Monosomy 22 in rhabdoid or atypical tumors of the brain

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Cytogenetic studies of three rare childhood brain tumors were performed. Two children presented with pure rhabdoid tumors. The third child had a tumor composed of a mixture of rhabdoid elements with neuroepithelial, epithelial, and mesenchymal tissue — an atypical teratoid tumor. All three tumors demonstrated monosomy 22 as the only cytogenetic abnormality. The cytogenetic findings suggest that loss of a gene or genes on chromosome 22 may be involved in the initiation or progression of these malignant tumors. Further studies on additional fresh tumor specimens are warranted; however, it is possible that cytogenetic studies may be used as an additional means of diagnosing rhabdoid or atypical teratoid tumors of the brain.

KEY WORDS • chromosome 22 • brain neoplasm • rhabdoid tumor • teratoid tumor • children

The rhabdoid tumor of the kidney is a well-known entity in the spectrum of renal tumors of childhood. It presents in infants and young children, and since it is usually resistant to irradiation and chemotherapy, it carries with it a dismal prognosis. It has been shown that rhabdoid tumors may also occur in other organ systems, such as the brain and soft tissues. In the brain, these malignant tumors may present as pure rhabdoid tumors, or they may consist of a mixture of rhabdoid cells with neuroepithelial, epithelial, and mesenchymal tissue. The latter tumors have been given the name "atypical teratoid tumors." Atypical teratoid or rhabdoid tumors of the brain characteristically present in infants less than 2 years of age. Like rhabdoid tumors of the kidney, they are extremely aggressive and patients often fail to achieve a clinical remission. To date, there have been no full reports on cytogenetic studies of rhabdoid tumors, and no clues as to the underlying mechanism responsible for these malignancies.

During the last several years, we have begun to define the chromosomal changes that characterize pediatric brain tumors. The goals of such studies are twofold. The first is to identify nonrandom abnormalities that can be used to classify specific types of brain tumors, and the second is to delineate those areas of the genome that would be most fruitful for molecular studies. As part of our ongoing cytogenetic studies of pediatric brain tumors, we have prepared karyotypes from three rare tumors: one atypical teratoid tumor and two pure rhabdoid tumors of the brain. The results of these studies are presented below.

Case Reports

Case 1

This 6-month-old baby boy was well until 2 days prior to admission, when he developed vomiting, irritability, and fever. A left abdominal mass was found on examination. Abdominal computerized tomography (CT) and ultrasonography confirmed the presence of a left-sided abdominal mass which arose from the lower pole of the left kidney. On October 30, 1986, a left radical nephrectomy was performed with simultaneous biopsy of surrounding lymph nodes. Pathology disclosed a rhabdoid tumor with infiltration into the regional lymph nodes. He was transferred to The Children's Hospital of Philadelphia on November 4. In addition to the abdominal findings, his examination was remarkable for irritability, macrocephaly, a head lag, truncal ataxia, and an intention tremor. A CT scan of the head demonstrated a large hyperdense polycystic mass filling the fourth ventricle which enhanced heterogeneously on intravenous administration of contrast material. There was some surrounding edema and obstructive hydrocephalus with dilatation of the third and
Monosomy 22 in rhabdoid or atypical teratoid tumors

fourth ventricles. On November 11, a posterior fossa craniectomy and exploration disclosed a large reddish tumor within the cerebellar vermis and fourth ventricle. The tumor was grossly totally resected. Postoperative CT did not reveal residual tumor. Myelography and lumbar cerebrospinal fluid (CSF) examination performed 7 days later disclosed no evidence of tumor cell dissemination. The child was treated with high-dose cyclophosphamide, VP-16, cis-platinum, and vincristine. Follow-up CT on December 13 showed no evidence of recurrent intracranial disease.

One month later, the patient became irritable and began to vomit. Ultrasound studies of the abdomen showed recurrent tumor with ascites. A CT scan of the head after intravenous contrast enhancement showed diffuse subarachnoid enhancement of the posterior fossa and tentorium, with a discrete enhancing mass along the right tentorium. This was considered to represent leptomeningeal disease. No further treatment was given and the child died 7 days later of respiratory failure secondary to massive ascites and gastrointestinal bleeding. A postmortem examination was refused.

Case 2

This 11-month-old boy was brought to The Children's Hospital of Philadelphia in December, 1988, with a 3-month history of developmental slowing. He was the product of an uncomplicated full-term pregnancy, labor, and delivery. His early developmental milestones were normal, but in the month prior to evaluation he had stopped attempting to walk, and fell when attempting to sit. One week prior to admission he had become irritable, began waking in the middle of the night, and seemed lethargic. Intermittent vomiting began 2 days prior to evaluation.

