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Two Malignant Peripheral Primitive Neuroepithelial Tumor Cell Lines Established From Consecutive Samples of One Patient: Characterization and Cytogenetic Analysis

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A 6-year-old girl presented with a tumor of the right shoulder involving bone, adjacent soft tissue, and regional lymph nodes. The conventional histologic diagnosis was ambiguous, initially suggesting lymphoma. After relapse on lymphoma therapy, reevaluation with additional multiple diagnostic techniques performed on the biopsy tissue and on two cell lines derived from the biopsies established the diagnosis of a primitive neuroepithelial tumor of bone and soft tissue. This was strongly supported by 1) focal rosette formation by the tumor cells and positive immunostaining for neuron-specific enolase and synaptophysin, with absent staining for leukocyte common antigen; 2) at the ultrastructural level, formation of cellular processes containing microtubules, a paucity of neurosecretory granules, absence of synaptic junctions, formation of long "intermediate" junctions between cells, and, in culture, widespread development of rosettes; 3) marked surface positivity to W 6/32 and negativity to HSAN 1.2 antibodies; and 4) elevated expression of *MYC* and lack of overexpression of *MYCN* oncogenes. Numerical and structural abnormalities were present in the karyotype, but the expected t(11;22)(q24;q12) was not present in the tumor-involved marrow or in either of the established tumor cell lines, although there was an interstitial deletion of 11q involving breakpoints in q21 and q23. *Genes Chrom Cancer 4:195–204 (1992).*

INTRODUCTION

The poorly differentiated, round-cell pediatric tumors, which include lymphoma, rhabdomyosarcoma, neuroblastoma, Ewing's sarcoma, and central and peripheral primitive neuroepithelial tumors (PNET), present a problem in differential diagnosis. The term peripheral PNET is used to describe a group of tumors of presumed neural-crest origin that arise outside the brain, spinal cord, and sympathetic nervous system and have been reported under various terms such as peripheral neuroepithelioma, peripheral medulloepithelioma, malignant small-cell tumor of the thoracopulmonary region in childhood (Askin's tumor), and ectomesenchymoma (Boccon-Gibod, 1984; Moll et al., 1987; Coffin and Dehner, 1989; Henderson et al., 1989; Llombart-Bosch et al., 1989; Marina et al., 1989; Schmidt et al., 1989). These tumors have features in common with neuroblastoma and extraosseous Ewing's sarcoma, and their diagnosis and nosology (Dehner, 1986; Triche, 1986; Rubinstein, 1987) are still surrounded by controversy. Recent evidence from ultrastructural, immunologic, cytogenetic, molecular-genetic, and biochemical analyses suggests that there is an overlapping spectrum of neural differentiation in this group of tumors (Cavazzana et al., 1987).

In the past, it has been difficult to differentiate between classic neuroblastoma and PNET. Compelling data have recently been presented for distinguishing these two tumors, based on findings that neuroblastoma patients are usually under 5 years old, their tumors secrete catecholamine in >95% of cases, and more than 70% of neuroblastomas exhibit a deletion or an unbalanced translocation of the short arm of chromosome 1 (1p). In addition, double minute chromosomes (dmin) and homogeneously staining regions (HSRs) are a common feature in neuroblastoma (Christiansen and Lampert, 1988; Brodeur and Fong, 1989; Triche and Cavazzana, 1989). In contrast, PNET occurs in older patients; these tumors only rarely secrete catecholamines; and a nonrandom translocation, t(11;22)(q24;q12), has been reported (Whang-Peng et al., 1984, 1986; Lopez-Gines et al., 1988). The cytogenetic analysis often has diagnostic relevance in small round-cell tumors of children, as reported recently by Fletcher et al. (1991). In addition, it has been demon-

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strated that the immunophenotypes and the expression of two oncogenes differ between neuroblastomas and PNET (Donner et al., 1985; Thiele et al., 1987). We report here the characterization of two tumor cell lines that did not exhibit the common translocation t(11;22) that were established from a patient who fulfilled the criteria for PNET.

MATERIALS AND METHODS

Case Report

A 6-year-old girl presented with pain and swelling of the right shoulder. The right supraclavicular and axillary lymph nodes were clinically enlarged. Imaging showed minimal bone destruction of the spine of the right scapula, surrounded by soft-tissue swelling. Technetium bone scan and gallium scans showed increased uptake in the scapula and its spine. Biopsy of a supraclavicular node was initially interpreted as non-Hodgkin's lymphoma, and she was started on a chemotherapy protocol appropriate for this tumor, with slow response of local symptoms and signs. Pretherapy staging showed no evidence of metastatic spread to the skeleton, lungs, or bone marrow.

