Chromosome Abnormalities in Pediatric Brain Tumors

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ABSTRACT

Recurrent, site-specific chromosome translocations and other cytogenetic abnormalities are being described in ever-increasing numbers and types of human tumors. Primary brain tumors are the most common pediatric solid tumor and differ from those of adults in both histology and clinical behavior. We examined chromosomes from 21 primary pediatric brain neoplasms grown in short-term tissue culture, including 6 astrocytomas, 10 primitive neuroectodermal tumors, and 5 other tumors. Karyotypes from 3 of 5 astrocytomas were abnormal, as were those of 9 of 10 primitive neuroectodermal tumors. Numerical abnormalities were found in 6 tumors and structural aberrations in 12 tumors. Deletions, additions, and translocations involving the short arm of chromosome 1 were observed in 5 tumors, with chromosome breakpoints ranging from 1p1 to 1p3. An isochromosome of the long arm of 17, i(17q) was the most frequent site-specific structural abnormality, found in 1 anaplastic astrocytoma and 2 recurrent cerebellar primitive neuroectodermal tumors, one with islands of anaplastic astrocytoma. These results differ from reported chromosome studies of adult brain tumors, suggesting that pediatric brain tumors may differ from those of adults when examined at the genetic level. Additional chromosomal and molecular studies of brain tumors from children are warranted to define these differences.

INTRODUCTION

Recurrent, site-specific chromosomal translocations and other nonrandom cytogenetic abnormalities are being found in ever-increasing numbers and types of human tumors. Molecular studies have demonstrated that the 9;22 translocation of chronic myelogenous leukemia results in production of an aberrant bcr-abl fusion protein (1-4). While the cytogenetically similar chromosomal translocation in acute lymphocytic leukemia differs at the molecular level (5, 6), it too results in expression of several unique abl-derived tyrosine kinases which, however, are distinct from the aberrant protein of chronic myelogenous leukemia (7). In lymphomas, the 8;14 and variant 2;8 and 8;22 translocations of Burkitt’s lymphoma result in c-myc gene deregulation (8–10). These results strongly suggest that specific chromosome rearrangements are important in tumor initiation or maintenance. Similar clues regarding the location of genes important in solid tumor pathology are now being sought by cytogenetic examination of solid tumor specimens.

Primary brain tumors are the most common solid tumor occurring in children (11). Pediatric brain tumors differ from those of adults in both histology and clinical behavior (12). Comparison of chromosomal findings of pediatric and adult brain tumors may delineate additional significant differences between tumors of children and adults. Here we report results of chromosome analysis of 21 primary pediatric brain neoplasms and show that analysis of cytogenetic changes in these tumors can quite often be accomplished using current optimal solid tumor protocols.

METHODS

All tumors from children undergoing diagnostic or therapeutic surgery for brain tumors at The Children’s Hospital of Philadelphia during a 10-mo period were eligible for cytogenetic study when sufficient excess tissue remained after that required for analysis by the pathologists. Tumors were placed in transport media (RPMI 1640 with 15% fetal calf serum) immediately upon removal at surgery and were brought to the cytogenetics laboratory where they were usually processed within 1 h of arrival. One specimen (Case 7, a medulloblastoma metastatic to bone marrow) was sent from an outside hospital and cultured the following day.

Tumors were dissected aseptically into 1- to 2-mm pieces. Specimens which were not easily dispersed mechanically were further disaggregated in 0.8% collagenase type II (Cooper Biomedical) in RPMI 1640 for 2–5 h until reduced to small clumps of cells. Cells were then centrifuged to remove them from collagenase and seeded into 25-cm² tissue culture flasks in RPMI 1640 (GIBCO) with 15% fetal calf serum (GIBCO), 50 units penicillin, 100 ¿g streptomycin, 2 mM glutamine and cultured at 37°C in 5% CO₂/95% air. At least two cultures from each specimen were initiated whenever possible.