On examination he was macroencephalic, had a right head tilt, sat with difficulty, and exhibited a bilateral intention tremor. The remainder of his neurological examination was normal. A CT scan showed a large hyperdense polycystic mass filling the fourth ventricle. Magnetic resonance (MR) imaging demonstrated the same mass, which was of decreased signal intensity on T1-weighted and increased signal intensity on T2-weighted images. On December 13, a suboccipital craniectomy and posterior fossa exploration disclosed a reddish lobular mass filling the fourth ventricle and attached to the left side of the brain stem. The tumor was grossly totally resected. Following surgery, no residual disease was seen on CT. Ten days later, the patient underwent myelography and lumbar CSF cytological examination, which disclosed no evidence of disseminated disease. Ultrasonic studies of the abdomen were normal. Postoperatively, he required a permanent ventriculoperitoneal shunt. He was treated with five cycles of intravenous cis-platinum, high-dose cyclophosphamide, vincristine, high-dose methotrexate, and oral 1-(2-chlorethyl)-3-cyclohexyl-1-nitrosourea (CCNU), which was completed in March, 1990. While he was on chemotherapy his neurological examination returned to normal, he was walking unaided, and his CT and MR studies showed no evidence of recurrent disease. In June, 1990, however, MR studies showed recurrent tumor in the brain and spinal cord.

Case 3

This 1-year-old girl was brought for evaluation of bilateral leg paralysis of acute onset. An MR image of the brain and spine was remarkable for widening of the thoracolumbar spinal canal, with lesions at the T-12 and L-2 vertebral levels. A posterior fossa tumor was also evident. The patient underwent subtotal resection of the spinal masses, which were thought to be drop metastases from a malignant tumor of the posterior fossa. Other complications from the tumor included a left facial nerve paralysis and incomplete neurogenic bladder. She received three courses of chemotherapy consisting of vincristine, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), procarbazine, hydroxyurea, cis-platinum, cytosine arabinoside (Ara-C), and cyclophosphamide.

Follow-up CT of the brain showed extension of the tumor into the right ambient cistern. An MR image of the spine showed recurrent lesions in the spinal cord at the T-10 to L-1 level. The patient was then started on radiation therapy and an experimental protocol of VP-16, l-fosfamide, and Mesna, with no response. She died 5 months from the time of diagnosis. Tissue from the posterior fossa tumor was obtained at autopsy for pathological and cytogenetic studies.

Pathological and Cytogenetic Studies

Pathological Studies

The histological features of the tumors from Cases 1 and 3 were similar. The neoplasms consisted of medium to large round cells with prominent pink cytoplasm (on hematoxylin and eosin staining). Nuclei were eccentrically placed and nucleoli were prominent. The individual cells were often outlined by a thin, bright pink membrane. In places, cells grew in a dense strand and whorl pattern. Mitotic figures were frequent and there was a prominent fibrovascular stroma. Immunoperoxidase studies gave strongly positive results for epithelial membrane antigen and vimentin, focal positivity for cytokeratin, and only rare cells containing glial fibrillary acidic protein (GFAP) and desmin. These two tumors were diagnosed as pure rhabdoid tumors.

The tumor from Case 2 was composed of cells displaying two patterns. Large regions consisted of the same type of fairly big cells with distinct cytoplasmic margins, pink cytoplasm, and round nuclei with prominent nucleoli as described above. The other component displayed a population of spindle-cell forms growing in a windblown pattern. Mitoses were fairly frequent among the large round cells. Results of the immunoperoxidase studies showed strong positivity for epithelial membrane antigen and vimentin (primarily in the mesenchymal-appearing areas), intermediate positivity...
for cytokeratin and GFAP in both the mesenchymal and nonmesenchymal portions, and negative staining for neurofilament and desmin. The final diagnosis was atypical teratoid tumor.

**Cytogenetic Analysis**

Tumor tissue from Cases 1 and 2 was obtained during surgical resection at The Children's Hospital of Philadelphia. Tumor tissue from the third child was obtained at necropsy through the Cooperative Human Tissue Network in Columbus, Ohio (Case 89-02-P01). Upon receipt in the laboratory, the tissue was minced with sterile, disposable scalpel blades in Dulbecco's modified essential medium supplemented with 20% fetal bovine serum, 2 mM glutamine, and antibiotics. Three T-25 flasks were used to initiate short-term cultures. For Case 2 tissue, a direct chromosome preparation was made by exposing cells to 0.01 μg/ml colcemid for 1 hour at 37°C, followed by hypotonic treatment with 0.075 M KCl for 35 minutes. Cells were fixed in 3:1 methanol:acetic acid, and dropped onto chilled wet slides which were then air-dried. Slides were banded with trypsin-Wright's stain, and all usable metaphases were analyzed. Chromosomes were harvested from the short-term cultures as described above. Karyotypes were arranged and designated according to the International System for Cytogenetic Nomenclature, 1985.

