Chromosome 17 Abnormalities in Pediatric Neuroblastic Tumor With Abundant Neuropil and True Rosettes

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Abstract

Although as a group, embryonal central nervous system tumors share a common background of primitive round cells, numerous distinctive histologic features allow for further subclassification. One tumor with a unique microscopic appearance is the recently described pediatric neuroblastic tumor with abundant neuropil and true rosettes (PNTANTR). We report 2 additional cases of this unusual tumor; both arose in 4-year-old children, one a midpontine tumor and the other a large cerebral lesion. The tumors contained hypercellular sheets of undifferentiated cells, broad zones of neuropil, and scattered perivascular, Homer Wright, and multilayered ependymoblastic-like rosettes. Isochromosome 17q was detected in multiple samples from one tumor, while the other tumor showed polysomy 17. No deletions of INI1 or amplifications of MYC or MYCN were detected. This report adds 2 cases to our experience of PNTANTR and is the first to demonstrate isochromosome 17q, a molecular alteration typical of medulloblastomas.

Embryonal tumors represent the largest group of malignant brain tumors in children. The World Health Organization classification of central nervous system (CNS) tumors recognizes 5 main histologic subtypes, all of which contain a population of primitive cells with a variable capacity for divergent differentiation.1 Medulloblastomas and supratentorial primitive neuroectodermal tumors (PNETs) are at one end of the spectrum, representing prototypical “small blue cell tumors” with generally monotonous cell populations showing variable degrees of neuronal and, less commonly, glial differentiation. Variants of these tumors have been recognized, with histologic classification dependent on the degree of nodularity and nuclear anaplasia. Three additional embryonal tumors (atypical teratoid/rhabdoid tumor [AT/RT], ependymoblastoma, and medulloepithelioma) harbor distinguishing microscopic features in addition to areas resembling PNET. Although all of these lesions show some degree of histomorphologic similarity, they diverge markedly in their biologic behavior and in their underlying molecular alterations.

Pediatric neuroblastic tumor with abundant neuropil and true rosettes (PNTANTR) is a recently recognized CNS embryonal tumor that displays a unique combination of histologic features overlapping cerebral neuroblastoma (supratentorial PNET with advanced neuronal differentiation) and ependymoblastoma. The largest published series to date describes 7 such lesions, all arising in children 1 to 3 years old and following an aggressive clinical course.2 We describe the clinicopathologic and radiologic features in 2 additional children with PNTANTR. Fluorescence in situ hybridization (FISH) was undertaken to determine whether PNTANTRs show molecular similarity to any of the more conventional CNS embryonal tumors.
Case Reports

Case 1

A 4-year-old boy was examined because of progressive ataxia. Magnetic resonance (MR) imaging showed a 3.9 × 3.7 × 2.9-cm, well-marginated, midpontine lesion that was heterogeneously hypointense on T₁- and T₂-weighted images, with numerous small central and peripheral cysts. The tumor softly enhanced postcontrast. MR spectroscopy demonstrated a markedly elevated choline peak. Spinal imaging and cerebrospinal fluid (CSF) cytology were negative. Surgical debulking was followed by radiation therapy and high-dose chemotherapy. Subsequent MR imaging revealed subtotal resection, with foci of intratumoral necrosis and hemorrhage. The lesion was stable at 19 months of follow-up.

Case 2

A 4-year-old girl was examined because of worsening headaches and visual and gait disturbances and underwent emergency surgery for transtentorial herniation. Postoperative MR showed a large, left parieto-occipital cavity with enhancing hypercellular residual tumor adjacent to a partially thrombosed superior sagittal sinus, together with tumor in the medial right

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**Image 1** A and B (Case 1), Sagittal T₁-weighted (A) and axial fluid attenuated inversion recovery (FLAIR) (B) magnetic resonance images showed a large, well-marginated midpontine lesion that was heterogeneously hypointense on T₁- and T₂-weighted images and softly enhanced after contrast. It contained numerous small central and peripheral cysts. C and D (Case 2), Sagittal T₁ postcontrast (C) and axial T₂-weighted (D) images demonstrated extensive residual hypercellular enhancing tumor along the surgical margin and invasion of the splenium of the corpus callosum.
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er [Image 1C] and [Image 1D]. Spinal imaging and CSF cytology were negative. Despite 3 debulking procedures, radiation therapy, and high-dose chemotherapy, subsequent imaging studies showed progressive disease with extension across the midline via the corpus callosum. The patient died of disease 6 months after initial examination.

