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CASE REPORT

Cytogenetic and Histopathologic Studies of Congenital Supratentorial Primitive Neuroectodermal Tumors: A Case Report

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Primitive neuroectodermal tumors (PNET) represent about 25% of primary central nervous system tumors in childhood, but congenital PNETs are rare. Cytogenetic studies and studies on molecular pathology have identified several genetic alterations in medulloblastoma, but molecular investigations on supratentorial PNETs are infrequent. We present a male newborn with a large congenital PNET of the right cerebral hemisphere and the molecular analysis of the tumor. Tumor tissue was investigated by routine histology and immunohistochemistry. Fluorescence in-situ hybridization was carried out on native tumor tissue to investigate deletions on chromosome 17p and to analyze c-Myc or N-Myc amplifications. Histologic examination revealed a primitive neuroectodermal tumor with massive extension covering almost the entire right hemisphere. Genetic analysis of the native tumor tissue of our patient excluded a deletion of chromosome 17p. An amplification of the c-Myc or N-Myc oncogene was absent using fluorescence in-situ hybridization. Despite unremarkable genetic analysis in our case prognosis was poor, suggesting that there are additional, yet unknown constitutional genetic aberrations in the pathogenesis of congenital supratentorial PNET. (Pathology Oncology Research Vol 7, No 1, 67–71, 2001)

Keywords: PNET, primitive neuroectodermal tumor, brain neoplasm, congenital brain tumor, cytogenetic studies

Introduction

Primitive neuroectodermal tumors (PNETs) are highly malignant embryonal tumors which arise mainly in the cerebellum (80%), commonly described as medulloblastoma, but are histologically indistinguishable from supratentorial PNET (stPNET).[3,7,19] Histopathology shows a predominant population of small cells which contain only scanty cytoplasm. Focally there may be areas resembling neuronal, glial, ependymal, muscular or melanotic differentiation in the tumor.[19]

Supratentorial PNET represent about 13% to 20% of all PNET in several series on childhood brain tumors, including stPNET,[1,2,4,8,10,12,17] accounting for about 1-2% of all childhood brain tumors. Congenital brain tumors are rare childhood tumors when considered as tumors present at birth and then being congenital by definition. However, when childhood brain tumors, arising within the first year of life are also considered as probable or possible congenital brain tumors many more tumors fall into this group.[9] Definitely, congenital supratentorial PNETs have been reported only occasionally.[10]

Several factors contribute to an increased risk of recurrence and poor clinical outcome of PNET: an age less than 3 years[8,26] and the presence of spinal or CSF-metastases.[5] Histologic features, especially glial differentiation, have also been correlated to poor outcome.[12]

There are several reports on chromosomal alterations in childhood PNET/medulloblastoma and their putative role in tumorigenesis. A variety of chromosomal gains and losses have been demonstrated, a gain of genetic material on chromosome 17q in form of an isochromosome i(17q)[25] or loss of heterozygosity on chromosome 6q, 16q, or 17p.[18] A consecutive loss of tumor suppressor genes leading to tumor promotion especially in the region 17p was discussed.[21,25] Furthermore, mutations of

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Abbreviations: StPNET – supratentorial primitive neuroectodermal tumor; FISH – fluorescence-in-situ-hybridization

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the tumor suppressor gene p53 have been identified in the 17p region in rare cases.[16] An amplification of the c-Myc or N-Myc oncogene in medulloblastoma was rarely demonstrated.[18,28]

The rarity of PNET at sites other than the posterior fossa has precluded larger cytogenetic studies on stPNET.[15] In the presented case we analyzed the heterozygosity on chromosome 17p and a possible amplification of the c-Myc oncogene on chromosome 8q21 and N-Myc in the native tumor.

Case report

After uneventful pregnancy the male newborn was delivered by cesarean section due to delayed progress of delivery. Postnatal adaptation was complicated and assisted ventilation became necessary for one day. A macrocephalus with a prominent and tense anterior fontanella and widened cranial sutures were present. The head circumference was 4 cm above the 97th percentile (41cm). The muscular tone especially of the left hemisphere was elevated, a facial palsy and a spontaneous Babinski sign was present on the left side. Cranial ultrasound and computed tomography revealed an enormous tumor of the right hemisphere with signs of necrosis and hemorrhage in its central areas.