For the tumor in Case 1, chromosomes were harvested on Days 3 and 7. No metaphases were obtained from the 3-day culture; the 7-day preparation yielded a high number of well-banded metaphases. Forty-seven metaphases were analyzed. Seven metaphases were normal, 46,XY; the remaining metaphases were abnormal with a simple karyotype, 45,XY,-22. Five metaphase spreads were photographed and arranged. No additional abnormalities were detected.

A direct preparation of the tumor in Case 2 yielded an adequate number of metaphases for analysis. Seventeen cells were analyzed, with a range of 38 to 45 chromosomes per cell; the modal number was 45. All metaphases were abnormal with a stemline karyotype of 45,XY,-22. Chromosome preparations from a 3-day culture demonstrated better quality banding. Fifteen cells were analyzed, and all of them had an abnormal karyotype, 45,XY,-22. Two metaphases from each of these harvests were photographed and arranged, and no additional abnormalities were observed. A later harvest from the second passage (Day 15) was also successful; however, all of the metaphases obtained demonstrated normal karyotypes.

Case 3 tumor cells were harvested after 3 days in culture. Seventeen metaphases were analyzed, with a range of 40 to 46 chromosomes per cell and a modal number of 45 chromosomes per cell. Four metaphases were photographed and arranged. All metaphases were missing one chromosome 22. One cell demonstrated a random t(15;16). A representative karyotype is shown in Fig. 1.

**FIG. 1.** Representative G-banded karyotype from the rhabdoid tumor of the brain from Case 3, demonstrating monosomy 22.

J. A. Biegel, et al.
Monosomy 22 in rhabdoid or atypical teratoid tumors

Discussion

There is debate among pathologists as to the histogenesis of rhabdoid tumors. It has been suggested that there is a common primitive precursor cell that gives rise to rhabdoid tumors in various tissues but utilization of ultrastructural and immunohistochemical techniques to date have not provided clear information upon the nature of this precursor cell. A combined molecular-cytogenetic approach to a study of these tumors at various sites may be helpful in determining whether similar regions of the genome are altered in each of these tumors.

We have described the cytogenetic findings in two malignant rhabdoid tumors and one atypical teratoid tumor of the brain in three infants. All three tumors demonstrated monosomy 22 as the only chromosomal abnormality. To our knowledge, there are no full published reports of cytogenetic studies in rhabdoid tumors. However, we are aware of one primary rhabdoid tumor of the brain and one of soft tissues, each of which demonstrated an abnormal chromosome 22.4,9 Two previously described atypical teratoid tumors had normal karyotypes.7

One of us (L.B.R.) has observed that rhabdoid tumors of the brain in children are often misclassified by standard histological techniques as medulloblastoma or primitive neuroectodermal tumor (unpublished data). In contrast to the monosomy 22 in rhabdoid or atypical teratoid tumors reported here, the most common cytogenetic abnormality observed in primitive neuroectodermal tumors of the central nervous system is an isochromosome 17q.1,3,7 Furthermore, involvement of chromosome 22 has rarely been noted in primitive neuroectodermal tumors of the brain.1,3,7 Although the data presented here are limited, it appears that monosomy of chromosome 22 may be a nonrandom change in rhabdoid or atypical teratoid tumors. These findings therefore suggest that cytogenetics may prove useful in the differential diagnosis of rhabdoid or atypical teratoid tumors of the brain. Clearly, a larger number of tumors require study before this marker can be used in a clinical setting.

Monosomy 22 is also the common chromosomal abnormality in both pediatric and adult meningiomas, occurring in approximately 70% of cases.11 In a smaller proportion of these tumors, deletions of the distal long arm have been described. Cytogenetic studies of a limited number of acoustic neurinomas have also demonstrated monosomy 22 as a nonrandom change.12

The gene for the central form of neurofibromatosis, NF-2, has been mapped to the long arm of chromosome 22 by linkage analysis in families.13 Patients with NF-2 are at an increased risk for developing a variety of tumors, including meningiomas and bilateral acoustic neurinomas. Molecular genetic analysis of matched normal and tumor tissue from patients with acoustic neurinomas, meningiomas, and spinal neurofibromas has demonstrated tumor-specific loss of alleles on chromosome 22.3,14 These findings have implicated a common pathogenetic mechanism of tumorigenesis in these three benign nervous system tumors, and suggest the presence of one or more tumor suppressor loci on chromosome 22q.14 The development of malignant rhabdoid or atypical teratoid tumors of the brain could be due to a different mutation of the same gene(s) involved in meningioma, acoustic neurinoma, and spinal neurofibroma, or alternatively, may involve a distinct locus on chromosome 22.

Finally, several other nonrandom, tumor-associated chromosomal breakpoints have been regionally localized on chromosome 22,8 suggesting the presence of additional genes involved in tumorigenesis on this chromosome. In order to identify and determine the role of specific genes involved in the initiation or progression of these diverse tumors, additional molecular and cytogenetic studies of fresh tumors are warranted.

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References


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