While still on lymphoma chemotherapy, she developed clinical and marrow evidence of tumor recurrence at 9.5 months after diagnosis. Examination of the tumor cells suggested neuroblastoma or PNET. The first cell line (PER-352) was established from marrow at this time. Urinary catecholamines (VMA and HVA) were normal, and computed tomography (CT) scanning failed to demonstrate a suprarenal or other primary tumor. Serum neuron-specific enolase (NSE) was elevated to 95 μg/liter (normal <15 μg/liter). Retrospective analysis of serum from the time of original diagnosis showed that NSE had been 108 μg/liter initially, falling to 8 μg/liter after chemotherapy.

She developed a further clinical recurrence with cervical lymphadenopathy and a subcutaneous scalp deposit while undergoing chemotherapy consisting of cis-platinum, adriamycin, cyclophosphamide, and VP16. The second cell line (PER-364) was established from a lymph node biopsy at this time. Evidence that the tumor was PNET (see below) led to the institution of Iphosphamide plus VP16 chemotherapy plus local radiotherapy to both the cervical and the scalp tumors, with a temporary clinical response. Serum NSE levels again rose at the time of this secondary recurrence. Eventually, the tumor became refractory to all therapy, including carboplatinum administration. Bone pain developed, and she was treated with palliative home care but died 21 months after the diagnosis. Postmortem examination was not performed.

Establishment of Cell Lines

Tumor cell lines PER-352 and PER-364 had been established from biopsy samples of the first and second recurrences 5 months apart (see above). The method for establishing long-term cell cultures from bone marrow biopsies has been described in detail (Kees et al., 1987). The same method was used for the lymph node sample, except that we put the tissue through a stainless-steel sieve to obtain a single-cell suspension.

The cells were cultured in 24-well Nunclon plates (catalogue No. 1-43982) in a humidified 37°C incubator containing 5% CO^2 in air. Fifty percent of the medium was replaced by fresh medium every third day, and cultures were split provided they had reached cell concentrations of 3×10^6/ml. Both cell lines have been in continuous culture for 13 months, and all results reported here have been obtained from cells cultured for 4–12 months. The cell lines were regularly tested for the presence of *Mycoplasma* by means of an agar culture technique (Schneider and Stanbridge, 1975) and were shown to be free of contamination.

Characterization of Cell Lines

Population doubling time

Cells were seeded at 2×10^5/ml in duplicate cultures and were counted every second day.

Light and electron microscopy

Tissue for histologic assessment was fixed in buffered formol saline and processed routinely to paraffin blocks. Five-micrometer sections stained with haematoxylin and eosin were examined by light microscopy. For electron microscopy, biopsy tissue fragments (1 mm^3) and albumin pellets prepared from the tissue cultures were fixed in 2.5% phosphate-buffered glutaraldehyde, postfixed in 1% osmium tetroxide, and processed routinely, and sections were examined in a Philips 410 TEM.

NSE secretion

Culture supernatants were collected from logarithmically growing cultures and were analyzed with the radioimmunoassay NSE RIA 100 from Pharmacia (Uppsala, Sweden).

Immunophenotyping

Immunohistochemical studies were carried out on frozen sections of biopsy material and on the two cell lines. The following antibodies were included: antihuman cytokeratin (No. M717), antisynaptophysin (No. M776), and antileukocyte common antigen (M701), all

from Dakopatts (Copenhagen, Denmark) and antineurofilament 200 kD (No. 814342) from Boehringer Mannheim Biochemica (Mannheim, Federal Republic of Germany). These antibodies were used in combination with a modification of the biotin-streptavidin method as described by Hsu et al. (1981) or with the A.P.A.A.P. technique (Cordell et al., 1984).

More detailed immunophenotyping of the cell lines was performed with a panel of monoclonal antibodies (mcabs), which are specified in Table 1. These antibodies were kindly made available by Dr. J. Kemshead, London (mcabs 1–6) (Cotmore et al., 1981; Allan et al., 1983; Kemshead et al., 1983a, b), by Dr. R.C. Seeger and Dr. C.P. Reynolds, Los Angeles (mcabs 7–10) (Donner et al., 1985), by Dr. W.F. Bodmer (mcab 11) (Parham et al., 1979), and by Dr. T. Matsumura, Kyoto (mcabs 12–15) (Matsumura et al., 1988). Immunofluorescence analysis was performed as described previously (Kees et al., 1987), and staining was determined under a Zeiss fluorescence microscope.