The midline was shifted to the left and the cerebral ventricles were enlarged due to compression of the aqueduct (***Figure 1a***). Due to the obvious brain damage, young age and thus poor prognosis no oncological or surgical treatment was carried out. The boy died after 4 days due to respiratory failure. Post mortem examination of the brain revealed a stPNET which had compressed and infiltrated the right hemisphere except a small part of the frontal lobe (***Figure 1b***).

Materials and Methods

Histopathology and immunohistochemistry

Specimen for light microscopy was fixed in 10% buffered formalin and was subjected to routine procedures for paraffin embedding. Four-micrometer-thin sections were prepared from paraffin blocks and were stained with hematoxylin-eosin. Immunohistochemical characterization on paraffin sections was performed using the standard avidin-biotin-peroxidase-complex (Biogenix, USA) method. Primary antibodies antibodies to glial fibrillary acidic protein (GFAP), synaptophysin, neurofilaments, S100-protein, neuron-specific-enolase (NSE), desmin, epithelilal membrane antigen bcl-2 (all antibodies by DAKO, Denmark), keratins (KL1, Immunotech, France), MIB-1 (Dianova, Germany), Cam5.2 (Becton Dickinson, Belgium), were used for immunostaining after pretreatment of slides by microwave heating in citrate buffer for antigen retrieval.

Genetic analysis

Fluorescence ***in-situ*** hybridization (FISH) was performed on interphase nuclei that were prepared from deep frozen tumor tissue. PNET tissue was cut in small pieces and incubated in pronase (100 µg/ml). Cells were washed extensively in phosphate buffered saline, fixed in 1% paraformaldehyde and dehydrated in ethanol. YAC 677H10 was used as a specific probe for chromosome 17p13.3.[29] It has previously been shown to be deleted in all medulloblastomas and PNET with loss of heterozygosity (LOH) of chromosome 17p.[22] Cosmid cos-Myc 72 encompasses the c-Myc gene and was used for detection of c-Myc amplification in the tumor tissue.[11] YAC-DNA was amplified using interspersed long-

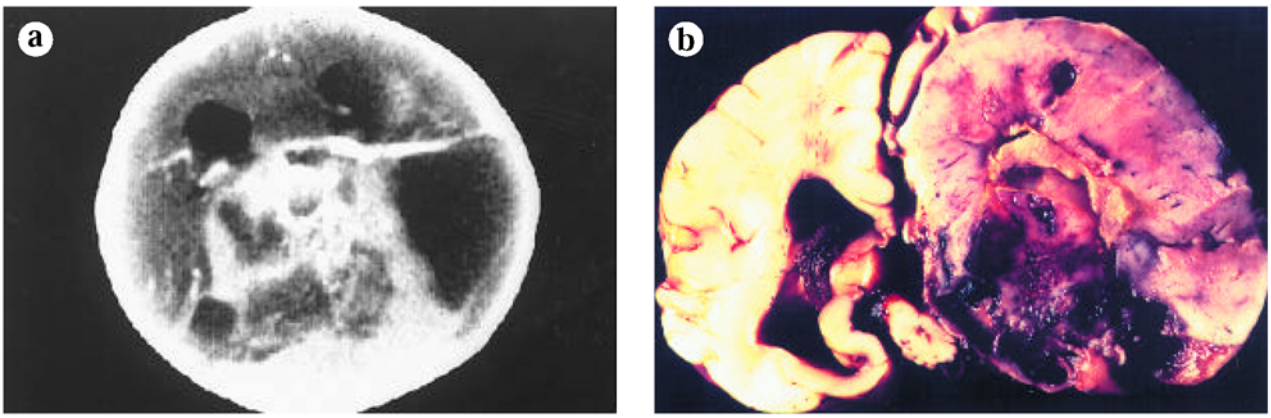

Figure 1. *Radiologic and macroscopic appearance of large congenital stPNET* ***a)*** *Contrast-enhanced computed tomography of the skull shows the massive tumor of the right hemisphere with a complex morphology of cystic and solid areas in the tumor with intralesional hemorrhage. The circulation of the cerebrospinal fluid is impaired presenting as hydrocephalus because of compression of the aqueduct.* ***b)*** *The entire right hemisphere consists of a greyish fleshy tissue with a multicoloured appearance with necrotic, hemorrhagic and cystic areas on cut surface.*