Materials and Methods

Formalin-fixed, paraffin-embedded specimens were received as consultation cases at St Jude Children’s Research Hospital, Memphis, TN. Sections from all blocks were stained with H&E for histomorphologic evaluation; Gomori reticulin stain was performed on select sections. We cut 5-µm-thick sections and mounted them on poly-l-lysine–coated slides for use in immunohistochemical and FISH analysis. Clinical data and follow-up were obtained by chart review in accordance with institutional review board approval.

Immunohistochemical and FISH Analyses

Immunohistochemical staining was performed using an indirect avidin-biotin detection system (Ventana IVIEW DAB, Ventana, Tucson, AZ) with an automated immunostainer (Ventana). Pretreatment included blocking of endogenous biotin using Ventana AB Block and epitope retrieval with citrate EDTA (CC1; Ventana). Antibodies included those directed toward glial fibrillary acidic protein (GFAP; 6F2, dilution 1:1,000; DAKO, Carpinteria, CA), synaptophysin (supplied prediluted; Ventana), chromogranin (LK2H10(3), supplied prediluted; Ventana), neurofilament protein (68/200, dilution 1:100; DAKO), and Ki-67 (MIB-1, dilution 1:200; DAKO).

Dual-color FISH assays were performed as previously reported.2 Commercial fluorochrome-labeled probes included chromosome enumeration probe CEP8 and a locus-specific probe targeting 17q25.2 (RP11-87G24, Invitrogen, Carlsbad, CA), 2q35 (contig of CTD-2034E7 and CTD-2355C2, Invitrogen), MYCN (355H10, Invitrogen), and 22q13.3 (RP3-402G11, Invitrogen) were labeled with rhodamine, and those targeting 22q11.23 (contig of RP11-269A19 and 384O8, Invitrogen), and 17p13.3 (RP11-4F24, Invitrogen) were labeled with fluorescein isothiocyanate. Probe pairings were as follows: 17p/17q, INII/22q13.3, MYC/CEP8, and MYCN/2q35. All probes were diluted 1:50 in DenHyb buffer (Insitus, Albuquerque, NM) for dual-target hybridization, and 4,6-diamidino-2-phenylindole (0.5 µL/mL; Insitus) was used as a nuclear counterstain.

Samples in which the majority of nuclei contained signals were considered informative, and 100 to 200 nonoverlapping, intact nuclei were scored for the number of fluorescent signals by 2 reviewers (C.F. and J.D.). Cutoffs for copy number abnormalities were based on counts from nonneoplastic control specimens (normal brain from autopsy cases) for each probe. Interpretation of INII deletion required more than 37% of tumor nuclei containing 1 signal (mean + 3 SDs in control samples). Because cells with 3 signals were not detected in normal control samples, the presence of 3 17q signals paired with 1 or 2 17p signals in greater than 5% of cells was interpreted as gain of 17q, such as that encountered in isochromosome 17q, or i(17q). In addition, specimens were considered amplified for MYC or MYCN when they demonstrated nuclei containing 10 or more MYC or MYCN signals or a MYC/CEP8 or MYCN/2q35 ratio greater than 2.

Results

Tumor samples from both cases showed similar histomorphologic features. Broad islands and nodules of hypocellular neuropil [Image 2A] and [Image 2B] were interrupted by cellular sheets of primitive embryonal cells (small round blue cells). Although these histomorphologic features in isolation are quite typical of cerebral neuroblastoma, the present lesions harbored the additional distinctive feature of scattered true rosettes showing pseudostratification of primitive cells around well-formed lumina; some of these were empty and resembled ependymoblastic rosettes (Image 2B) [Image 2C] and [Image 2D], whereas others had enlarged lumina containing granular or mucinous material [Image 2E]. True rosettes are not considered a feature of cerebral neuroblastoma, and, likewise, broad neuropil-rich islands are not seen in ependymoblastoma. The combination of these findings is, however, quite typical of PNTANTR, as previously reported.2

Additional features we encountered in the present PNTANTRs included occasional papillary or pseudopapillary foci [Image 2D], neoplastic ganglion cells [Image 2G], and GFAP-positive tumor cells [Image 2H]. Rare Homer Wright and perivascular pseudorosettes were present. The mitotic rate was variable from region to region, being higher in hypercellular regions and quite low in regions rich in neuropil. Absent were tubular or papillary neuroepithelial arrangements delineated by basement membranes, a feature typical of medulloblastoma. Synaptophysin [Image 2I], chromogranin, and neurofilament stains were diffusely positive, and Ki-67 confirmed a brisk proliferation rate in both lesions. No cells with a rhabdoid phenotype were detected.

i(17q), a molecular alteration encountered in a significant subset of medulloblastomas but not typical of other conventional CNS embryonal tumors, was demonstrated by FISH in multiple tumor samples from case 2 [Image 3A]. Case 1 showed polysomy (generally tetrasomy) of chromosomes 2, 8, 17, and 22 [Image 3B] (showing tetrasomy 17).
Amplifications of MYC or MYCN (as seen in an aggressive subset of medulloblastomas) or deletions of INI1 (the molecular signature for AT/RT) were not detected in either case.

