Central neurocytoma: morphological, flow cytometric, polymerase chain reaction, fluorescence in situ hybridization, and karyotypic analyses

Case report

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The results of cytogenetic and molecular genetic analysis of a central neurocytoma are presented. Central neurocytomas are intriguing neoplasms that exhibit primarily neuronal, but also glial characteristics, which indicate an origin from a pluripotential neuroglial precursor. The authors describe an intraventricular neurocytoma in an 11-year-old boy that showed anaplastic features with widespread necrosis and mitoses, as well as extensive calcification and foci that exhibited marked neuronal differentiation with clusters of ganglion cells. Immunohistochemical examination showed prominent synaptophysin and neurofilament positivity and focal glial fibrillary acidic protein positivity. Electron microscopy revealed abundant neuritic processes with microtubules and dense core granules as well as mature ganglion cells. Flow cytometry studies revealed increased S (7.8%) and G2M (9.7%) phase components. Molecular and cytogenetic studies were undertaken to assess whether there were similarities to two other tumor types that exhibit neuronal differentiation, the neuroblastoma and medulloblastoma. Polymerase chain reaction and fluorescence in situ hybridization (FISH) analysis revealed no evidence of amplification of the MYCN oncogene or chromosome 1p deletion, which are common in neuroblastomas. Chromosomal analysis by G banding revealed a complex karyotype, with counts in the near-diploidy range (45–48). Two chromosomes 1 appeared normal on G banding and FISH analysis, with p58 signals present on the distal p arm of both chromosomes 1; however, three additional copies of distal 1q were present in rearrangements with 4 and 7. Although the histological findings indicate a kinship to the neuroblastoma and medulloblastoma, the central neurocytoma appears to have a different karyotypic profile, although more cases need to be assessed using molecular genetic analysis.

Key Words • neurocytoma • polymerase chain reaction • fluorescence in situ hybridization • brain neoplasm • cytogenetic analysis

Since the initial description of the “central neurocytoma” by Hassoun, et al., a number of reports have documented the histological, immunohistochemical, and ultrastructural features and clinical outcome of this intriguing neoplasm. Usually found in an intraventricular location, these tumors show clear cells intersected by a vascular meshwork, an appearance reminiscent of oligodendroglioma. Tumors with these histological features have also been reported in an extraventricular location in the central nervous system. Although generally associated with a favorable prognosis, a few cases with aggressive courses have been reported.

Although a number of publications address the morphological, immunohistochemical, and clinical aspects of this entity, there are only rare reports on molecular genetic and karyotypic studies of this tumor. We describe a central neurocytoma with anaplastic features detected with polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), and karyotype analyses.

Case Report

History. This 11-year-old right-handed boy had emigrated from Ukraine to Canada 1 year prior to the present admission. One year previously, at another medical institution, the patient had undergone neurosurgical treatment of a traumatic epidural hematoma caused by a head injury suffered while riding his bicycle. A head computerized tomography (CT) scan obtained at that time showed a large right-sided epidural hematoma. The patient recovered completely after evacuation of the hematoma. A postoperative CT scan was obtained before he was discharged from the hospital. In addition to a small residual...
collection at the operative site, a small calcification was identified in the region of the right thalamus (Fig. 1 left). According to his medical history, no additional studies were performed at that time.

One year later, the patient started to complain of transient headaches and unsteady gait and he was referred to an ophthalmologist. The ophthalmologist diagnosed severe papilledema, and the patient was sent to the local hospital. Physical examination at admission showed papilledema, diplopia on left lateral gaze, and gait unsteadiness. A head CT scan revealed a large mass lesion within the right lateral ventricle (Fig. 1 center). After this finding was made, the patient was referred to The Hospital for Sick Children.

Examination. On arrival at our institution, the patient was awake and alert. Funduscopic examination revealed florid papilledema with bilateral retinal hemorrhages. Visual acuity was 20/70 on the right and 20/100 on the left. The pupils were equal with normal light reflexes. Diplopia was confirmed on left lateral gaze. No other cranial nerve deficits were present. Although obvious ataxia was present, motor strength was normal. Tendon reflexes were mildly increased on the left side. Magnetic resonance imaging was performed at this time and revealed a large right-sided intraventricular lesion (Fig. 1 right), which enhanced on administration of gadolinium.

Operation. The patient underwent surgery the next day. A right-sided occipitoparietal craniotomy was performed. A soft, friable, vascular tumor was found occupying much of the parietal lobe, and a gross-total excision was performed. Immediately postoperatively, the patient experienced a mild motor weakness involving his left leg and was transiently confused. During the ensuing days, the patient’s condition improved remarkably, and he quickly became ambulatory. However, his vision continued to deteriorate: 3 days postoperatively, visual acuity was 1/10 on the right and 1/20 on the left. A postoperative CT scan demonstrated no definite residual enhancing tumor and no signs of hydrocephalus.