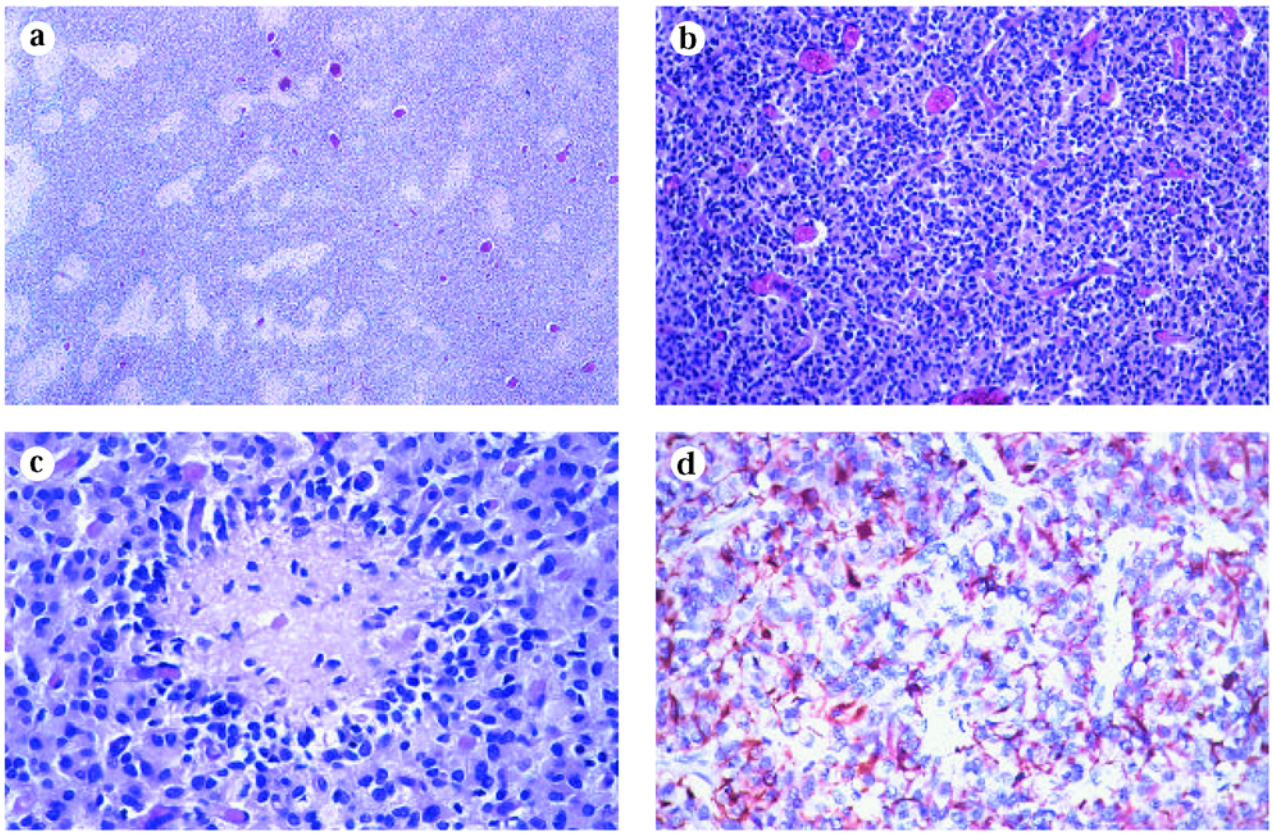

Figure 2. *Histopathology of congenital stPNET* ***a)*** *Low magnification shows a small cell tumor infiltrating adjacent brain, leaving out round and irregular shaped islands of brain tissue (Hematoxylin-eosin, original magnification x 30).* ***b)*** *The tumor is of high cellularity, consists of small tumor cells and is well vascularized with a dense network of small capillary vessels (Hematoxylin-eosin, original magnification x 120).* ***c)*** *Higher magnification shows an island of brain tissue which is surrounded by infiltrating tumor cells arranged in a palisading pattern (Hematoxylin-eosin, original magnification x 240).* ***d)*** *Immunohistochemistry shows expression of GFAP in numerous tumor cells (original magnification x 240).*

range polymerase chain reactions (IRS-LR PCR) as previously described.[30] For fluorescence ***in-situ*** hybridization the PCR product of cosmid DNA was labeled with digoxigenin-11-dUTP by nick translation and used as probes for FISH to human interphase nuclei.[30] The N-Myc gene was analyzed using a commercially available DNA-probe (Appligene/Oncor, Heidelberg, Germany). Hybridized probes were detected via fluorescein-conjugated anti-digoxigenin antibody and the interphase nuclei were counterstained with 4,6-diamino-2-phenylindole-dihydrochloride (DAPI).

