of necrosis were sometimes seen. The tumors generally lacked entrapped, non-neoplastic tissue, but dural infiltration and subpial involvement were evident in some cases. Choroid plexus infiltration

was seen in 1 case. Two tumors were composed mainly of cords and nests of hyperchromatic cells with marked nuclear polymorphism, large nucleoli, cell-cell wrapping as seen in anaplastic medulloblastoma, and atypical mitoses. Both these tumors lacked well-differentiated neuropil-like matrix, but did feature ependymoblastic rosettes (Fig. 2E). Interestingly, the recurrent lesions observed in case 19 showed similar anaplastic features, but lacked the telltale true rosettes (Fig. 2F).

The MIB-1 proliferation index ranged from 10% to over 80% in areas of high cellularity. The ependymoblastic rosettes were also highly proliferative (Fig. 3A). IHC showed almost all the undifferentiated cells to be negative for synaptophysin and neurofilaments, whereas the streaming neuropil stained intensely for these markers of neuronal differentiation (Fig. 3B). NeuN immunopositivity was intense in the nuclei of neurocytic tumor cells in the neuropil regions. Immunostains for the glial marker GFAP highlighted scattered cells with a morphology consistent with reactive astrocytes in most tumors. A few cases contained rare embryonal appearing tumor cells expressing GFAP. A sarcomatous, reticulin-rich component of elongated spindle cells (Fig. 3C) showing diffuse immunoreactivity for smooth muscle actin (Fig. 3D) and focal desmin staining was present in case 22. Immunostains for epithelial membrane antigen were negative in those lesions in which they were performed. In contrast, all tumors tested showed strong and diffuse nuclear immunoreactivity for hSNF/INI1. Cytogenetic studies were performed in 2 cases, showing extra copies of chromosomes 2 and 8 in case 15, and trisomy of chromosome 2 in case 22.

DISCUSSION

We consider the pediatric “embryonal brain tumor with abundant neuropil and true rosettes” to be a unique entity, a subtype of CNS PNET. The reversed male to female ratio of ETANTR patients, with female predominance, is quite different from that of other CNS embryonal tumors. More than twice as many of the tumors in our series occurred in girls ($n = 20$) as boys ($n = 9$). This contrasts greatly with medulloblastoma, ependymoblastoma, medulloepithelioma and AT/RT, in which males outnumber females or the percentage of the two is roughly equal.^{5,10} The relative incidence of ETANTR is not clear, but in our experience it is more common than ependymoblastoma or medulloepithelioma.

The tumor affects infants, often under 2 years of age, with preferential location in the frontal lobes or posterior fossa. Neuroradiologically, ETANTR are distinctive compared with other embryonal tumors. The lesions are in most cases well-demarcated and hyperintense on T2 imaging,^{4,9} and, in contrast to most CNS PNET, generally enhance focally or not at all. The proton nuclear magnetic resonance analysis, as shown in Figure 1 and reported by Fuller et al,⁴ is typical of a highly cellular tumor.¹²

We observed some variation in the microscopic appearance of ETANTR in our series. Histopathologically, ETANTR is distinctive in the combination of abundant neuropil and ependymoblastic true rosettes, although in a few cases, the undifferentiated component predominated, neuropil being scant. This pattern was especially pronounced in some recurrent tumors. For example, case 19, showed progressive loss of neuropil and ependymoblastic rosettes as well as an increase in the large cell (anaplastic) component

when compared with the primary tumor (Fig. 2F). The appearance of the rosettes varied somewhat, with some showing empty lumen, whereas in others the lumen was filled with granular debris. Prior ultrastructural analyses have identified zonula adherens/intermediate junctions between the cells forming the true rosettes, and some basal bodies in the apical cytoplasm, but no fully formed cilia.^{1,3}

ETANTR are microscopically distinct from other CNS PNET. Their extensive neuropil formation distinguishes ETANTR from ependymoblastomas, which also contain ependymoblastic rosettes. Although the occasional finding of large, pleomorphic cells and/or a mesenchymal component raises the problem of differentiating ETANTR from AT/RT, no rhabdoid cells were noted in our tumors. In addition, epithelial membrane antigen immunostains are negative, and strong nuclear immunoreactivity for INI-1 protein was seen in all cases examined.^{7,8} Finally, they lack the long, distinctive surfaces of medulloepithelioma.

The prognosis of children with ETANTR is extremely poor. Patients in our series were often treated postoperative chemotherapy alone or in combination with radiotherapy. Chemotherapy regimens varied but often included cisplatin, vincristine, and cytoxan. Of the 25 cases with available clinical follow-up, 19 patients (76%) died of disease at a short median survival of only 9 months. This is a similar or worse prognosis than is associated with other embryonal tumors occurring in young children. In a recent report on 42 AT/RT patients, 64% died, the median survival being 16 months.⁶ Similarly, in a series of 22 supratentorial PNET patients, 37% experienced a progression-free survival.¹¹ Only 2 children in our series remain disease-free over 1 year after resection. At present, it is unclear whether prognostically favorable subgroups of ETANTR exist.

Only limited cytogenetic data are available, thus making it difficult to determine whether they exhibit a unique genomic alteration as compared to other embryonal tumors. Fuller et al⁴ reported the molecular analysis of 2 ETANTR cases; 1 showed the presence of isochromosome 17q (i17q), a feature common to medulloblastoma, and with the other tetrasomy of chromosomes 2, 8, 17, and 22. Neither tumor showed *N-MYC* or *c-MYC* gene amplification or alteration of *hSNF5/INI-1*. Interestingly, the 2 tumor cases in our study in which cytogenetic analysis was performed revealed gains of chromosomes 2 and 8, as well as trisomy of chromosome 2. In yet another case, Dunham et al¹ found neither amplification of *N-MYC* or *c-MYC*, nor isochromosome 17q (i17q) or changes in chromosomes 2, 8, or 17 by FISH analysis. Overall, this limited molecular characterization suggests that ETANTR could have more frequent gains of chromosome 2 than other defined embryonal neoplasms, such as AT/RT and medulloblastoma.