Postoperative Course. One week postsurgery, the patient was discharged from the hospital, but a few days later he was readmitted for continued visual loss in the left eye. The papilledema was less pronounced than before, and steroid medication and acetazolamide were prescribed. Opening pressure on lumbar puncture was 170 mm H2O. Xanthochromic cerebrospinal fluid was obtained, and cytological examination revealed the presence of malignant cells. Based on the tissue diagnosis of anaplastic neurocytoma, focal irradiation to the initial tumor volume and irradiation of the craniospinal axis were initiated. After 3 weeks, the patient’s vision started to improve, although constricted visual fields were still present.

Laboratory Analyses

The excised tumor tissue was submitted for histological, immunohistochemical, and ultrastructural studies. After confirmation of tumor type by frozen section, samples were submitted for flow cytometry, PCR, FISH, and karyotype analyses.

Histological, Immunohistochemical, and Electron Microscopy Studies

The tissue was processed for conventional histological examination with formalin fixation and paraffin embedding, and immunohistochemical staining was performed using the avidin-biotin complex or peroxidase antiperoxidase techniques with the following antibodies: glial fibrillary acidic protein (GFAP), polyclonal, 1:200, synaptophysin (monoclonal, 1:5), phosphorylated neurofilaments
(low-, mid-, and high-molecular-weight subsets, monoclonal: 1:100), nonphosphorylated neurofilaments (monoclonal, 1:50), neuron specific enolase (NSE, polyclonal; 1:250), MIB-1 (monoclonal, 1:50), epithelial membrane antigen (EMA, monoclonal, 1:10), and low-molecular-weight cytokeratin (monoclonal, 1:30).

For electron microscopy, tissue was fixed in universal fixative (equal parts of 4% formaldehyde and 1% glutaraldehyde) and postfixed in 1% osmium tetroxide, dehydrated in a graded alcohol series and propylene oxide, and embedded in epon. One-micrometer-thick sections were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a transmission electron microscope.

**Flow Cytometry**

Flow cytometry analysis was performed according to published protocols to determine the ploidy and proliferative rate (S/G/M phase) in both control and tumor tissues.11

**Differential PCR Assay for Analysis of MYCN Amplification**

We extracted DNA from frozen tumor tissue by using conventional proteinase digestion followed by standard phenol–chloroform extraction. One hundred nanograms of target DNA was used, and the CF gene was used as a single-copy gene control. A differential PCR assay for MYCN amplification was performed according to previously published protocols.12,26

**Karyotype and FISH Analysis**

Tumor tissue was minced, cultures were grown, and cells were harvested after 4 and 5 days by using established methods.29 Metaphase chromosomes were examined using G banding (12 metaphases),24 which consisted of a light and dark pattern and was produced by treating metaphase chromosomes with trypsin and then staining with Giemsa. The FISH analyses were performed using commercially available probes according to the manufacturers’ instructions; FISH analysis utilized DNA sequences tagged with a fluorochrome to form probes that were hybridized to metaphase chromosomes or interphase nuclei. The FISH probes used contained DNA sequences that were either specific for a gene/locus or derived from an entire chromosome (chromosome-specific paint). A fluorescent signal corresponded to the presence of the sequence in the chromosome. Structural abnormalities were characterized by FISH analysis using chromosome paints for chromosomes 1, 4, 7, 8, 20, and Y (10 metaphases on FISH studies for each of the chromosome paints). In addition, metaphase spreads (10 for each probe) were examined using FISH with locus-specific probe p58<sub>c</sub> and p58 within 1p36 and the probe for the MYCN gene.

Interphase nuclei from cultured of tumor cells and control cells were examined by FISH with probes for chromosome region 1p36 and locus MYCN. We used the IMR32 neuroblastoma cell line with 40-fold MYCN amplification in two homogeneously staining regions and one p58 signal per nucleus as a positive control for assessing the chromosome 1p deletion and MYCN status. Normal human lymphocytes (specimens with two copies of p58 and MYCN per nucleus) were used as a negative control. A minimum of 100 interphase nuclei were scored for FISH analysis per probe.

The karyotype presented in this paper was also confirmed by spectral karyotyping analysis of the metaphase chromosomes. Spectral karyotyping is a new genome-wide FISH technique that results in a multicolor karyotype.22 This technique involves chromosome-specific painting probes combinatorially labeled with one to four fluorochromes from a selection of five fluorochromes. Detection of the fluorescence was achieved using a bio-imaging system. This technique results in a unique color for each chromosome in the karyotype and the identification of any interchromosomal rearrangements.

