Establishment and Characterization of a Human Primitive Neuroectodermal Tumor Cell Line from the Cerebral Hemisphere

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Abstract. The primitive neuroectodermal tumors (PNET) comprise a class of malignant nervous system neoplasms that afflict children. These tumors consist of cells that are morphologically identical to the primitive neuroepithelial cells normally seen in early stages of neural embryogenesis, supporting the notion that PNET result from a disturbance in the process of normal neuronal or glial differentiation. In the central nervous system, PNET occur most commonly in the cerebellum (medulloblastomas), but only occasionally in the cerebral hemispheres. We report here the establishment and characterization of a new human cell line (PFSK) derived from a PNET from the cerebral hemisphere of a child. The growth characteristics of PFSK cells were typical of an immortalized, transformed cell line. Cytogenetic and molecular genetic studies showed that three different sublines were present. In one of these sublines, sequences from chromosome 17 had been lost during establishment in culture. Immunocytochemical studies showed that PFSK cells expressed nestin, an intermediate filament protein normally expressed by neuroepithelial stem cells during neurogenesis. The PFSK cells did not express antigens typically found in terminally differentiated neurons or glia, indicating that this tumor cell line might represent neuroepithelial stem cells prior to commitment to a neuronal or glial lineage.

Key Words: Medulloblastoma; Nestin; PFSK; Primitive neuroectodermal tumor.

INTRODUCTION

The primitive neuroectodermal tumors (PNET) comprise a class of malignant nervous system neoplasms that afflict children. These tumors consist of cells that are morphologically identical to the primitive neuroepithelial cells normally seen in early stages of neural embryogenesis. Primitive neuroepithelial cells proliferate rapidly before differentiating along various pathways to form the subpopulations of cells (neurons, glia) that make up the mature nervous system. The presence of differentiated cell types within certain PNET supports the notion that these tumors may result from a disturbance in the process of normal neuronal or glial differentiation (reviewed in 1 and 2). In the central nervous system, PNET occur most commonly in the cerebellum, where they are referred to as medulloblastomas. Occasionally they are found in the cerebral hemispheres. Although central PNET grow rapidly in vivo and form highly malignant brain tumors, they seldom survive as immortalized cell lines in culture (reviewed in 3). The few examples of permanent PNET cell lines from the brain have all been derived from cerebellar medulloblastomas (4, 5).

Differentiation of primitive neuroepithelial cells proceeds through various stem cell intermediates, each with a different developmental repertoire. Immunofluorescent staining using antibodies to cell type specific antigens has provided a means of identifying some of these cellular intermediates. In the developing rat central nervous system, for example, the intermediate filament protein nestin appears in neuroepithelial stem cells during neurogenesis but disappears from these cells upon their differentiation into post-mitotic neurons (6). Nestin-specific antibodies may, therefore, serve as molecular markers for neuroepithelial stem cell intermediates prior to neuronal or glial differentiation.

We report here the establishment and characterization of a novel human cell line (PFSK) derived from a primitive neuroectodermal tumor from the cerebral hemisphere of a child. The PFSK cells formed solid tumors efficiently in nude mice, and cells derived from the rodent xenografts retained the morphologic and histologic features of the primary tumor cells. Cytogenetic and molecular genetic studies revealed that three different sublines were present. In one of these sublines, sequences from chromosome 17 had been lost during establishment in culture. Immunocytochemical studies showed abundant nestin expression in PFSK cells but no expression of antigens typically found in terminally differentiated neurons or glia, indicating that this tumor cell line might represent neuroepithelial stem cells prior to commitment to a neuronal or glial lineage.
MATERIALS AND METHODS

Patient History

A 22-month old boy presented with a two-week history of lethargy and vomiting. Radiographic examination (computed cranial tomography, magnetic resonance imaging, and cerebral angiography) showed a large mass within the right frontal lobe of the brain. At craniotomy, a hemorrhagic tumor was found invading the dura mater at the skull base. Histopathologic examination showed an undifferentiated primitive neuroectodermal tumor. The tumor was subtotally removed and chemotherapy with vincristine, cisplatin, cytoxan, and methylprednisolone was administered. Postoperatively, a radiographic examination of the spinal axis showed no signs of spinal metastasis. Despite treatment the tumor recurred within three months following surgery. A magnetic resonance scan of the brain at that time showed recurrent tumor occupying both cerebral hemispheres. The child soon lapsed into coma and died four months after the diagnosis was made.