Results

Gross pathology and histology

The entire right hemisphere except the anterior part of the frontal lobe consisted of a greyish fleshy tissue. On cut surface, the tumor demonstrated a multicoloured appearance of greyish tissue with hemorrhagic, cystic and necrotic areas (***Figure 1b***).

Hematoxylin-eosin stains showed a small cell tumor of high cellularity, well vascularized with a dense network of small capillary vessels (***Figure 2a, 2b***). The tumor demonstrated an unusual infiltration pattern of adjacent brain, leaving out round and irregular shaped areas of brain tissue which were surrounded by tumor cells arranged in a palisading pattern (***Figure 2a, 2c***). Numerous tumor cells showed expression of GFAP (***Figure 2d***), S100-protein, vimentin and NSE by immunohistochemistry. Mitotic figures were infrequent and the proliferation index (MIB-1) was about 5% of all tumor cells. No expression was detected of synaptophysin, neurofilaments, keratins, desmin, EMA, CD34, cyclin D1 and bcl-2 in tumor cells.

Genetic analysis

Two specific signals were detected after fluorescence ***in-situ*** hybridization of tumor cell interphase nuclei using YAC 677H10 as a specific probe for chromosome arm 17p indicating preservation of both alleles.

Similarly, two distinct signals were observed after FISH with cosmid cos-Myc 72 and a commercially available clone for N-Myc confirming, that the c-Myc and the N-Myc oncogene were not amplified in the tumor cells.

Discussion

Supratentorial PNETs seem to be more aggressive and do not respond to treatment as well as medulloblastoma.[27] Recently prognostic factors including clinical data and genetic analysis of stPNET have been reported: the pineal location and a lower M-stage were predictors of a better outcome.[12] Younger children more frequently had advanced disease and worse outcome.[12] In contrast, a more favorable outcome of congenital stPNET had been reported by Haddad, who found a survival of 2 and 9 years in children with complete tumor removal and chemotherapy.[8]

In addition to clinical staging, the differentiation of medulloblastoma / PNET also seems to be of prognostic importance as tumors with glial differentiation, like in our case, were found to show a worse outcome.[17,19]

There are conflicting data on the clinical outcome and prognosis and the impact of genetic lesions in childhood PNET and medulloblastoma and their putative role in tumorigenesis. The loss of heterozygosity (LOH) of chromosome 17p is the most frequently described chromosomal aberration, present in up to 40–50 % of medulloblastomas.[22] Previous suggestions, that biallelic loss of the tumor suppressor gene TP53 located on chromosome 17p13.3 may play a role in tumorigenesis were not confirmed by different investigators, as less than 15% of medulloblastomas display biallelic inactivation of the TP53 gene.[16] Uniform impact of LOH of chromosome 17p on the prognosis of the patients could not be demonstrated.[6] The YAC clone 677H10 used for FISH of chromosome 17p in our study encloses the TP53 gene. As both alleles were preserved in the stPNET of our patient, it seems improbable, that biallelic inactivation of the TP53 plays a role in pathogenesis of the tumor presented.

Amplification of the c-Myc oncogene was demonstrated in less than 10% of medulloblastoma[18,28] suggesting a minor role in tumorigenesis. However, we recently found an association of c-Myc amplification as double minutes with early relapse and fatal outcome in childhood medulloblastoma.[23] The early arising, highly malignant stPNET, described in this study harbored two distinct copies of the c-Myc oncogene without any hint for amplification. A previously reported molecular analysis of medulloblastomas occuring simultanously in monocygotic twins also lacked aberrations of chromosome 17p and amplifications of c-Myc.[24] Other investigators have shown that increased expression of N-Myc may be an indicator for poor prognosis in medulloblastoma and PNET.[14] In addition, different patterns in the expression of the neurogenic basic helix-loop-helix class of transcription factors have been implicated in the pathogenesis of neuroectodermal tumors recently.[20] Despite unremarkable genetic analysis in our case prognosis was bad, suggesting that there may be additional, yet unknown, constitutional genetic aberrations in the pathogenesis of congenital stPNET.

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