Loss of Heterozygosity on 6q, 16q, and 17p in Human Central Nervous System Primitive Neuroectodermal Tumors

Gregory A. Thomas¹ and Corey Raffel²

The Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah 84132 [G. A. T.] and the Division of Neurosurgery, Childrens Hospital of Los Angeles and the Department of Neurosurgery, University of Southern California School of Medicine, Los Angeles, California 90027 [C. R.]

ABSTRACT

The loss of genetic material from specific chromosomal locations in a given tumor type has been taken for evidence of the importance of tumor suppressor genes at these loci in the genesis of the tumor. The primitive neuroectodermal tumor of the central nervous system has such a loss on 17p in one-third of tumors. In this report, a detailed analysis of 17p loss in 23 tumors has been performed using 10 probes mapping to this region. In addition, an analysis for allelic deletion on chromosomes 6q, 16q, and 22q has been performed. Six of the 23 tumors showed loss of markers on 17p, and the area of common loss spanned 17p11.2 to 17pter. Five of the 23 tumors showed loss of markers on 6q, and 3 showed loss on 16q. No tumor lost markers on 22q. Only one tumor showed loss at more than one location. These data suggest that primitive neuroectodermal tumors either are a heterogeneous group of tumors with more than one mechanism leading to a tumor or that more than one recessive oncogene may play a role in the genesis of these tumors.

INTRODUCTION

PNETs³ are central nervous system tumors that occur predominately in the pediatric population. PNETs may occur anywhere in the brain but have a propensity for the posterior fossa. Cellular differentiation along glial or neuronal lines may occur within the tumor (1). Five-year survival with current therapy combining operative resection, radiation therapy, and chemotherapy is about 60% (2).

Recessive oncogenes may play a role in the development of a number of pediatric tumors (for review, see Ref. 3). These genes are thought to normally function as regulators or suppressors of cell division (4). Mutation through a variety of mechanisms may cause inactivation of both copies of a tumor suppressor gene, leading to potentially unrestricted growth. The best studied recessive oncogene is the retinoblastoma susceptibility gene, RB-1 (5, 6). Mutations of both copies of the RB-1 gene have been documented in retinoblastoma, and insertion of the gene has been shown to suppress the transformed phenotype in retinoblastoma and osteosarcoma cell lines (7).

The loss of a specific region of one chromosome in a given tumor type suggests that the region contains a recessive oncogene important in the development of that tumor. The technique of Southern blot analysis can be used to compare an individual's constitutional genotype with that of his tumor by examining the pattern of RFLPs seen in constitutional and tumor DNA. Loss of constitutional heterozygosity in tumor DNA at a given locus, called a loss of heterozygosity in the tumor, indicates that a portion of the patient's genome containing that locus has been deleted from the tumor. Tumor-specific loss of genetic material may signal the presence of a recessive oncogene in the deleted DNA fragment. RFLP analyses have been used frequently to detect loss of DNA in a growing number of pediatric tumor types (8-11).

Data defining specific genomic deletions in PNETs have come from two sources. Cytogenetic studies have shown that deletion of the short arm of chromosome 17 is the most common abnormality detected (12-15). In addition, losses from chromosomes 6q, 16q, and 22 have been seen (13, 14). Using an RFLP analysis, we have shown that 3 of 9 tumors have lost genomic material from 17p (16). No loss was identified on chromosomes 1p, 7q, 10, 11p, or 13q.

To further define the region of deleted DNA on the short arm of chromosome 17, we have now examined 23 tumors by RFLP analysis with 10 probes mapping throughout 17p. In addition, we have looked for tumor-specific DNA loss on chromosomes 6q, 16q, and 22. The results of these analyses are presented here.

MATERIALS AND METHODS

The patient material used in this study come from two institutions. Those numbered PN1-PN7 come from the University of Utah Medical Center; the remaining patients were treated at Childrens Hospital of Los Angeles. The patients represent a consecutive series treated for PNET between July 1988 and December 1989 at the two institutions. Normal DNA isolation, tumor DNA isolation, and Southern blot analysis for the Childrens Hospital of Los Angeles patients were performed as described previously (16). The same techniques on the University of Utah patients were performed as previously described (17)

The probes used in this analysis are listed by clone and locus in Table 1. Probes EW502, EW503, EW504, and EW505 were obtained from Dr. Mark White. All other probes were obtained from the Repository of Human DNA Probes and Libraries (American Type Culture Collection, Rockville, Md.). A number of the probes used detect more than one RFLP with one or more enzymes.

RESULTS

Of the 23 patients involved in these studies, 19 had a PNET of the posterior fossa (medulloblastoma); the remaining patients had supratentorial intracerebral tumors. Three tumors were recurrences after radiation therapy and chemotherapy; two recurrences were local in the posterior fossa and the third was an intramedullary metastasis to the cervical spinal cord in a patient whose original tumor was in the posterior fossa (Table 2).

The 10 probes, detecting 19 RFLPs, used to analyze deletions on the short arm of chromosome 17 are listed, along with their physical map location, in Table 1. Six of the 23 tumors (26.1%) showed loss of alleles on 17p when compared to the constitutional genotype of the patient from whom the tumor arose.