Establishment of the PFSK Cell Line

A portion of the surgical specimen was minced with a scalpel blade and pieces measuring 1 mm in diameter were placed into Ham's F10 culture medium supplemented with fetal calf serum (10%), penicillin (100 units/ml), and streptomycin (100 µg/ml). Initially, the cells proliferated in a monolayer; but after ten days in culture, foci of cells growing as multilayered aggregates were observed on the monolayer surface. Foci were picked from the monolayer with Pasteur pipettes, transferred to microtiter wells, and subsequently expanded as individual sublines. Two of these focus-derived sublines, designated PFSK-1 and PFSK-2C, were characterized further as described in this report. The population of monolayer cells from which PFSK-2C was derived was passaged independently as a third subline designated PFSK-2 and characterized further. After 14 passages, the culture medium was changed to RPMI supplemented with 10% fetal calf serum. All experiments described in this report were carried out on cells cultured in RPMI medium. The PFSK cells were found to be free of Mycoplasma contamination when stained with Hoechst compound 33258 (Flow Laboratories; McLean, VA).

Growth Curve for the PFSK Cell Line

The PFSK cells (10^4) were seeded in triplicate into multiwell plates containing RPMI medium supplemented with fetal calf serum (10%) and incubated at 37°C under 5% CO₂ and 95% air. At successive 24-hour intervals, cells from three wells were removed by trypsin treatment and the number of cells in each well were counted with a hemacytometer. The growth curve for each PFSK subline over a 16-day time period was determined by plotting the logarithm of the average cell density versus incubation time. The population doubling time was calculated as the time required for the cell density to double in the middle of the log phase of the growth curve.

Growth of Cells in Soft Agar

Anchorage-independent growth of PFSK cells was determined by the method of Hamburger and Salmon (7) modified only by the use of RPMI medium supplemented with fetal calf serum (10%). Viable cells (10^6) were suspended in culture medium (2 ml) containing 0.3% agar (Difco [Detroit, MI] Bactoagar) and plated over a layer of 0.5% agar in the same culture medium. Cell viability was assessed by Trypan blue staining prior to plating. Cultures were incubated at 37°C in an atmosphere of 5% CO₂ and 20% O₂. Colonies were counted by viewing in a microscope at 125x magnification eight days after plating. Any colonies containing more than 20 cells were scored as positive. The efficiency of plating was defined as the number of colonies formed as a percentage of viable cells plated.

Tumorigenicity of PFSK Cells in Nude Mice

Four athymic nude mice (BALB/c-nu/nu) were injected subcutaneously with suspensions of PFSK cells (10^6) from the three different sublines: PFSK-1 (passages 37 and 44), PFSK-2 (passage 38), and PFSK-2C (passage 49). Within four weeks following injection, solid tumor nodules developed at the injection sites. The mice were sacrificed by cervical dislocation and the tumor nodules were excised.