Discussion

As a group, malignant embryonal tumors of the CNS constitute a significant proportion of brain tumors encountered in the pediatric population. Although all arise from a background of undifferentiated cells, the occurrence of a variety of divergent patterns of differentiation has permitted further subclassification. Distinct entities within this diverse category of aggressive small round cell tumors currently recognized by the World Health Organization include medulloblastoma, ependymoblastoma, supratentorial PNET, medulloepithelioma, and AT/RT. Eberhart et al illustrated a series of unusual CNS embryonal tumors showing combined features of ependymoblastoma and cerebral neuroblastoma. These PNTANTRs occurred in 7 children, ages 1 to 3 years, and were shown as predominantly solid by MR imaging. All but one arose in the frontal lobe; the seventh involved the tectal plate. A 1.5-year-old boy with a cerebellar tumor and a 2-year-old boy with a frontal lobe mass are 2 additional cases cited in an addendum to the article by Eberhart et al. None of the patients in their series had metastatic disease at initial examination, although all tumors were aggressive, with 6 of 9 patients dying of disease within 5 to 14 months of diagnosis.

We report 2 additional cases of this microscopically distinctive tumor, including the first documented occurrence of PNTANTR arising as a well-marginated midpontine lesion and the other a large parieto-occipital mass. Both patients were 4 years old and exhibited no imaging or CSF evidence of disseminated disease at initial examination. Their tumors...
showed variable postcontrast enhancement on MR imaging. The pontine lesion additionally harbored multiple small cysts, an imaging finding encountered in only 1 patient in the series by Eberhart et al. Following subtotal resection, both patients received radiation therapy and chemotherapy. One patient (case 1) remained in stable condition at 19 months of follow-up, whereas the second patient (case 2) experienced rapid tumor progression and died of disease in just 6 months despite extensive multimodality therapeutic intervention.

The tumors in both of our cases were composed of undifferentiated round cells in hypercellular sheets, focally interrupted by broad zones of neuropil containing sparse neoplastic cells. Scattered throughout were multilayered true rosettes reminiscent of ependymoblastic rosettes and occasional perivascular and neuroblastic (Homer Wright) rosettes. Neuropil and tumor cells were immunopositive for synaptophysin, chromogranin, and neurofilament protein in our cases. We detected neoplastic ganglion cells and GFAP+ tumor cells; however, we also encountered areas showing a papillary or pseudopapillary architecture (Image 2D) and rosetted structures containing mucinous material (Image 2E), features not previously described in the series by Eberhart et al. True rosettes were present within hypocellular and hypercellular regions of the tumors.

As stated, PNTANTRs share histologic features of cerebral neuroblastoma and ependymoblastoma. It is not surprising then that at least 2 tumors with morphologic features similar to those we have described herein have appeared in the literature classified as these other entities. Specific molecular alterations have been found to be associated with several CNS embryonal tumor variants. For example, i(17q) is encountered in approximately 40% of medulloblastomas, and amplifications of MYC and MYCN occur with increased frequency in large cell and anaplastic medulloblastomas. Deletions or mutations of the INI1 gene have been observed in the vast majority of AT/RTs. By FISH analysis, we were able to demonstrate the first example of i(17q) in a PNTANTR (case 2). This finding would suggest that despite arising at locations outside the cerebellum, PNTANTR may be related more closely to medulloblastoma than to supratentorial PNET because i(17q) is distinctly uncommon in the latter. In addition, the lack of detectable deletions involving INI1 in our 2 tumors affirms that PNTANTRs are unlikely to be unusual forms of AT/RT.

This report adds 2 cases to our experience of PNTANTR and is the first to demonstrate i(17q), an uncommon molecular alteration in embryonal tumors arising outside the cerebellum. Their histologic appearance is quite distinctive with overlapping features of ependymoblastoma and cerebral neuroblastoma; a papillary or pseudopapillary architecture and enlarged rosetted or pseudoglandular structures containing mucinous material may be encountered. Although this and
previous reports suggest that PNTANTRs are biologically aggressive tumors, further experience will be necessary to determine the best clinical management for these patients.

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