**Results**

**Morphological, Immunohistochemical, and Electron Microscopy Studies**

The gross specimen consisted of multiple pieces of tan, vascular, firm tissue with focal regions of gritty calcification, measuring 4.5 × 2 × 1 cm in aggregate. The markedly calcified portion of the specimen was processed after decalcification. The tumor revealed a variety of histological patterns, including cells exhibiting clear perinuclear haloes (Fig. 2 upper) and a fine vascular meshwork and other foci exhibiting increased cellularity and Homer Wright rosettes. Much of the tumor was represented by a poorly differentiated cellular component with extensive necrosis and many mitoses. A smaller tumor component with marked neuronal differentiation had a neuropil-like background and bi- and multinucleated ganglion cells (Fig. 2 center). There was extensive tumor calcification.

Immunostaining revealed marked synaptophysin, neurofilament, and NSE positivity and scattered GFAP positivity in tumor cells. Staining for cytokeratin and EMA was negative. The cellular component of the tumor exhibited distinct MIB-1 labeling (Fig. 2 lower), with a labeling index greater than 50%, indicating very high proliferative activity.

Electron microscopy revealed extensive neuronal differentiation in the form of recognizable ganglion cells with vesicular nuclei, neurosecretory granules, microtubules, neurofilaments, abundant rough endoplasmic retic-
Cytogenetic/FISH analysis of neurocytoma

ulium, and synaptic contacts. The less differentiated elements showed more primitive-appearing cells with poorly developed intercellular junctions and processes containing microtubules, neurofilaments, mitochondria, and dense core neurosecretory granules. Glial differentiation was not evident in the small sample taken for electron microscopy studies.

Flow Cytometry Studies

The tumor appeared to be diploid on flow cytometry studies, with increased S and G2M phases of 7.8% and 9.7%, respectively (results not shown).

Differential PCR Analysis

There was no evidence of MYCN amplification on the differential PCR assay (results not shown).

Karyotype and FISH Analysis

Of 100 nuclei examined in the tumor sample by using karyotype and FISH analyses, 89% of nuclei with signals showed two signals per interphase nucleus for the MYCN probe, whereas 98% of nuclei with signals showed two p58 signals per interphase nucleus. The positive control sample exhibited discrete domains of yellow/green fluorescence consistent with homogeneously staining regions with the MYCN probe and also showed one signal with the p58 probe in the majority of nuclei examined. Background hybridization was not apparent. The FISH analysis on 10 metaphases also revealed no evidence of MYCN amplification or deletion of the distal portion of the short arm of chromosome 1 by p58 analysis.

Twelve metaphases were examined using G banding at a band level of approximately 400. The tumor showed the following karyotype (Fig. 3): 45–48,X,-Y,der(4)t(1;4)(q21;p16)t(1;7)(q32;p15),+der(8)t(8:?)(p21;?),der(16)t(16:?)(q2;?), der(18)t(18;20)(q23; q11)[cp12].

Both chromosomes 1 appeared normal by G banding and FISH analysis using the probe p58 for 1p36; however, distal 1q was present as three additional copies on two structurally altered chromosomes. Using the chromosome paints for 1, 4, and 7, chromosome 4 was found to be a derivative 4 with different-sized portions of 1q attached at each end, and one chromosome 7 was discovered to be a derivative 7 with part of the distal 1q replacing part of the distal 7p. Additional structural abnormalities included a derivative 8 (confirmed by FISH with the chromosome 8 paint) with an extra piece of unknown origin present as an extra chromosome replacing part of the distal p arm, one derivative 16q+, and one derivative 18q+ with an extra piece from chromosome 20 (confirmed using FISH analysis with chromosome 20 paint). The Y chromosome was absent.

Discussion

After the introduction of the term central neurocytoma by Hassoun, et al.,11 to describe a distinctive intraventricular tumor, several reports have appeared in the neurosurgery, pathology, and radiology literature documenting its morphological appearance, immunophenotype, and generally favorable outcome.14,16,18,21,25,26–33 Before its recognition as a distinctive entity, many of these tumors were presumably labeled as oligodendrogliomas because of the
characteristic histological appearance of clear cells intersected by a fine vascular meshwork. Similarly, some of these tumors, especially the anaplastic variants, were likely labeled as cerebral neuroblastomas. Some authors have used the terms neuroblastoma and neurocytoma synonymously, referring to neurocytoma as primary cerebral neuroblastoma. Despite discrepancies in nosology, the neurocytoma is generally encountered as a lateral ventricular tumor in a young adult that commonly presents with obstructive hydrocephalus, and has a good prognosis after surgical excision.

Neurocytomas with histological features of anaplasia are rare, but have been documented in the literature. Robbins, et al., reported aggressive tumor behavior in one patient whom histological studies of the tumor showed no anaplastic features. Eng, et al., reported two cases of recurrent neurocytoma with no histological evidence of anaplasia, but widespread ventricular dissemination and seeding of the spine. Thus, even tumors without obvious anaplastic features may be associated with more aggressive behavior and the postoperative prognosis of central neurocytoma may not always be as favorable as previously believed. Our patient received postoperative radiation therapy based on the documentation of anaplastic features on histological studies.