Immunocytochemistry

Immunofluorescence staining using antibodies to the high, middle, and low molecular weight neurofilament proteins (NF-H, NF-M, NF-L), neuron-specific enolase (NSE), galactocerebroside (GC), and nestin was carried out on PFSK cells grown on coverslips coated with poly-L-ornithine. The cells were fixed with paraformaldehyde (4% v/v) in 0.1 M sodium borate (pH 9.3) for 20 minutes at room temperature, washed three times in phosphate-buffered saline (PBS, pH 7.4), and then permeabilized by immersion in a solution containing Triton X-100 (0.1% v/v) and normal goat serum (5% v/v) for 45 minutes at room temperature. The cells were incubated in the presence of the primary antibody for 60 minutes at room temperature and then washed three times with PBS. Fluorescein-conjugated secondary antibodies (goat anti-mouse IgG for NF and NSE and goat anti-rabbit IgG for GC and nestin) were diluted 1:80 in PBS and then applied to the cells for 60 minutes at room temperature. The cell preparations were washed three more times in PBS, mounted with glycerol/PBS (1:1 v/v), and viewed with a Nikon fluorescence microscope. The anti-nestin antibody used was a polyclonal rabbit antiserum raised against a bacterially expressed mouse nestin cDNA (diluted 1:500). The antibodies to NF-H, NF-M, and NF-L were purified mouse monoclonal antibodies (diluted 1:4) obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). The anti-GC antibody was a polyclonal rabbit antiserum (diluted 1:50) obtained from Chemicon (Temecula, CA). The antibody to NSE was a polyclonal rabbit antiserum (diluted 1:100) obtained from Accurate Chemicals (San Diego, CA). The secondary antibodies were purified Fab immunoglobulin fragments conjugated to fluorescein isothiocyanate obtained from Organon Teknika (Westchester, PA).

Immunofluorescence staining using antibodies against glial fibrillary acidic protein (GFAP) and vimentin was carried out as described above except that the cells were fixed and permeabilized by immersion in methanol (−20°C) for 10 minutes prior to application of the primary antibody. The anti-GFAP and anti-vimentin antibodies were purified mouse monoclonal antibodies (each diluted 1:5) obtained from Boehringer Mannheim Biochemicals.

In immunofluorescence experiments using monoclonal anti-
Fig. 1. Panel A—primary tumor tissue, H&E. Panel B—PFSK-1 cells after 18 months in culture (passage 64), phase contrast. Panel C—tissue from nude mouse xenograft derived from injection with PFSK-2C cells, H&E. Scale bars, 20 micrometers. H&E, hematoxylin and eosin.
body A2B5, the antibody was applied to unfixed cells for 60 minutes at room temperature, then removed by washing with PBS prior to addition of the secondary antibody. The A2B5 hybridoma line was obtained from the American Type Culture Collection (Rockville, MD). Supernatant from confluent cultures of A2B5 hybridoma cells was applied to the cells undiluted as primary antibody.

**DNA Extraction and Southern Transfer Analysis**

Extraction of genomic DNA from human tumor samples, cell lines, and peripheral blood leukocytes; Southern transfer analysis; and radiolabeling of DNA markers was carried out as described previously (8). The following polymorphic DNA markers and restriction enzymes were used in the restriction fragment length polymorphism (RFLP) analysis: YNZ22 (Taql or MspI), YNH37.3 (Taql), MCT35.1 (Rsal), HHH202 (Rsal), THH59 (PvuII), TBQ7 (PvuII), TB10.171 (PvuII), TBB2 (Taql), MCT128.1 (MspI), MLJ14 (Rsal). Original references for these markers have been cited previously in reference 9.

**Cytogenetic Analysis**

Cytogenetic analyses were performed on the established cell lines at various passages after recovery from the frozen state. Cells received fresh medium the day before processing. Colcemid (0.05 µg/ml) and ethidium bromide (5 µg/ml) were added to the cells together for two hours before harvest. Cells were detached by brief trypsin treatment and added to the previously removed supernatant medium. After centrifugation in a clinical centrifuge for five minutes at 300 rpm, a 4:1 mixture of potassium chloride (0.075 M) and sodium citrate (0.034 M) was added for 12 minutes as hypotonic treatment. Cells were fixed in methyl alcohol:acetic acid (3:1). After aging for three days, chromosomes were trypsin-banded and three to five karyotypes were constructed from each subline. Ten to twenty cells were then counted and examined for the presence of derivative chromosomes common to each subline.

**RESULTS**

**Morphology, Histology, and Growth Characteristics of the Primitive Neuroectodermal Tumor Cell Line, PFSK**

Histopathological examination of the primary tumor tissue removed at surgery showed numerous undifferentiated cells with densely basophilic chromatin interspersed within an eosinophilic fibrillar stroma (Fig. 1A). After establishment in culture, the tumor cells assumed a round to polygonal shape with short cytoplasmic processes (Fig. 1B). Certain differences were seen on comparing the morphologic features of the three PFSK sublines. Within the PFSK-1 subline, multinucleated cells with a broad, flat morphology were occasionally seen. The PFSK-2 cells tended to grow in clumps and were poorly adherent to the surface of the culture flask. The PFSK-2C cells were smaller in size and displayed a more uniform appearance.