Cultures were observed daily for evidence of cell growth and harvested for metaphase chromosome preparations as soon as mitotic cells were observed. Cells were exposed to colcemid, 0.01–0.03 ¿g/ml for 1–24 h depending on the number of mitotic cells seen, treated with prewarmed 0.075 M KCl for 30 min at 37°C, and fixed in 3 changes of 3:1 methanol:acetic acid. Slides were made, air-dried, and aged at least 1 wk at room temperature before staining with Wright’s stain and G-banding with trypsin (13) or phosphate buffer (14). At least 20 metaphases and 4 karyotypes from each tumor were analyzed whenever possible.

The pathological classification of all tumors was performed by one of us (L. B. R.) except for the tumor from Patient 7, which was sent from an outside hospital. Classification was based on the revision of brain tumor nomenclature described at the Pediatric Brain Tumor Workshop of 1984 (15). Two of these classes are further explained here. Atypical teratoid tumors consist of a mixture of neuroepithelial, epithelial, and mesenchymal cells of various types and in varying proportions. They also generally contain a population of cells similar to those found in rhabdoid tumors. They do not resemble any of the ordinary germ cell tumors and because of their mixed cellular elements were given the unusual name of atypical teratoid tumors. To date they seem to be a tumor of infancy. PNETs are a group of central nervous system tumors which occur most commonly in childhood and are primarily composed of primitive or undifferentiated neuroepithelial cells. Much histological heterogeneity is often present. The term PNET, not otherwise specified, describes poorly differentiated neuroepithelial cells, while PNET with astrocytes, rosettes, etc. describes the presence of more differentiated cells within the tumor.

RESULTS

Twenty-one brain tumors were received for chromosome analysis during the period of study. Histological subtypes included 6 astrocytomas, 10 PNETs, 2 atypical teratoid tumors, 1 mixed glioma, 1 ependymoma, and 1 meningioma. We have...
included Case 10 in the group of PNETs. This was a recurrent tumor which had features of anaplastic astrocytoma; 7 yr earlier at initial presentation the tumor appeared to be a PNET with islands of anaplastic astrocytoma. Thirteen tumors were newly diagnosed, 6 were recurrent disease, and 2 were second primary tumors (pineoblastomas in children with hereditary retinoblastomas).

No metaphases were obtained in 3 tumors, in 6 we were unable to detect abnormalities, and 12 had abnormal karyotypes with or without normal cells present. The chromosomes of 3 of 5 astrocytomas were abnormal, as were those from 9 of the 10 PNETs. Karyotype results are summarized in Tables 1–3.

The majority of tumors were near diploid. Two tumors had 2 distinct populations of cells (Cases 2 and 7), while 2 tumors (Cases 10 and 13) had only a hypotetraploid cell population. Five tumors had clonal numerical abnormalities, with additions and losses of apparently normal chromosomes. Additions of chromosome 7 were noted in 2 tumors, both anaplastic astrocytomas, and gains of chromosomes 3, 10 and 13 were each noted once. Loss of chromosome 16 was observed in 2 tumors, an anaplastic astrocytoma and an ependymoma, and losses of chromosomes 6, 10, and 14 were each noted once.

Twelve tumors had structural chromosome abnormalities; 5 tumors had more than one. The most frequently nonrandom structural abnormality was an isochromosome for the long arm of 17, i(17q). This abnormality was found in 3 tumors, including a newly diagnosed anaplastic astrocytoma (Fig. 1), a recurrent tumor, which at the time of original diagnosis was classified a PNET with islands of anaplastic astrocytoma and at recurrence appeared to be predominantly an anaplastic astrocytoma (Fig. 2), and a cerebellar PNET (recurrent medulloblastoma) (Fig. 3). An i(17q) was never the solitary abnormal finding.