Isolation and analysis of nucleic acids

DNA was isolated by standard phenol-chloroform extraction and ethanol precipitation. The conditions for restriction enzyme digestion of DNA, electrophoresis, blotting of DNA, hybridizations, and autoradiography have been described previously (Kees et al., 1989). RNA was extracted by the method of Chomczynski and Sacchi (1987). Samples were denatured by incubation with glyoxal, electrophoresed on 1% agarose gels, and transferred to Zeta-Probe membrane (Bio Rad Laboratories, Richmond, CA).

TABLE 1. Cell Surface Marker Analysis of Cell Lines PER-352 and PER-364 by Immunofluorescence Analysis

	Mcab	PER-352	PER-364
1	HI1	+ +/+ + +[a]	+ + +
2	UJ 181.4	+	+
3	UJ 127.11	+/+ +	+
4	UJ 223.8	+ + +	+ + +
5	Anti-Thy	+ + +	+ + +
6	UJ 13.A	+ + +	+ + +
7	459	+ +/+ + +	+/+ +
8	390	+ +/+ + +	+
9	HSAN 1.2	−	−
10	HNK.1	−	−
11	W 6/32	+ +/+ + +	+ +/+ + +
12	KP-NAC 2	−	−
13	KP-NAC 8	+ +	+/+ +
14	KP-NAC 9	+/+ +	+
15	KP-NAC 10	−	−

[a] − to + + + indicates intensity of staining.

DNA probes and analysis of autoradiographs

MYCN sequences were detected with the pNb-1 probe (Schwab et al., 1983), kindly provided by Dr. G.M. Brodeur (St. Louis, Mo). In addition, a *MYC* probe (Amprobe RPN.1315X from Amersham International), a clone encoding the rat glyceraldehyde-3-phosphate-dehydrogenase *(GAPDH)* gene (Fort et al., 1985), and a probe for the *Bam*HI K region of the Epstein-Barr virus (EBV;M-ABA) (Polack et al., 1984) were utilized. Densitometric scanning of autoradiograms from DNA and RNA blot analyses was performed only on autoradiograms in which the band intensity as a function of the time of exposure was in the linear range. We used the autoradiographs from RNA blots hybridized with the *GAPDH* probe to monitor the amount of RNA in each lane.

Chromosome preparation

Chromosomes were harvested after treatment with Colcemid at a concentration of 0.8 μg/ml for 1.5 hr, from fluorodeoxyuridine-synchronized cultures, according to methods previously described (Webber and Garson, 1983). G-banding was performed by the trypsin-Giemsa technique (Seabright, 1971) and C-banding according to Salamanca and Armendares (1974). Multiple karyotypes for each cell line were constructed from photographs of metaphase cells. Karyotypes were designated according to the classification of the ISCN (1985). Chromosome abnormalities were defined as clonal if two or more metaphase cells had identical structural abnormalities or extra chromosomes or if three or more metaphase cells had identical missing chromosomes.

RESULTS

Biopsy Tissues

Light microscopy

Both lymph node biopsy specimens showed identical features. Sheets of mononuclear cells infiltrated nodal and soft tissue predominantly without any pattern. The cells had round to oval vesicular nuclei, containing one to three small nucleoli. The cytoplasm was scanty and pale, and cell borders were not clearly defined. Focally, in the first biopsy, rosettes of Homer-Wright and Flexner were seen. There were numerous mitotic figures and a high rate of necrosis of individual cells (Fig. 1A, upper inset). Tumor cells contained small amounts of glycogen, and they revealed positive staining for NSE, synaptophysin, and neurofilament protein. There was no staining for leukocyte common antigen or cytokeratins.

Electron microscopy

Similar features were observed in both biopsies. Most of the infiltrating cells were closely apposed, round or polyhedral, and appeared undifferentiated (Fig. 1A). They possessed rounded or cleaved euchromatinic nuclei with well-developed nucleoli. The cytoplasm was scant and electron lucent. There were few organelles, including scattered polyribosomes, small Golgi fields, scattered elements of smooth endoplasmic reticulum (SER), and few randomly distributed microtubules of ~25 nm diameter. Very rarely, isolated, rounded, membrane-bound dense-core granules were seen in the perikaryon (Fig. 1A, lower inset). More commonly found were small collections of pleomorphic lysosome-like granules, often related to the Golgi apparatus. Cytoplasmic glycogen was prominent in the second biopsy specimen.

Blunt cytoplasmic processes of irregular caliber arose from some cells and were insinuated between cell bodies (Fig. 1B). Long and often sinuous junctions resembling intermediate junctions, but of much greater length, were often formed between cell processes (Fig. 1B, inset) or between processes and cell bodies. Desmosomes were not found, and there were no definite synaptic contacts. Basal lamina was not present. Within these processes, microtubules were evident (Fig. 1B) but were never organized into compact parallel bundles; there were no neurosecretory granules and no definable filaments. Rosettes were not found despite intensive scrutiny of semithin and ultrathin sections.

