FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 42, No. 4, 2004
pp. 229-234

Heterogeneity of extraparenchymal primitive neuroectodermal tumors within the craniospinal axis

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Abstract: Four cases of primitive neuroectodermal tumors (PNETs) with unusual localization (three intraspinal extramedullary and one pontocerebellar) are reviewed. Histologically, they were small round blue cell tumors with diverse patterns. Immunohistochemically, all tumors were positive for at least two neuronal markers, two cases were Mic-2 positive and one showed glial differentiation. The paraffin-embedded tumor specimens were examined by interphase FISH using dual-color probes specific for *EWS*, *HER-2* and *BCR* loci. Molecular cytogenetic study revealed the presence of *EWS* rearrangement in two cases and the presence of i(17q) in one tumor. Three tumors exhibited 22 disomy and one was 22 polyploid. Extraparenchymal PNETs within craniospinal axis are heterogeneous from the clinical, histological, immunohistochemical and molecular point of view. These PNETs can be of a central or peripheral type. Multidisciplinary approach is of a basic importance in differential diagnosis of such cases.

Key words: PNET - Ewing's sarcoma - Medulloblastoma - FISH - *EWS* gene, i(17q)

Introduction

Primitive neuroectodermal tumors (PNETs) comprise a heterogeneous group of neuroepithelial small round blue cell tumors. They can develop within or outside of the central nervous system (CNS), being described as central or peripheral PNETs. PNETs reveal a spectrum of patterns evident at morphological, immunohistochemical and molecular levels [1, 17, 21, 26].

The most frequently diagnosed central PNET is cerebellar medulloblastoma, which shows isochromosome 17q as the most characteristic cytogenetic abnormality [2, 4]. The other central PNETs are tumors morphologically undistinguishable from medulloblastoma, located within cerebral hemispheres, pineal gland and pituitary [17]. These PNETs show a spectrum of non-random chromosomal structural aberrations or losses, detected by karyotyping or by loss of heterozygosity (LOH) allelotyping, which include deletions of chromosomes 5q, 6, 9, 10, 11, 16q and 22 [2, 3, 21]. LOH on chromosome 22 is also a feature of some other CNS tumors, such as meningiomas, ependymomas, gliomas, and atypical rhabdoid tumors [3, 4].

The peripheral PNETs belong to the pPNET/ Ewing sarcoma group of tumors [9, 26]. They develop usually in bones and less frequently in soft tissue of the lower extremities, as well as within paravertebral and retroperitoneal regions [20, 24, 26] . This group of neoplasms is characterized by the presence of abundant Mic-2 surface protein and common t(11;22)(q24;q12) translocation [9, 17, 25]. Due to this translocation, in 85% of cases, specific gene rearrangement occurs, leading to *EWS*/*FLI1* gene fusion [1, 19, 26]. In rare cases, *EWS* translocates to other gene partners, creating as a consequence different chimeric fusion proteins [17, 26, 29].

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Table 1. Clinical data of patients with PNETs

Patient	1.	2.	3.	4.
Age/ sex	26 years/ male	13 years/ female	26 years/ female	9 years/ female
Site of primary tumor	Intra-dural C4-6, C5 root infiltration	Epidural C7-Th11, dural infiltration	Cerebello-pontine angle	Epidural Th11-L2, para-vertebral
Treatment	Partial excision, radiotherapy	Open biopsy, radio-therapy, chemotherapy	Total excision radiotherapy	Subtotal excision, chemotherapy
Metastases	Dissemination via CSF	Spine, lungs, bone marrow	Not detected	Not detected
Follow-up	Died 3 months after diagnosis	Died due to dissemination after 5 years	3.5 years, no symptoms	3.5 years, no symptoms

Extraparenchymal PNETs localized within the craniospinal axis are very rarely encountered. Only few such cases are reported, indicating the heterogeneous nature of these neoplasms [7, 18, 28]. We review herein our own four cases.

Materials and methods

All cases were diagnosed at the Department of Pathology, Medical University of Gdańsk. Clinical history was available in each case. Patients 1, 2 and 3 have been reported previously in separate case studies [14-16]. Clinical data of all patients are summarized in Table 1.

Formalin-fixed, paraffin embedded tumor sections were examined histologically by routine H-E staining.

Immunohistochemical evaluation was performed according to standard streptavidin technique with the appropriate positive and negative controls, using LSAB method. The following antibodies (DAKO Glostrup, Denmark) were employed: MIC-2 (CD99), leukocyte common antigen (LCA), vimentin, synaptophysin, neuron-specific enolase (NSE), glial fibrillary antigen (GFAP), cytokeratin AE1/AE3, Cam 5.2, epithelial membrane antigen (EMA), desmin, α-smooth muscle actin, sarcomeric actin, myoglobin , chromogranin, neurofilament protein, NB84 and CD56 (NCAM- Novocastra).