The PFSK cells have been in culture continuously for over two years (85 passages). From the growth curves, the population doubling time of the PFSK cell line was determined to be 30 hours for sublines 1 and 2C and 48 hours for subline 2. After 16 days of incubation, the cells had reached a very high density (8.7 × 10^5 cells/cm^2). When viewed under a microscope at this high density, the cells were clumped into disorganized, loosely adherent aggregates with no signs of contact inhibition. We concluded from these observations that the PFSK cell line had transgressed the density limitations of growth.

To ascertain that PFSK was a transformed cell line, we tested PFSK cells for their ability to form colonies in soft agar. After eight days of incubation in soft agar, cells from sublines 1 (passage 35), 2C (passage 42), and 2 (passage 36) formed colonies with plating efficiencies of 17%, 19%, and 5%, respectively. To provide further evidence for transformation, athymic mice were injected subcutaneously with suspensions of PFSK cells from the three different sublines. Within four weeks, solid tumor nodules were observed in each of the four animals injected. After the appearance of solid tumors, the xenograft tissue was excised and a portion was fixed in paraformaldehyde, embedded in paraffin, and stained with hematoxylin and eosin. The histologic appearance of the nude mouse xenografts was very similar to that of the primary tumor tissue (Fig. 1C). The abundance of mitotic figures and multinucleated cells gave witness to a rapid rate of cell division.

Portions of two of the xenografts were placed into culture medium and cells derived from these explants have been proliferating stably in culture for more than 20 passages as tertiary tumor cell lines (PFSK-MT2 and PFSK-MT2C). Moreover, the morphology of these xenograft-derived cell lines was identical to that of the progenitor cell lines. We concluded from these results that PFSK was an immortalized cell line with growth characteristics that defined a fully transformed phenotype, specifically, anchorage-independent growth, tumorigenicity in nude mice, and lack of contact inhibition.

**Immunocytochemistry**

We carried out a series of immunocytochemical experiments using antibodies specific for antigens expressed by neuroectodermal cells during their differentiation into mature neurons and glia. The discovery of nestin, an intermediate filament protein expressed by neuroectodermal stem cells of the developing rat central nervous system, provided an in vitro marker for the PFSK cell line (6). We incubated paraformaldehyde-fixed PFSK cells with an antibody raised against a bacterially expressed mouse nestin cDNA and visualized the immune complexes by immunofluorescent staining. Abundant nestin immunoreactivity (IR) was detected in all three PFSK sublines and in the two xenograft-derived cell lines. The intracellular pattern of nestin staining was wavelike with
the nestin-specific antiserum showed only faint nonspecific staining in the nuclei of certain cells (Fig. 2B). No IR was found on PFSK cells with monoclonal antibody A2B5 under conditions that detect a cell surface antigen expressed on glial precursor cells and on certain postmitotic neurons (11–13).

In experiments designed to detect antigens expressed only in terminally differentiated neural cells, no IR was detected in PFSK cells with antibodies against NF-H, NF-M, NF-L, GFAP, or GC. These results indicated that PFSK cells did not express antigens typically found in the three major cell types of the mature central nervous system: neurons (NF), astrocytes (GFAP), and oligodendrocytes (GC) (14–16).

Immunoreactivity was detected in all PFSK cells with antibodies specific for NSE, an isoenzyme present in normal neurons and neuroendocrine cells as well as in a variety of neoplastic cell types, and vimentin, an intermediate filament protein expressed in many cultured cells as well as in many developing and differentiated tissues (reviewed in 17). Taken together, the results of these immunocytochemical studies indicated that PFSK cells had the antigenic profile of a neuroepithelial stem cell, a finding that supported the notion that the original tumor may have occurred as a result of some breakdown in the normal process of neural differentiation with persistence of undifferentiated neuroectodermal stem cells in the brain.