Characterization of Cell Lines

Cell lines PER-352 and PER-364 grew as large aggregates of 50–400 cells in tissue culture. They did not adhere to plastic, nor did they form neurites or extensions. The population doubling times for both lines were very similar (Table 2). Immunocytochemistry and oncogene analysis revealed the same pattern for both lines (Table 2), which was identical to the pattern obtained for tissue sections from the patient. Neurofilament protein (200 kD subunit) and synaptophysin were present, whereas cytokeratin and leukocyte common antigen were not detected. The cell lines did not show any amplification of the *MYCN* oncogene. The RNA analysis revealed that *MYC* expression was increased slightly, whereas *MYCN* expression was not elevated. DNA from both lines was tested for the presence of genes coding for EBV. Whereas a strong band was obtained for an EBV-transformed cell line, QIMR B95-8 (Scully et al., 1984), neither PER-352 nor PER-364 gave any signal (data not shown).

We used a panel of monoclonal antibodies against a variety of cell membrane antigens on cells of neuroectodermal origin to characterize the cell lines further. This immunophenotyping (Table 1) revealed similar profiles for both lines, although, in some instances, distinct differences in the intensity of staining were observed (e.g., with antibody 390). It is important to note that both lines showed strong staining with the W 6/32 antibody (recognizing HLA-A, -B, -C), whereas antigens recognized by HSAN 1.2 and HNK.1 antibodies were not expressed on cells of either line. This pattern of surface marker expression is characteristic for PNET cells, in contrast to the results obtained for neuroblastoma cells (Donner et al., 1985; our unpublished results).

Ultrastructural Features of Cell Lines

The two cell cultures showed identical ultrastructural features. The cells grew in sheets and, in many areas, were polarized around central "lumens," forming distinct rosette-like structures (Fig. 1C). A spectrum of organelle development and complexity was evident. Where cells grew as solid clusters, the cytoplasm was often poorly developed and contained merely lakes of glycogen, ribosomes, scattered mitochondria, and sparse microtubules. Rare, primitive intercellular attachments were formed, and there was no cell surface specialization. Cells forming rosette-like structures possessed more cytoplasm, and they often assumed pyramidal or columnar shapes (Fig. 1D), frequently with basally orientated nuclei. Broad

Figure 1. **A:** This tumor cell has a deeply cleft nucleus, with a prominent nucleolus. The cytoplasm is sparse and contains few organelles. There are rare microtubules (straight arrows), elements of the SER (curved arrows) with associated smooth vesicles (arrowheads), scattered mitochondria, and polyribosomes. Some of the cytoplasmic clarity may be due to extraction of glycogen during processing. Two ill-defined, small, membrane-bound dense granules of uncertain nature (open arrows) are present; one of these is present in a neighboring tumor cell. × 15,600. **Upper inset:** A diffuse infiltrate of mononuclear cells in the node. Rosettes of Homer-Wright (straight arrow) and Flexner (curved arrows) types are present focally. Haematoxylin and eosin. × 480. **Lower inset:** Detail of a round, membrane-bound granule of ~100 nm external diameter. It possesses a central dense core separated by only a thin lucent rim from the investing membrane. × 46,500. **B:** A broad, tapering cytoplasmic process is insinuated between two other tumor cells, forming junctions with them. Numbers of microtubules are evident, focally showing quasiparallel arrangements (straight arrows). Smooth vesicles (curved arrow) and profiles of SER are evident. × 29,900. **Inset:** Detail of a long, sinuous junction between a cell process and an adjacent tumor cell body. Condensed subplasmalemmal microfilaments are evident on both sides of the junction. × 35,650. **C:** Low-power view of a central rosette-like structure, displaying a "lumen" (L) into which irregular microvillus-like processes project. The tumor cells display pleomorphic euchromatinic nuclei with prominent nucleoli. Supranuclear concentration of organelles is evident, including prominent pleomorphic mitochondria. Intercellular junctions (arrowheads) are prominent at the cell apices surrounding the lumens. × 6,600. **D:** Part of a rosette-like structure with a central "lumen" (L), onto which broad cytoplasmic processes converge. Microvilli project into the "lumen." Bundles of microtubules and aggregates of glycogen particles (arrowheads) are prominent in the processes. Apically, cell processes are united by junctions as illustrated previously. × 24,200.