Interphase fluorescence *in situ* hybridization (FISH) was performed on nuclei isolated from paraffin-embedded tissue, according to the protocol described previously [12]. The following probe sets were used: biotin-labeled cosmid G9 and digoxigenin-labeled cosmid F7 (their corresponding loci flank *EWSR1*/22q12 region) DNA probes and dual-color Spectrum-Orange LSI HER-2/neu (17q11.2)/Spectrum Green CEP17 (17p11.1-q11.1; Vysis Inc., Stuttgart, Germany) DNA probes. A locus- specific LSI22q (BCR) probe was used for chromosome 22-copy enumeration.

Hybridization and detection was performed as previously described [9]. Hybridization signals were visualized using an epifluorescence microscope (Leica DMRB, Wetzlar, Germany) equipped with a cooled CCD camera and processed by image analysis software (QUIPS, Vysis, IL, USA).

Results

Pathology

All cases were highly cellular, composed of mitotically active small blue cells with high nucleo-cytoplasmic ratio. Differential diagnosis of PNET included neuroblastoma, lymphoma and rhabdomyosarcoma in children, and lymphoma, poorly differentiated small cell sarcomas and metastatic small cell carcinoma in the adults.

Cases 1, 2, 3 had monomorphic cellular composition with different tissue patterns: solid cohesive, creating sheets and in case 3 complex-lobular and microcystic. Case 4 had two cellular populations: small cells in solid cohesive areas and larger cells in a nested pattern with rosettes and necrosis. All cases had rich delicate vasculature, made of small vessels without parietal proliferation (Fig. 1).

Histological and immunohistochemical features of tumors are summarized in Tables 2 and 3 (Fig. 2).

Immunostaining for cytokeratin, chromogranin, neurofilaments, Cam5.2, EMA, desmin, ASMA, sarcomeric actin, myoglobin, LCA and NB84 were all negative.

FISH analysis

The results of interphase FISH studies are presented in Table 4. In summary, FISH revealed the presence of *EWS* rearrangement in two cases (case 2 and 4) and the presence of i(17q) in one tumor (case 3). Case 4 displayed also overrepresentation of chromosomes 17 and 22. Most likely, the rearrangement of *EWS* in cases 1 and 4 indicated the presence of t(11;22), since this variant is most common in PNET/ES family of tumors. The presence of i(17q) in case 3 suggested the occurrence of central PNET of medulloblastoma type (Fig. 3).

Discussion

The combination of clinical, histological and immunohistochemical features allows categorization of the majority of PNETs. However, in some cases an immunohistochemical profile is not always specific enough, requiring the molecular approach in search for a specific gene rearrangements, for instance toward *EWS* gene involvement [5, 17, 25, 26].

In the presented cases, diagnosis of PNET was established on the basis of histological and immunohistochemical features of the tumor tissue. Further diagnosis