Molecular Genetic Analysis

Previous reports have suggested that genes on chromosome 17 may be important in the genesis of cerebellar medulloblastomas (18, 19). To determine if sequences from chromosome 17 had been lost from PFSK cells, we carried out a RFLP analysis wherein genomic DNA from the PFSK cells at different passages was analyzed on Southern blots with polymorphic DNA markers for loci on chromosome 17. DNA from the patient’s original tumor and peripheral blood leukocytes were analyzed simultaneously. Markers for five loci on chromosome 17 (YNZ22, YNH37.3, MCT35.1, HHH202, and THH59) showed loss of heterozygosity in DNA from one subline, PFSK-1, at passages 39 and 73 but not at passage 22. Figure 3 shows the results obtained using marker HHH202. None of these markers showed loss of heterozygosity in DNA from the primary tumor, PFSK-2 cells (passages 23, 40, 73), PFSK-2C cells (passages 29, 46, 81), or from xenograft cell lines PFSK-MT2 or PFSK-MT2C. We concluded that sequences on chromosome 17 had been lost during establishment in culture of only one of the sublines, PFSK-1. The fact that the loci identified by these markers spanned both p and q arms of chromosome 17 indicated that one entire copy of chromosome 17 had probably been lost.

To ascertain that the PFSK sublines originated from cells in the primary tumor specimen, we compared the

Fig. 2. Immunofluorescence micrographs showing expression of nestin intermediate filament protein by PFSK-1 cells. Cells were grown on coverslips coated with poly-L-ornithine and then labeled with rabbit anti-nestin antiserum, diluted 1:500 (Panel A) or non-immune rabbit serum, diluted 1:500 (Panel B), followed by goat anti-mouse IgG conjugated to fluorescein isothiocyanate. Scale bars, 20 micrometers.
Fig. 3. RFLP analysis showing loss of heterozygosity for a locus on chromosome 17 (HHH202) in PFSK cells (subline 1) during establishment in culture. Genomic DNA (5 μg) from the following sources was digested with RsaI, electrophoresed through a 1.0% agarose gel, transferred to a nylon filter, and hybridized to 32P-labeled DNA probe, HHH202: patient's peripheral blood leukocytes (lane 1) and primary tumor (lane 2); PFSK-1 cells at passages 22 (lane 3), 39 (lane 4), and 72 (lane 5); PFSK-2 cells at passage 73 (lane 6); PFSK-2C cells at passage 81 (lane 7); nude mouse xenograft-derived cell lines PFSK-MT2 (lane 8) and PFSK-MT2C (lane 9). The filter was exposed to X-ray film for 96 hours to generate the autoradiogram shown. The length of each restriction fragment is expressed in kilobase pairs.

RFLP patterns of DNA from the original tumor, the patient's peripheral blood leukocytes, the three PFSK sublines, and the two xenograft-derived lines with the following five polymorphic DNA markers for loci on other chromosomal arms: TBQ7 (10p), TB10.171 (10q), TBB2 (11p), MCT128.1 (11q), and MLJ14 (14q). The lengths of the restriction fragments obtained with each marker were identical in all of these DNA samples, indicating that the PFSK cells came from the patient's tumor rather than from some adventitious source (data not shown).

Genetic abnormalities associated with other neuroectodermal tumors of childhood include amplification of the cellular oncogenes N-myc in neuroblastoma tumors and c-myc in medulloblastoma cell lines (5, 20). To determine whether the c-myc or N-myc genes were amplified in PFSK, we hybridized EcoRI-digested genomic DNA prepared from cell lines PFSK-1 (passages 39 and 73), PFSK-2 (passages 40 and 73), PFSK-2C (passages 45 and 81), PFSK-MT2 (passage 5), and PFSK-MT2C (passage 6), as well as from the patient's primary tumor and peripheral blood leukocytes with a c-myc specific probe, pHSR-1 (21), an N-myc specific probe, pNB1 (20), and with a probe specific for human DNA encoding the immunoglobulin joining region, JH (22). The JH probe served to standardize the amount of DNA in each lane. The c-myc and N-myc probes hybridized to DNA fragments 13.5 and 2.0 kilobase (kb) in size, respectively, from comparable amounts of DNA from leukocytes, primary tumor, and the PFSK cell lines with similar intensity, indicating that neither c-myc nor N-myc was amplified or rearranged in the PFSK cell lines or in the primary tumor (data not shown).