Deletions, additions, or translocations involving the short arm of chromosome 1 were observed in 5 tumors. The breakpoints varied between 1p1 and 1p3 and in one case could not be assigned with greater specificity than to the involved region because of the quality of chromosome banding obtained. Involvement of 1p was observed in three astrocytomas: addition of unidentified material to 1p3 (Case 10); a der(1)t(1;14) (p36;q24) in Case 15; and a der(1q15q) in Case 12. An ependymoma from Case 18 contained an extra copy of a deleted 1, a 1p− chromosome which appeared to be deleted within 1p1.
or proximal 1p2. The PNET from Case 8 contained two derivative chromosomes 1, the first a 1p− chromosome with deletion within 1p13-21, and the second a 1p+ chromosome with addition of unidentified material at 1p36, possibly forming an aberrantly banding region. These abnormalities of chromosome 1 are illustrated as partial karyotypes in Fig. 4.

Abnormalities were also observed. Fig. 5 shows a cerebellar PNET with monosomy 6 and an interstitial deletion of 3q in the region q24-q26. The two patients with pineoblastoma occurring following hereditary retinoblastoma were of particular interest, but the quality of chromosomes obtained from these two tumors limited the findings to those abnormalities of chromosome 1 described above for Patient 8 and the observation of a 1q+ chromosome and a Dq+ chromosome in the tumor cells from Patient 9. Involvement of chromosome 13, which could include deletions within 13q or monosomy 13, could not be confidently determined in these two instances.

Clinical correlation of chromosome abnormalities with patient survival will be of interest. At the time of this writing 5 patients have died, 3 are alive with disease, and 12 patients are disease free. The number of patients in this study is too small and the mean length of follow-up (less than 12 mo) is too short to determine meaningful correlations between survival and karyotype.
See Tables 1 and 2 for descriptions of the derivative chromosomes of Patient 8. The abnormal chromosome is indicated by an arrow in each set. It is anaplastic astrocytoma (Patient 12); a. PNET, pineoblastoma with islands of anaplastic astrocytoma (Patient 10); b. astrocytoma, fibrillary type; c. ependymoma (Patient 18); d. recurrent PNET; e. astrocytoma, fibrillary type short arm of chromosome 1. a, ependymoma (Patient 18); b. recurrent PNET; c. astrocytoma, fibrillary type; d. anaplastic astrocytoma (Patient 12); e. PNET, pineoblastoma (Patient 8). The abnormal chromosome 1 is indicated by an arrow in each set. See Tables 1 and 2 for descriptions of the derivative chromosomes 1.

**DISCUSSION**

The majority of brain tumors occur in two age peaks, one in childhood (ages 3–12 yr) and the other in older adults (50–70 yr) (12) and both the histology and clinical behavior of these two groups of tumor differ significantly. More than half of the pediatric central nervous system lesions are infratentorial in location, while most adult brain tumors are supratentorial (11). However, the majority of primary central nervous system tumors at all ages are neuroectodermal in origin (16). In children, the most common histological types are astrocytomas and primitive neuroectodermal tumors (often called medulloblastomas) (16, 17). Many of the latter type are composed of embryonic or poorly differentiated cells which often present with unusual histological patterns of growth. Significant heterogeneity of cell types is often present in these neoplasms. Considerable variation in the histological pattern within a given tumor or from one tumor to another is common. Consequently, there has been a tendency to create new, descriptive names for tumors which are not easily classified (16, 17), a practice which makes it difficult to study the literature of pediatric brain tumors. These facts must be borne in mind when one considers the cytogenetics of these neoplasms.