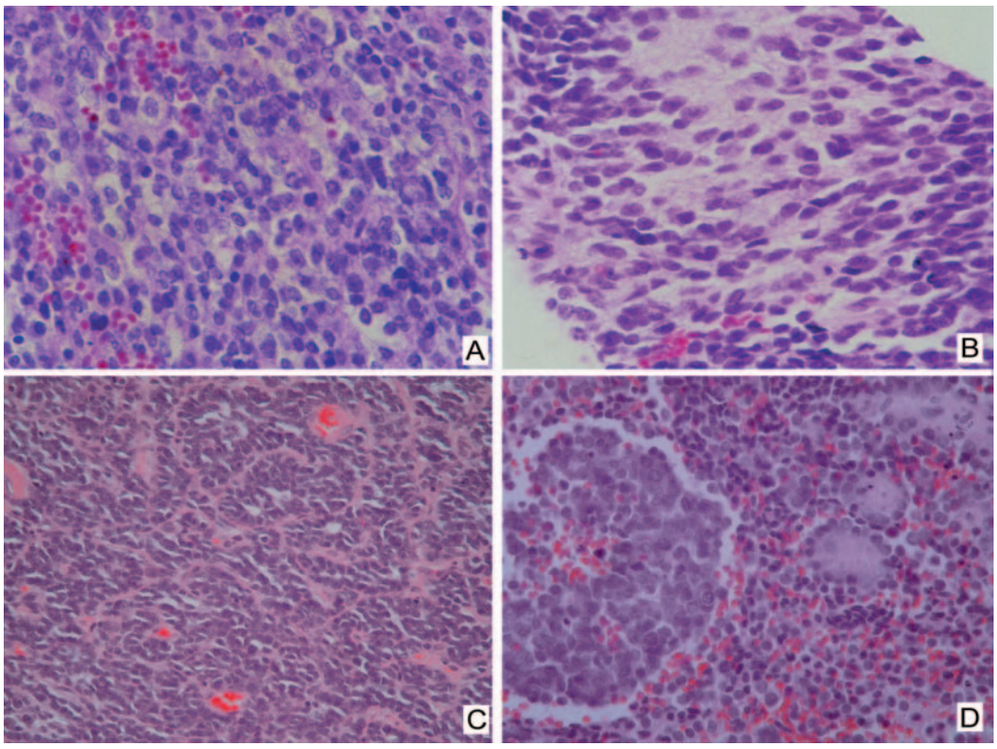

Fig. 1. Histological pictures of tumors: **A** - case 1, **B** - case 2, **C** - case 3, **D** - case 4; H-E.

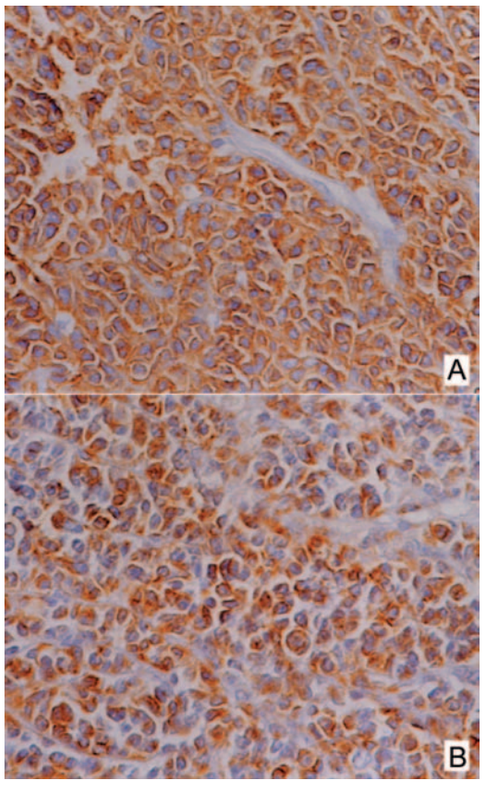

Fig. 2. Immunohistochemical staining: **A** - case 4, strong membraneous CD99 reactivity; **B** - case 3, cytoplasmic GFAP deposits.

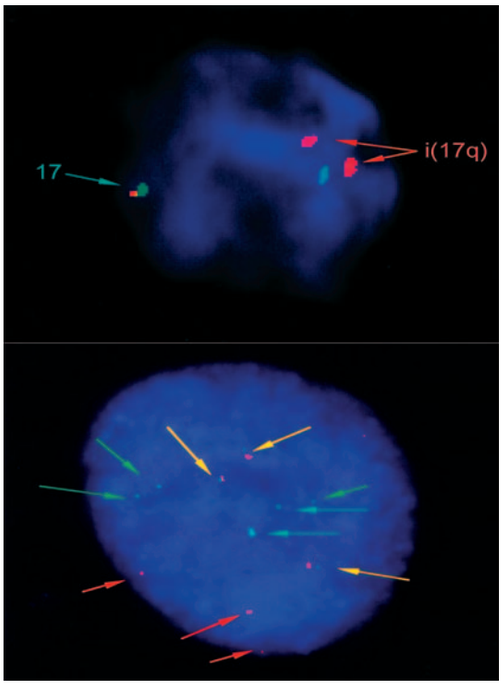

Fig. 3. FISH. Upper half - case 3: isochromosome 17q (association of two red signals 17q11.2 with green signal p11.1-q11.1). Lower half - case 4: increased number of signals from chromosome 22 (green arrows); fusion (yellow arrows) of red (cos F7) and green signals (cos G9) prove rearrangement of EWS region.

Table 2. Histological features of the examined tumors

Case	1.	2.	3.	4.
Cells	Round, elongated	Round, oval	Round, carrot-shaped	Round- small and large
Tissue pattern	Solid cohesive	Sheets, lobules	Lobular, microcystic	Solid, nested
Rosettes	Scattered	Scattered	Scattered	Scattered
Necrosis	Focal	–	Focal	Focal
Vessels	Delicate	Delicate	Delicate	Delicate
PAS staining	–	–	–	Focal

Table 3. Immunostaining of the examined tumors

Case	1.	2.	3.	4.
NSE	Focal	+	+	+
Synaptophysin	Focal	+	–	+
GFAP	–	–	+	–
NCAM	–	+	+	–
S100	+	+	+	+
Mic-2	–	+	–	+
Vimentin	–	–	–	Focal