Cytogenetic Analysis

Cytogenetic analysis of tumor sublines PFSK-1, PFSK-2, and PFSK-2C showed three distinctly different karyotypes (Table 1). The karyotype of the PFSK-1 subline was hypotetraploid with numerous chromosomal abnormalities, both numerical and structural (Fig. 4). Subline PFSK-2 was pseudodiploid with chromosomal abnormalities not seen in PFSK-1 such as translocations t(11;11), t(3;10), and t(17;22) (Fig. 5). The karyotype of PFSK-2C cells differed from normal only by the presence of trisomy of chromosome 8. Each subline appeared to be karyotypically homogeneous, and few cytogenetic changes were observed on comparing the karyotypes from each subline at two different passages. Comparing the karyotypes of tumor lines PFSK-2 and PFSK-2C with those of their derivative xenograft lines PFSK-MT2 and PFSK-MT2C showed that few chromosomal changes had taken place during passage through the nude mouse.

DISCUSSION

In this report, we describe the establishment of a new human cell line (PFSK) derived from a primitive neuroectodermal tumor from the cerebral hemisphere. The growth characteristics of PFSK cells were typical of an immortalized, transformed cell line. Immunocytochemical studies showed that PFSK cells expressed the intermediate filament protein nestin but lacked the antigenic
features of terminally differentiated neurons or glia, indicating that the PF5K cells may represent neuronal or glial precursor cells.

Primitive neuroectodermal tumors which arise in the cerebral hemispheres have histopathological features that closely resemble medulloblastomas—related but more common malignant neuroepithelial tumors that occur in the cerebellum. The observations that cerebral PNET occur in younger children and have a worse prognosis compared to cerebellar medulloblastomas indicate that these two tumor types are different clinical entities (23). Moreover, there are molecular genetic differences between PF5K and medulloblastoma cell lines. Amplification or rearrangement of the cellular oncogene c-myc has been reported in DNA from several human medulloblastoma cell lines (5). The fact that the primary tumors from which some of these cell lines were derived did not show this genetic abnormality indicated that c-myc amplification may have played a role in establishing the medulloblastoma cells in culture. We did not find amplification or rearrangement of the c-myc gene in DNA from the PF5K cell line, the primary tumor, or the rodent xenografts. These observations indicated that the genetic events that led to immortalization in culture proceeded along different pathways in the cerebellar medulloblastoma cell lines compared to the cerebral hemisphere PNET cell line PF5K.

Chromosome 17 mutations have been implicated in the genesis or progression of cerebellar medulloblastomas. A common cytogenetic abnormality observed in this type of tumor is isochromosome 17q wherein the q arm of chromosome 17 is duplicated and the p arm is deleted (18). An RFLP analysis showed loss of heterozygosity on chromosome 17p in one-third of informative patients with cerebellar medulloblastomas (19). Our RFLP studies showed loss of heterozygosity for multiple loci on chromosome 17 in late passage PF5K-1 cells but not in early passage cells or in the primary tumor, indicating that loss of one of the two parental copies of chromosome 17 had occurred during establishment of this particular subline in culture. Four copies of chromosome 17 were seen, however, on the karyotype of tetraploid PF5K-1 cells indicating that the remaining parental copy of chromosome 17 had been duplicated. Another chromosome 17 abnormality observed in the PF5K cell line was a 17;22 translocation with a breakpoint on 17 band q21 seen in

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Fig. 4. Karyotype of PF5K-1 cells at passage 75.

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subline PFSK-2. The fact that loss of heterozygosity for chromosome 17 was not seen in PFSK-2 cells indicated that either no genetic material had been lost from chromosome 17 during the translocation or a small deletion was present that was not detected by the markers used in this study.