With respect to the progressive visual loss in our patient after removal of the cerebral mass lesion, this is not an uncommon phenomenon in this clinical setting. Chronically high intracranial pressure leads to papilledema and optic nerve injury. Even with removal of the mass lesion, the damage to the optic nerve can be progressive and vision can continue to decline. This is particularly true in children who present with posterior fossa mass lesions and obstructive hydrocephalus. In such cases, it is thought that continued administration of high-dose steroid medications even after tumor resection may prevent some of this visual deterioration.

In the present case, the anaplastic, hypercellular, less-differentiated tumor component was predominant, with extensive necrosis and a high mitotic index. In our patient, a very high MIB-1 labeling index, well above 50%, as well as high S and G2 M components on flow cytometry, attest to a high proliferative potential. This is also supported by the patient’s clinical history, which indicates a dramatic radiographically confirmed progression, judging from a comparison of the CT scan obtained for a traumatic epidural hematoma treated 1 year earlier and the CT study obtained at the present admission. The calcific specks observed in the earlier scan indicate that a more indolent process might have been ongoing before the phase of rapid tumor growth and sudden clinical deterioration.

A spectrum of cell differentiation was evident in our patient, ranging from the highly anaplastic proliferative component to an intermediate element with clear cells and extensive calcification, and a mature component with neuropil and ganglion cells and lack of mitotic activity. In a recent report, Schwaizer and Davies described a patient who underwent a partial resection of an intraventricular neurocytoma and a second resection approximately 18 years later, at which time the tumor was found to have foci of ganglioglioma. Similar to our case, expression of neuronal and glial markers has been reported in this tumor type by other authors.

Reports of molecular genetic or karyotypic analyses in neurocytomas are sparse. Using FISH analysis, Taruscio, et al., reported gains of chromosome 7 in three of nine neurocytomas. In our patient, one chromosome 7 was present as a derivative 7, with a portion of the distal 7p replaced by a portion of the distal 1q. Aneuploidy of chromosome 7 was not evident in our case.

![Fig. 3. Chromosomal analysis showing representative G-banded karyotype from the tumor: the derivative chromosomes 4, 7, 16, and 18 are placed to the right of the normal homologs; the derivative chromosome 8 is placed to the right of the two normal homologs of chromosome 8. The origin of mar 1 and mar 2 were not determined. There is a random loss of chromosome 2 in this metaphase spread.](image-url)
Cytogenetic/FISH analysis of neurocytoma

In light of the prominent neuronal differentiation and areas reminiscent of ganglioneuroblastoma, we performed molecular genetic studies to evaluate if two abnormalities commonly found in neuroblastomas, and that is, 1p deletion and MYCN amplification, were present in our case. The FISH and PCR assays failed to show evidence of MYCN amplification and 1p36 deletion in this case.

In addition to neuroblastomas, we have observed MYCN amplification in medulloblastomas with prominent neuronal differentiation. Rouah, et al. have also previously reported MYCN amplification in central primitive neuroectodermal tumors (PNETs) with neuronal differentiation. High copy numbers of MYCN are associated with an adverse outcome in neuroblastomas. In the case of neurocytoma reported here, there was no evidence of MYCN amplification on differential PCR and FISH analysis. The study by von Deimling, et al. also indicates that central neurocytomas may not be associated with high copy numbers of MYCN.

There was also no evidence in our patient of an isochromosome 17q, which is a commonly reported abnormality in primitive neuroectodermal tumors (PNETs) of the central nervous system. In a cytogenetic study of one case of neurocytoma, Cerdá-Nicolás, et al. reported a loss of chromosome 17, as well as various other abnormalities including -2, -22, -Y, and derivative chromosomes. In our case, the combination of cytogenetic abnormalities was different from those reported by Cerdá-Nicolás, et al., except for the loss of Y chromosome. Our karyotypic analysis of this one case of central neurocytoma reveals complex abnormalities that indicate no kinship to the peripheral neuroblastoma or central PNETs, but more cases need to be assessed. The observed abnormalities also do not resemble those commonly observed in other glial tumors of childhood.

Conclusions

We have presented the case of a central neurocytoma, which showed a spectrum differentiation ranging from a predominant anaplastic component with a greater than 50% MIB-1 proliferative index to a calcified mature tumor component with abundant ganglion cells on histological studies. This case was not associated with the genetic abnormalities commonly observed in neuroblastoma or medulloblastoma. Karyotypic analysis of our case revealed complex abnormalities that are not common in gliomas or PNETs. Genetic characterization of future cases will help to elucidate the molecular biology of this rare cerebral tumor.

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References


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