Table 4. FISH results in the examined tumors

Probes	Case			
	1	2	3	4
EWS flanking probes	No rearrangement	Rearranged	No rearrangement	Rearranged
Iso 17q	Absent	Absent	Present	Absent, 17 poliploidy
BCR(22q12)loci	22 disomy	22 disomy	22 disomy	22 poliploidy
Final diagnosis	PNET not otherwise specific	PPNET/ES	Central PNET-MB	PPNET/ES

was supported by FISH analysis, which revealed molecular differences between these tumors. Two tumors showed rearrangement of *EWS* gene characteristic for pPNET/ ES, one had iso17q typical for medulloblastoma, and in one no changes were found with the applied probes. Loss of heterozygosity in chromosome 22 was not revealed in any case. Case 4, however, showed *EWS* rearrangement together with polyploidy of chromosomes 17 and 22.

Several extraparenchymal CNS PNETs of leptomeningeal, dural and of nerve root origin have been reported up to now [7, 10, 13, 28]. Only in some of them molecular analysis was performed despite of the fact that this information carries therapeutic and prognostic implications [7, 13]. The paper of Dedeurwaerdere *et al.* [7] yields a current review of literature concerning dural intracranial and intraspinal cases with t(11;22). Previously Deme *et al.* [8] presented the review of 13 cases of spinal intramedullary and intradural PNETs from the literature, but cytogenetic data were unavailable there. Similarly, most of several reported pontocerebellar medulloblastomas in adults lack cytogenetic or molecular analysis, so it seems that some of them are in fact intracranial cases of PNET/ES [18, 27].

All our patients were under 40 years of age, similarly to the literature reports [7, 10, 18, 28]. Three of our tumors had intraspinal extramedullary location. Case 1 probably originated from cervical nerve root or spinal dura. Cases 2 and 4 were lumbar epidural tumors (the first with dural implants and systemic metastases, and the second with extension into the paraspinal muscles). The intracranial case 3, which was clinically manifested as meningioma seemed to be in fact an extracerebellar medulloblastoma. Two patients died due to neoplastic dissemination, and two, who were free of metastases at the clinical presentation, are alive and asymptomatic. Although the long clinical course is reported in up to 45% to 60% of PNET-ES cases, general prognosis is bad [23, 24]. An aggressive growth with extension into the paraspinal muscles and progression with CSF seeding or

distant metastases is characteristic for these neoplasms [8, 10, 20, 23]. Among patients with intracranial central PNETs, long-term survival is uncommon, although adult medulloblastomas show better prognosis [17, 22]. The known prognostic factor is the stage of the disease at the diagnosis [23, 24].

Neural differentiation is characteristic and diagnostic for most of PNETs and is also accepted as the feature distinguishing pPNET from ES in this family of tumors [13, 20, 24]. Schmidt *et al.* [24] defined pPNET by the presence of rosettes or immunoreactivity with at least two neuronal markers. All our tumors showed these features. Our pontocerebellar medulloblastoma presented in part an unusual microcystic pattern. The parallel expression of GFAP and neuronal markers revealed bipotential differentiation in this tumor. In the study of Nicholson *et al.* [21] on central PNETs, tumors with i17q were GFAP- negative. Amann *et al.* [1] examined relation of neuroglial marker expression and *EWS* gene fusion types in osseous PNET/ES. Only few of their cases were immunopositive for more than two neuroglial markers and a correlation of immunophenotype with molecular features was not found. The authors observed, however, some relations between fusion type and tumor histology. The morphology of our case 2 was quite typical for PNET, since case 4 showed multiple molecular changes together with complex histological features.

The Mic-2 gene product (CD99) is highly expressed in nearly all peripheral PNET-ES but it is not their specific marker [5, 11]. Central PNETs are CD99-negative [7, 13, 17]. Both our tumors with *EWS* rearrangement showed Mic-2 expression. In one of them, NCAM reactivity was found. This was rather an unusual finding, since commonly an inverse correlation exists between these two markers [11].

Lack of *EWS* rearrangement and Mic-2 expression in case 1 most likely excludes diagnosis of pPNET/ES [25, 26], although the presence of another translocation variant can not be ruled out [29]. It can be also a central type tumor with changes not detectable by our probes. Current literature on central PNET analysis with comparative genomic hybridization, spectral karyotyping and FISH [2, 3] shows involvement of different chromosomes including 7, 17, 10 and 22.

Extraparenchymal PNETs within craniospinal axis are heterogeneous from the clinical, histological, immunohistochemical and molecular point of view. In addition to immunohistochemistry, these tumors need to be diagnosed with molecular methods to resolve diagnostic difficulties and to optimize the therapy.

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Accepted April 26, 2004