Although the predominant cell type within PNET resembles the undifferentiated neuroepithelial stem cell normally seen in the developing nervous system, differentiated neurons or glia are sometimes found on histopathological examination of these tumors (24). Although the clinical significance of cytological differentiation in PNET is not clear, the potential for differentiation supports the hypothesis that PNET result from a disturbance in the normal process of neuronal or glial differentiation. Alternatively, disturbances in growth control mechanisms may exist that do not affect the differentiation potential of primitive neuroepithelial cells. Nevertheless, the absence of differentiated neurons or glial cells either among the PFSK cells in culture or within the primary tumor tissue suggested that PFSK is an undifferentiated PNET cell line. In fact, we have not been able to induce PFSK cells to differentiate in vitro in response to several exogenous agents (nerve growth factor, retinoic acid, dibutyryl cyclic AMP, isobutyl-methyl-xanthine).

Studies in the developing rat central nervous system have shown that neuroepithelial cells express nestin during neurulation, when the neural ectoderm folds up and fuses to form the neural tube (6). Later in development when the neuroepithelial cells differentiate to become neurons or glia, these cells stop producing nestin and express intermediate filaments characteristic of their differentiated state. In other experiments, cell lines composed of nestin-positive, neuroepithelial stem cells were established from neonatal rat cerebellum using a temperature-sensitive form of the SV40 T antigen as an immortalizing oncogene (10). The fact that these cells could be induced to differentiate into neurons, glial cells, or even muscle cells indicated that nestin expression was a marker for a multipotential stem cell (25). In light of these findings, our observations that PFSK cells expressed nestin but did not express proteins typically found in differentiated neurons or glia (NF, GFAP, GC) suggest that PFSK cells may represent an arrested stage of neural development prior to commitment to a neuronal or glial lineage.

Fig. 5. Karyotype of PFSK-2 cells at passage 60.
TABLE I
Cytogenetic Analysis of PFSK Sublines

<table>
<thead>
<tr>
<th>Subline</th>
<th>Passage</th>
<th>Karyotype</th>
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<tbody>
<tr>
<td>PFSK-1</td>
<td>59</td>
<td>81,XXY,Y(Xp;8q),del(1)(p22), -2,-3,del(4)(p14), -5, -8, -9, -9, -13, -14, -16, -17, -20,-22, +mar</td>
</tr>
<tr>
<td></td>
<td>75*</td>
<td>84,XXY,Y,t(Xp;8q),del(1)(p22), -3,del(4)(p14), -9, -10, -13, -14, -15, -16, -22</td>
</tr>
<tr>
<td>PFSK-2</td>
<td>60**</td>
<td>46,X,-Y,t(1;11)(p34;q13.3), t(3;10)(p11;q11.2), t(4;7)(p15.3;?), +8, del(8)(p12), (q12), (17;22)(q12;q13.1), +19,-21</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>47,X,-Y,t(1;11)(p34;q13.3), t(3;10)(p11;q11.2), t(4;7)(p15.3;?), +8, del(8)(p12), (q12), (17;22)(q12;q13.1)</td>
</tr>
<tr>
<td>PFSK-MT2†</td>
<td>3</td>
<td>47,X,-Y,t(1;11)(p34;q13.3), +2, t(3;10)(p11;q11.2), t(4;7)(p15.3;?), +8, del(8)(p12), (q12), (17;22)(q21;q13.1)</td>
</tr>
<tr>
<td>PFSK-2C</td>
<td>65</td>
<td>47,XY, +8</td>
</tr>
<tr>
<td>PFSK-MT2C††</td>
<td>12</td>
<td>47,XY, +8</td>
</tr>
</tbody>
</table>

* Karyotype shown in Figure 4.
** Karyotype shown in Figure 5.
†† From nude mouse injected with PFSK-2 cells at passage 49.
† From nude mouse injected with PFSK-2C cells at passage 38.

To date, PFSK is the only example of a cell line representing a PNET from the cerebral hemisphere. Although PFSK cells have genotypic features not present in the primary tumor cells, this cell line will hopefully serve as an important substrate in studies designed to elucidate the cause of this lethal form of childhood cancer and perhaps to gain some insight into the mechanisms of normal neuronal and glial differentiation.

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