We have presented results of chromosome analysis of a diverse group of pediatric brain tumors studied after short-term tissue culture. Metaphases for analysis were obtained from 18 of 21 tumors using routine cytogenetic methods. To our knowledge, only limited reports of chromosomes from pediatric brain tumors exist. Studies from the prebanding era include reports of 5 medulloblastomas (PNETs) from children with karyotypes varying from hypodiploidy to hypotetraploidy (18–20). Biegel et al. (21) have reported karyotypes from 6 cases of medulloblastoma of which 5 were from individuals 16 yr and younger. The primary abnormalities they found were nonreciprocal translocations and deletions involving chromosomes 1, 3, 5, 11, 17, 20. Douglass et al. (22) reported that 3 of 3 primitive neuroectodermal tumors from children (ages and sites of disease not specified) had abnormalities of chromosome 1, including deletion of the distal portion of 1p, multiple copies of a 1p− chromosome, and trisomy 1, +1p−. Latimer et al. (23) found no chromosomal abnormalities in two pediatric medulloblastomas and hyperdiploidy, −Y, and two marker chromosomes from a third. Yamada et al. (24) found normal chromosomes in craniopharyngiomas from a 3-yr-old male and a 16-yr-old female and in a pituitary adenoma from an 18-yr-old male. These workers also described an oligodendroblastoma from a 13-yr-old male, which was characterized by diploidy, and a t(21;22) when studied after 1 day in culture. Peripheral blood cells from that patient showed a normal karyotype. Bigner et al. (25) studied chromosomes of a giant cell glioblastoma from an 11-yr-old female, which was found to be near haploid and to include two copies of chromosomes 1, 7 or 7p+, and 18.

While the number of tumors we have studied thus far is smaller than the number of adult brain tumors which have been reported, comparison of our results with those from adult brain tumors suggests several differences in the chromosomal abnormalities of these two general groups of tumors. Reports of frequent involvement of 9p (Ref. 26), trisomy 7 (Refs. 27–31), and loss of chromosome 22 in both gliomas (32) and meningiomas (33–35) characterize adult brain tumors studied to date. While we did observe additional copies of chromosome 7 in two tumors and a deletion involving the short arm of chromosome 9 in one tumor, we did not observe monosomy 22 in this series of tumors. Instead, we found that one of the most frequent structural abnormalities was an isochromosome (17q) present in three tumors, including one medulloblastoma, one anaplastic astrocytoma, and one anaplastic astrocytoma occurring 6 yr after diagnostic and treatment of a PNET with islands of anaplastic astrocytoma. In three other PNETs (medulloblastomas) i(17q) has been noted (28) but has not been reported in adult astrocytommas.

Six structural abnormalities involving 1p were also observed in 5 tumors, including additions, deletions, and translocations, with breakpoints appearing to range from 1p1 to 1p3. While abnormalities involving 1p have occasionally been observed in adult gliomas (26–31), these findings are more reminiscent of deletions of 1p noted in neuroblastomas (35), which occur most often in children.

The significance of i(17q) is unclear. This structural abnormality has been reported in numerous neoplasms, including acute leukemias, refractory anemia, preleukemia, myeloscle-
Table 2 Results of chromosome analysis of astrocytomas

<table>
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<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Pathological diagnosis and tumor location</th>
<th>No. of days in culture</th>
<th>No. of metaphases</th>
<th>Modal Range</th>
<th>Stemline karyotype</th>
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<tr>
<td>11</td>
<td>7/12</td>
<td>F</td>
<td>Angiomatous astrocytoma, protoplasmic type; right temporal hemisphere</td>
<td>6</td>
<td>6</td>
<td>46</td>
<td>43-46</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
<td>M</td>
<td>Anaplastic astrocytoma; right temporal hemisphere</td>
<td>3</td>
<td>40</td>
<td>47-48</td>
<td>44-49</td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>M</td>
<td>Anaplastic astrocytoma; cerebellum</td>
<td>1</td>
<td>25</td>
<td>84-88</td>
<td>78-92</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>M</td>
<td>Astrocytoma, fibrillary type, with anaplastic features; 4th ventricle*</td>
<td>12</td>
<td>25</td>
<td>46</td>
<td>40-46</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>F</td>
<td>Astrocytoma, fibrillary type; basal ganglia*</td>
<td>26</td>
<td>12</td>
<td>46</td>
<td>43-46</td>
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<tr>
<td>16</td>
<td>2</td>
<td>F</td>
<td>Astrocytoma, pilocytic type; optic nerve*</td>
<td>None</td>
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<td></td>
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* Range in which 75% of counts were observed.