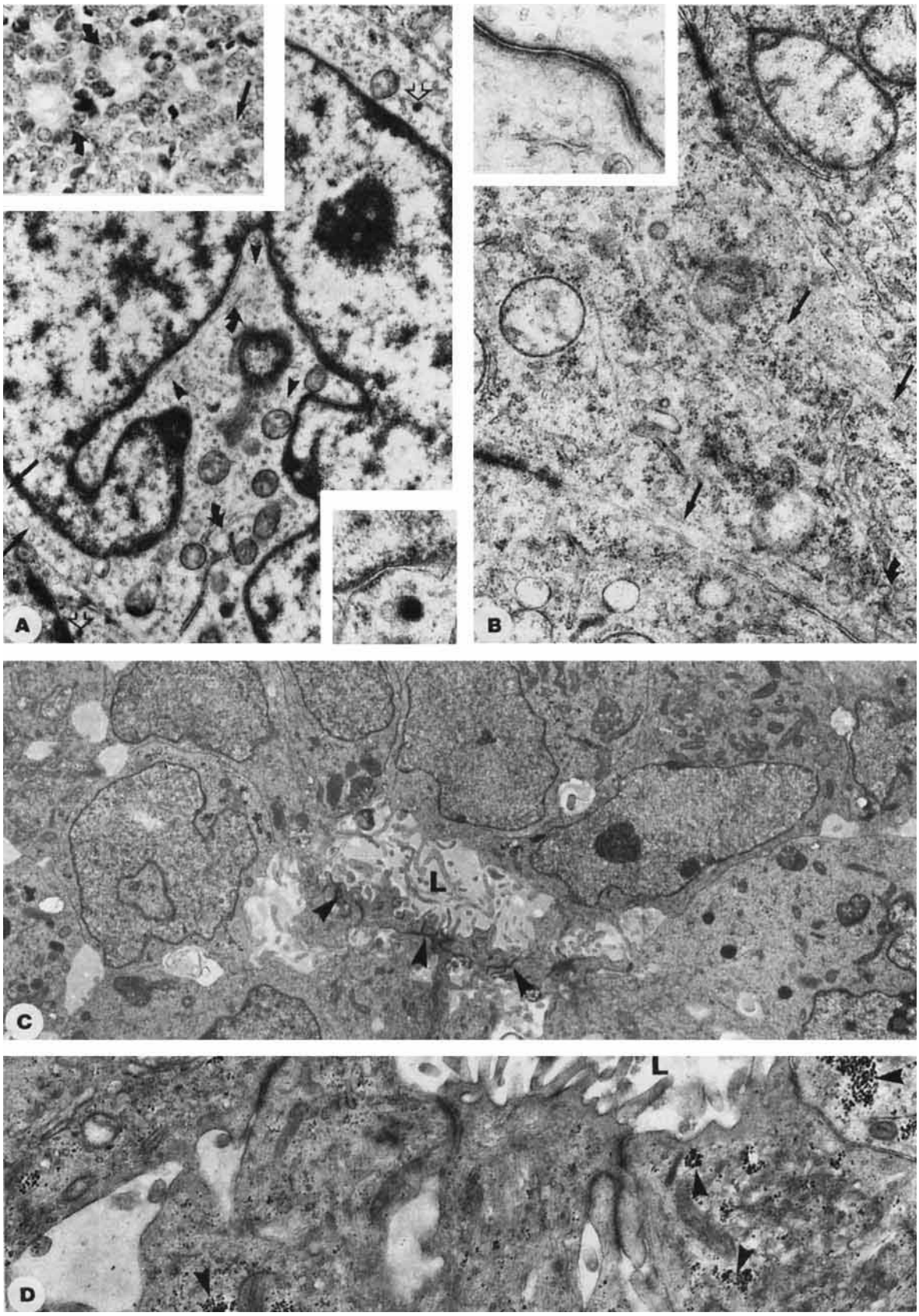

Figure 1.

cytoplasmic processes tapered toward the central "lumens," where adjacent processes were united apically by junctions having the same structure as seen in the in vivo biopsy specimens (Fig. 1D). Slender microvilli and broader microvillus-like processes projected into the "lumens," but cilia were not seen (Fig. 1D). Organelles were more abundant in these cells and comprised a well-developed Golgi apparatus, elaborate SER, abundant glycogen, pleomorphic mitochondria, and free lipid droplets. Microtubules were numerous and often were arranged in parallel bundles in the processes forming the rosettes (Fig. 1D). Neurosecretory granules and intermediate filaments were not found.