In summary, we believe that the data derived from this series of 29 cases and other published reports indicate that ETANTR exhibits specific histopathologic features and a characteristic neuroradiologic profile. As such, it appears to represent a distinct entity among embryonal tumors of the CNS (CNS PNET).

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FIGURE 1.

Neuroradiologic features of ETANTR: the MRI of case 19 showed a well-demarcated T2 hyperintense right frontal lesion (A). The proton nuclear magnetic resonance spectroscopy revealed a choline peak (Cho) and a high ratio of Cho/NAA suggesting a highly cellular tumor (B). Case 20 had a well-demarcated T2 hyperintense (C) and T1 hypointense lesion (D) in the pons. Case 29 showed a T2 hyperintense thalamic lesion (E) with no contrast enhancement (F). ETANTR indicates embryonal tumor with abundant neuropil and true rosettes; MRI, magnetic resonance imaging; NAA, N-acetyl aspartate.

FIGURE 2.

Histopathologic features of ETANTR: at high magnification, the hypercellular areas were composed by small hyperchromatic cells with round to oval nuclei and indistinct cell borders (A–B). Ependymoblastic rosettes, the distinctive feature of this tumor, were easily identifiable (C–D). In case 9, the tumor was composed of large elements with pleomorphic nuclei among which distinctive ependymoblastic rosettes were evident (E). The relapsing tumor of case 19 was also composed of large “anaplastic” elements with pleomorphic

nuclei, but lacking rosettes (F). ETANTR indicates embryonal tumor with abundant neuropil and true rosettes.

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FIGURE 3.

Immunohistochemical features of ETANTR: the neoplastic cells, composing the rosettes showed a high proliferation index and scattered mitotic figures (A). The neuropil-like matrix was intensely stained for neurofilaments (B). In case 22, the tumor showed also an unusual sarcomatous-like area composed by elongated spindle-like cells (C), positive for smooth muscle actin (D). ETANTR indicates embryonal tumor with abundant neuropil and true rosettes.

TABLE 1

Cases of ETANTR Presented in this Paper

No.	Age/Sex	Location	Type of Specimen	MRI Features	Treatment	Outcome/FU
1	24/F	R Frontal	Biopsy	Solid, hom enh	Res, Chemo	DOD, 6m
2	30/M	Lt Frontoparietal	Resection	Cystic	Res, Chemo, Rxt	DOD, 11m
3	42/M	Lt-R frontal	Resection	Solid	Res, chemo, Rxt	DOD, 14m
4	12/F	R Frontoparietal	Resection	Solid	Res	DOD, 11m
5	12/F	Pineal, tectal plate	Biopsy	Solid	Res chemo	DOD, 5m
6	24/F	Lt Frontal	Biopsy	Solid	Res, chemo	DOD, 30m
7	36/F	R Frontal	Resection	Solid	Res, chemo	DOD, 7m
8	36/M	NA	Resection	NA	Res, chemo, Rxt	NA
9	11/F	Spinal Cord (multiple)	Biopsy	nh enh	Chemo	DOD, 6m
10	22/F	Pons	Biopsy	Solid, no enh	Chemo	DOD, 7m
11	22/F	Cerebellum	Resection	NA	Res, chemo	DOD, 5m
12	36/F	Lt Frontal	Resection	Cystic	Res, chemo, Rxt	NED, 42m
13	10/M	Cerebellum + spinal mets	Resection	NA	Res, chemo	DOD, < 20m
14	15/F	Cerebellum	Resection	NA	Res, chemo	DOD, 15m
15	16/F	Midbrain + spinal mets	Biopsy	No enh	Chemo	DOD, 2m
16	39/M	R Frontal (dural)	Resection	Cystic, nh enh	Res	NED, 1m
17	36/F	Lt-R Frontal	Resection	Solid, nh enh	Res	RD, 8m
18	17/F	Brainstem	Biopsy	NA	Chemo	DOD, 2m
19	18/F	R Frontal	Resection	Solid, nh enh	Res, chemo, Rxt	DOD, 16m
20	18/F	Pons	Biopsy	Solid, nh enh	Res, chemo	NA
21	9/M	Cerebellum	Biopsy	Solid, no enh	Res, Rxt	RD, 8m
22	18/M	R Frontotemporal	Resection	Solid, nh enh	Res	DOD, 1m
23	12/F	Midbrain	Biopsy	Solid, no enh	Chemo	DOD, 6m
24	48/M	Lt Frontal (dural)	Resection	NA	Res	NA
25	48/F	R Frontoparietal	Biopsy	NA	Chemo, Rxt	DOD, 11m
26	24/F	R Parietal	Resection	NA	Chemo	NED, 18m
27	36/M	Lt Parietal (dural)	Resection	pa cystic, pat, enh	Chemo	NED, 4m
28	17/F	Cerebellum	Resection	pa cystic, no enh	Res, chemo	DOD, 4m
29	24/F	Thalamic	Resection	Solid, no enh	NA	NA

Age is shown in months.

Chemo indicates chemotherapy; DOD, dead of disease; enh, enhancement; ETANTR, embryonal tumor with abundant neuropil and true rosettes; F, female; FU, Follow-up; Lt, Left; < M, male; mets, metastases; m, months; NA, not available; NED, no evidence of disease; nh, not homogeneous; pa, partly; pat, patchy; R, Right; RD, relapsing disease; Res, surgical resection; Rxt, radiotherapy.