Table 3 Results of chromosome analysis of other pediatric brain tumors

<table>
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<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Pathological diagnosis and tumor location</th>
<th>No. of days in culture</th>
<th>No. of metaphases</th>
<th>Modal no.</th>
<th>Range</th>
<th>Stemline karyotype</th>
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<tr>
<td>17</td>
<td>8</td>
<td>F</td>
<td>Meningioma, transitional, location not specified*</td>
<td>None</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>M</td>
<td>Ependymoma; 4th ventricle</td>
<td>8</td>
<td>16</td>
<td>46</td>
<td>43-46</td>
<td>46.XY, +del(1)(p22),-16</td>
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<tr>
<td>19</td>
<td>13</td>
<td>M</td>
<td>Mixed glioma, ependymo-oligodendroglioma; cerebellum</td>
<td>16</td>
<td>18</td>
<td>46</td>
<td>44-46</td>
<td>46.XY</td>
</tr>
<tr>
<td>20</td>
<td>16/24</td>
<td>M</td>
<td>Atypical teratoid tumor; cerebellum</td>
<td>2</td>
<td>9</td>
<td>46</td>
<td>46</td>
<td>46.XY</td>
</tr>
<tr>
<td>21</td>
<td>4/12</td>
<td>F</td>
<td>Atypical teratoid tumor; cerebellum</td>
<td>5</td>
<td>43</td>
<td>45-46</td>
<td>43-46</td>
<td>46.XX (random losses)</td>
</tr>
</tbody>
</table>

* Range in which 75% of counts were observed.

* Recurrent disease.

Fig. 5. Karyotype of a newly diagnosed PNET from a 4-yr-old male (Patient number 6). 45,X,Y, -6, 3q-. Arrows, structurally abnormal chromosomes. Inset, pairs of chromosome 3 from two additional cells: the normal 3 is on the left and the abnormal 3 is marked with an arrow on the right.
CHROMOSOMES OF PEDIATRIC BRAIN TUMORS

rosis, lymphomas, and most frequently in the progressive phase of chronic myelogenous leukemia (36). It has also been observed less frequently in solid tumors, including carcinomas of the colon, cervix, prostate, ovary, and, interestingly, retinoblastoma (36). At present, it is not known whether a dosage effect of extra copies of genes from 17q may contribute to disease in these cases, but the roles of oncogenes c-erb-A1 at 17q11-12(37) and neu at 17q21-22 (37) and of growth hormone at 17q22-34 (37) must be considered. Alternatively, loss of genes present on 17p could represent the significant event. Perhaps presence of i(17q) indicates aggressive or progressive disease. Collection of survival data from cases with i(17q) will be required to verify this suggestion.

That the clinical course of disease correlates with specific chromosome abnormalities present in tumors has been shown most convincingly for subgroups of leukemias where specific translocations offer independent prognostic information in childhood acute lymphocytic leukemia (38, 39) and in subgroups of acute myelocytic leukemias (40). Whether chromosomal characterization of brain tumors in children will also provide prognostic predictors for response to therapy and overall survival remains to be defined.

Study of additional tumors is warranted. Description of specific chromosome abnormalities, determination of genes present at specific chromosome regions involved in such abnormalities, and determination of the clinical relevance of such observations all remain to be elucidated. Because a diversity of pathological classifications is used in the diagnosis of pediatric brain tumors, investigators should keep an open mind when comparing results of chromosome analyses between different histological types. As much clinical and pathological data as possible should be included with chromosome reports so that genetic subgroups of these tumors can be identified and compared.

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REFERENCES

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