Cytogenetic Analysis

The karyotypes of the tumor cells present in the bone marrow and in the two cell lines PER-352 and PER-364 are detailed in Table 3. Cytogenetic studies on the involved bone marrow were performed elsewhere, but karyotypes were constructed from G-banded metaphases provided to our laboratory. Three related clones had been identified, and we confirmed these from the five metaphases made available to us. All cells karyotyped were hypodiploid, and the basic complex karyotype was 45,XX, −1, −6, −16, del(1)(q32),del(11)(q21q23), +der(1)t(1;?)(p34;?), +der (6)t(6;?)(q24;?). There were common additional abnormalities that occurred in two separate clones, one being a missing X chromosome and the other a partial deletion at 8p21. The basic complex karyotype, but with a missing X, is shown in Figure 2.

Studies of the cell line PER-352, which had been derived from this bone marrow, showed five related clones. The major clone (72%) had the complex karyotype and a missing X chromosome, and there were four minor related clones, one of which had the deleted 8p. Additional unrelated numerical and structural abnormalities were found in three other clones, together with the complex karyotype. Structural abnormalities involved chromosomes 4, 5, 9, 10, 17, and 21 and included a dicentric translocation between 9p and 12q. The dicentric nature of this derivative translocation was confirmed by C-banding.

The cell line PER-364, which had been derived from an involved lymph node 5 months later than PER-352, showed three clones, all containing the complex karyotype. On this occasion, the major clone (71%) contained the dic t(9;12) present in a few cells in PER-352. There were two minor clones, one of which had also been present in PER-352; a new clone had a partially deleted Xq instead of the missing X chromosome.

DISCUSSION

This case exemplifies the diagnostic difficulty that the small round-cell tumors of childhood may pose (Triche, 1986). On morphologic grounds alone, the initial supraclavicular biopsy was interpreted as indicating non-Hodgkin's lymphoma. Inappropriate response to lymphoma chemotherapy prompted review of the light- and electron-microscopic findings, which, in conjunction with the immunohistochemical results, established a diagnosis of PNET. This underscores

TABLE 2. Characterization of Cell Lines PER-352 and PER-364

	PER-352	PER-364	Patient's Tissue
Doubling time (days)	7.9 ± 1.2	6.8 ± 0.7	
Immunohistochemical profile			
Cytokeratin	−	−	−
Neurofilament (200 kD)	+	+	+
Synaptophysin	+	+	+
Leukocyte common antigen	−	−	−
MYCN amplification[a]	0	0	ND[b]
Oncogene expression[c]			
MYCN	0	0	ND
MYC	30	34	ND
NSE secretion[d]	85.5 ± 24.4	115.5 ± 21.7	

[a]A neuroblastoma cell line, PER-106 (Kees et al., 1992), which shows 200-fold *MYCN* amplification, was used as positive control.
[b]ND, not determined.
[c]Cell line PER-106 (Kees et al., 1992) and a mesothelioma cell line, LO (kindly provided by Dr. M. Garlepp, Perth), were used as controls. The *MYC* expression measured for LO cells was given the arbitrary unit of 100, whereas the neuroblastoma cell line PER-106 showed no detectable *MYC* expression.
[d]NSE secretion in μg/liter. Leukemia cell lines were used as controls, and they showed NSE secretion levels between 2 and 4.5 μg/liter (average: 3.2 ± 1.3 μg/liter).

the importance of examining such tumors with multiple diagnostic modalities, including electron microscopy and immunohistochemistry (Mierau et al., 1985; Triche, 1986; Tsokos and Triche, 1987; Yunis et al., 1988).

Distinction between PNET and poorly differentiated neuroblastoma on ultrastructural grounds is difficult, and indeed some authorities do not make any distinction (reviewed by Henderson et al., 1989). We are in agreement with Mierau et al. (1985) and Tsokos and Triche (1987) that minimal criteria for establishing a diagnosis of neuroblastoma include the presence of well-formed neuritic cell processes containing many microtubules as well as classical 50 to 200-nm-diameter membrane-bound, dense-core granules compatible with neurosecretory granules. The latter authors report that even neuroblastomas undifferentiated by light microscopy can be diagnosed by electron microscopy. Ultrastructural scrutiny of the biopsy material from our case revealed a paucity of cell processes (in particular, absence of well-formed neuritic processes), rarity of typical neurosecretory granules (which occurred rarely only in cell bodies and not at all within processes), absence of typical asymmetric synaptic contacts, and presence of distinctive junctions rather than the rudimentary junctions usually present in neuroblastoma. In our opinion, these findings argue against a diagnosis of poorly differentiated neuroblastoma and are more in keeping with features reported in PNET (Jaffe et al., 1984; Schmidt et al., 1985; Erlandson, 1987; Llombart-Bosch et al., 1987, 1989; Tsokos and Triche, 1987; Henderson et al., 1989). Furthermore, with recognition that no single investigative technique will necessarily discriminate between neuroblastoma and PNET, and with acknowledgment that there may be overlapping findings in the two entities, the collective evidence derived from the various investigative modalities employed in this case strongly supports the diagnosis of PNET.

The primitive neuroepithelial features of this neoplasm, as assessed at the ultrastructural level, were maintained in culture. In addition to poorly differentiated cells, other cells possessing numerous microtubules in parallel bundles within broad cell processes were disposed around incompletely formed lumens, producing rosette-like structures most closely resembling primitive Flexner rosettes. These were seen focally at the light-microscopic level in the in vivo biopsies; Flexner rosettes have been described previously at the light-microscopic level in PNET (Hashimoto et al., 1983; Schmidt et al., 1985). Further discrimination between neuroblastoma and PNET was feasible in the cultured cell lines, and two experimental approaches gave corresponding results. The panel of monoclonal antibodies used can distinguish between neuroblastomas and other malignancies of similar morphology. With the exception of mcabs HNK.1 and W 6/32 (Table 1), the antibodies show a wide cross reactivity with different tumors of neuroectodermal origin. The pattern of surface marker expression obtained for the two lines is characteristic for PNET cells: HSAN 1.2 recognizes a marker expressed on most neuroblasts and stains PNET cells only weakly or not at all, whereas W 6/32 is not expressed on neuroblastoma but is present on PNET (Donner et al., 1985; Triche and Cavazzana, 1989). Thus, according to this surface marker expression, the lines are

TABLE 3. Chromosome Analysis of Tumor Cells and Derived Cell Lines

Specimen	No. of Cells Counted	Karyotypes
BM	5	44,X,−X,Cx[a] [2]
		44,X,−X,Cx,del(8)(p21) [2]
		45,XX,Cx,del(8)(p21) [1]
Cell line PER-352	54	44,X,−X,Cx [39]
		44,X,−X,Cx,del(8)(p21) [3]
		44,X,−X,Cx,t(9;17)(p13;p25) [4]
		44,XX,Cx,−9,−12,+der(9)dic(9;12)(p12;p12) [6]
		45,XX,Cx,−2,−4,−10,inv(5) (p14q13),t(21;21)(q21;q22),+der(4)t(4;?)(q22;?),+der inv(5)t(inv5;?)(q13;?),+der(10)t(10;?)(q26;?) [2]
Cell line PER-364	42	44,XX,Cx,−9,−12,+der(9)dic(9;12)(p12;p12) [30]
		45,XX,Cx,−2,−4,−10,inv(5)(p14q13),t(21;21)(q21;q22),+der(4)t(4;?)(q22;?),+der inv(5),t(inv5;?)(q13;?),+der(10)t(10;?)(q26;?) [8]
		45,X,del(X)(q23),Cx,−18,del(3)(q22),+der(18)t(18;?)(q22;?) [4]

[a]Cx = −1,−6,−16,del(1)(q32),del(11)(q21q23),+der(1)t(1;?)(p34;?),+der(6)t(6;?)(q24;?). [No. of cells analyzed in each clone].

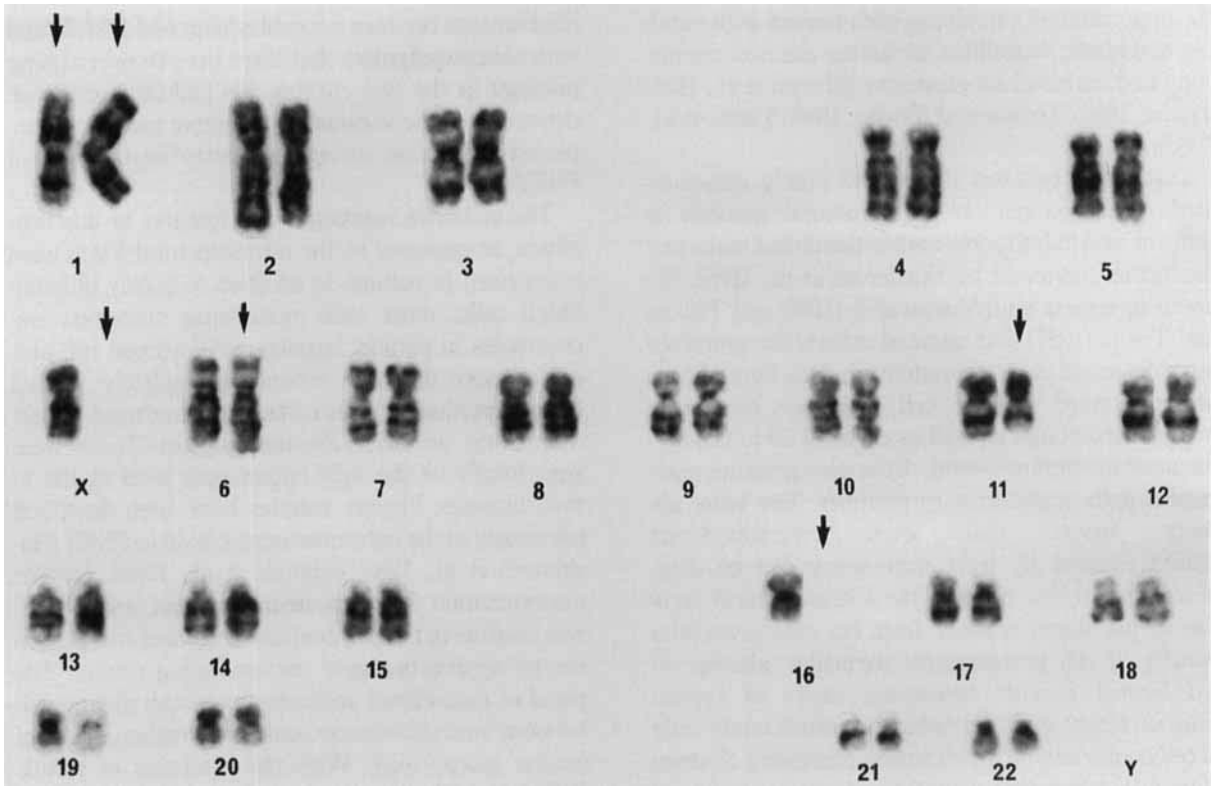

Figure 2. Karyotype of major cell line in PER-352: 44,X,−X,−1,−6,−16,del(1)(q32),del(11)(q21q23),+der(1)t(1;?)(p34;?),+der(6)t(6;?)(q24;?).

classified as PNET, a finding supported by the results obtained for *MYCN* and *MYC* expression. Both lines showed elevated levels of *MYC* expression, whereas *MYCN* was not overexpressed; this is the pattern of oncogene expression reported to be characteristic for PNET cells (Thiele et al., 1987; Triche and Cavazzana, 1989).

Cytogenetic analysis of the tumor cells found at diagnosis in the patient's bone marrow, and in cell line PER-352 derived from this marrow, showed loss of chromosome 16 and structural abnormalities of chromosomes 1, 6, and 11 to be common to both. A total of 23 PNET tumors and cell lines have previously been described cytogenetically (Whang-Peng et al., 1986; Potluri et al., 1987; Bown et al., 1988; Griffin et al., 1988; Lopez-Gines et al., 1988; Miozzo et al., 1990), and nine of these had abnormalities of chromosome 1. Abnormalities of chromosome 1 are common in solid tumors, and, in neuroblastoma, loss of 1p is the most common structural abnormality, being present in more than 70% of cases (Heim and Mitelman, 1987). Loss of heterozygosity in the region 1p36 has been correlated with *MYCN* amplification by one group of investigators (Fong et al., 1989), whereas another group (Weith at al., 1989) was unable to confirm this correlation, although a consensus deletion of 1p36 was found. The abnormalities of chromosome 1 found in PNET tumors and cell lines are, however, not as consistent as are those for neuroblastoma. The breakpoint on 1p in our tumor was at 1p34, but, because of the complex karyotype, it was not possible to decide whether the region distal to this breakpoint was lost.

The tumor and cell lines did not have the t(11;22)(q24;q12) previously described in PNET, and the breakpoint on the deleted 11 present in our karyotypes did not involve q24. Deletion of 6q has been described in four PNET tumors (Brown et al., 1988; Griffin et al., 1988), and in one of these the breakpoint was identified as 6q23. In PER-364, the karyotype had evolved further and included structural abnormalities of chromosome 5. Trisomy 5 (Whang-Peng et al., 1984) and i(5q) (Potluri et al., 1987) have been described previously in PNET. The appearance of a derivative dicentric 9;12 translocation is interesting; this abnormality has been described only in a variety of ALL usually associated with a poor prognosis (Carroll et al., 1987). The t(11;22)(q24;q12) in PNET is indistinguishable from the translocation observed in Ewing's sarcoma (Whang-Peng et al., 1984), although it is often possible to distinguish between these two tumors by histologic, ultrastructural, and immunohis-

tochemical analysis. It has been suggested that cytogenetic analysis could be used for separating the more primitive tumors of neuroectodermal origin that develop in peripheral sites into two groups, viz. those with a balanced t(11;22), for which the term peripheral neuroepithelioma could be reserved (Whang-Peng et al., 1986), and the remainder with different karyotypic changes which would be designated as PNET (Potluri et al., 1987). The karyotypes of our tumor and derived cell lines would be in agreement with this proposal.

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