---

¹ Received 6/21/90; accepted 10/30/90.
² The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
³ This work supported by American Cancer Society Clinical Oncology Career Development Award 89-189 (to G. A. T.).
² To whom requests for reprints should be addressed, at 1300 N. Vermont Ave., Ste. 906, Los Angeles, CA 90027.
² The abbreviations used are: PNET, primitive neuroectodermal tumor; RFLP, restriction fragment length polymorphism.
The tumor from patient 1 lost an allele from 6 informative RFLPs identified (D17S1, YNZ22.1, MCT35.1, EW502, p10–5). No other informative RFLPs were identified in this patient’s normal DNA.

The tumor from patient 4 lost an allele from all informative RFLPs identified (YNM37.3, YNZ22.1, p10–5). This patient’s normal DNA was homozygous with all the other probes used. The normal DNA from patient 14 was not informative at D17S1, YNM67, and p10–5. At 7 informative loci (YNH37.3, YNZ22.1, MCT35.1, EW502, EW503, EW504, EW505), an allele was lost from the tumor DNA. The tumor from patient 42 lost an allele at all informative RFLPs identified (D17S1, YNZ22.1, EW502, EW503, EW504, EW505, p10–5); the other probes used were not informative. The tumor from patient PN1 lost an allele at 6 informative loci used (YNH37.3, YNZ22.1, D17S1, p10–5, EW503, EW505) while retaining constitutional heterozygosity at YNM67 (Fig. 1). The remaining probes were uninformative. The tumor from patient PN6 lost constitutional heterozygosity at YNH37.3, YNZ22.1, EW504, EW502, MCT35.1, and EW503 but also retained constitutional heterozygosity at YNM67 (Fig. 1B). The other probes used were not informative. The results from the 6 tumors showing loss of constitutional heterozygosity on 17p are summarized in Fig. 2.

The constitutional and tumor genotypes from 23 patients with PNET were also compared for loci on chromosomes 6q, 16q, and 22q (Table 3). Six markers detecting 10 RFLPs were used to look for a reduction to homozygosity on 6q. DNA from 21 patients were examined with these probes, and all samples were informative at 1 or more loci. Five of these tumors showed a reduction to homozygosity with at least 1 marker. The tumor from patient 12 lost an allele at three loci (p2–2, pOR-3, and pJCZ30 (HindIII)) and was uninformative at the other loci examined. A loss of constitutional heterozygosity was seen in the tumor from patient 24 at the locus defined by pOR-4. Five other RFLPs examined were uninformative in this patient. The tumor from patient 41 lost an allele present in the patient’s constitutional DNA detected by pJCZ30 (HindIII). The only other probe used on this tumor, pHM2.6, was not informative. Patient 43 was constitutionally heterozygous for p2–2, pOR-3, and pHM2.6; however, no mutation was detected.

DISCUSSION

The data presented here confirm and extend our work on chromosome 17p deletions in childhood PNETs. Previously,
Table 3: Loss of constitutional heterozygosity on various chromosomes in PNET

<table>
<thead>
<tr>
<th>Patient</th>
<th>6q</th>
<th>16q</th>
<th>17p</th>
<th>22q</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RFLP</td>
<td>LOH</td>
<td>RFLP</td>
<td>LOH</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>0/2</td>
<td>2</td>
<td>2/2</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>0/4</td>
<td>6</td>
<td>0/5</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>ND</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>0/5</td>
<td>5</td>
<td>0/2</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>0/3</td>
<td>4</td>
<td>0/0</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>ND</td>
<td>4</td>
<td>0/2</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>0/4</td>
<td>5</td>
<td>0/3</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>1/2</td>
<td>5</td>
<td>3/4</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>3/3</td>
<td>5</td>
<td>1/5</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>0/1</td>
<td>4</td>
<td>0/3</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>2/2</td>
<td>2</td>
<td>0/2</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>1/1</td>
<td>2</td>
<td>0/2</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>0/4</td>
<td>2</td>
<td>0/4</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>0/2</td>
<td>2</td>
<td>0/2</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>0/4</td>
<td>8</td>
<td>0/6</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>0/4</td>
<td>8</td>
<td>0/7</td>
</tr>
<tr>
<td>17</td>
<td>6</td>
<td>0/1</td>
<td>8</td>
<td>0/2</td>
</tr>
<tr>
<td>18</td>
<td>6</td>
<td>0/3</td>
<td>8</td>
<td>0/7</td>
</tr>
<tr>
<td>19</td>
<td>6</td>
<td>0/4</td>
<td>8</td>
<td>0/7</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>0/4</td>
<td>8</td>
<td>0/5</td>
</tr>
<tr>
<td>21</td>
<td>6</td>
<td>0/4</td>
<td>8</td>
<td>0/8</td>
</tr>
</tbody>
</table>

* The number of RFLPs examined at each chromosomal location for each patient.
* LOH, loss of heterozygosity; listed as number of loci with allelic loss/number of informative loci. ND, not determined; NI, not informative.

we have shown that 3 of 9 tumors had allelic loss on 17p using 3 probes (16). In the current report, 6 of 23 tumors (including the initially described 3) show loss of DNA in this location.

In addition to the original 3 probes, the tumors were examined with 8 more probes to define a small critical deletion on 17p. The data obtained can be used to identify the subchromosomal location of a possible recessive oncogene on 17p, because such a gene must be contained in the smallest common region of chromosomal loss (Fig. 2). Five of the 7 tumors showing loss of heterozygosity on 17p lost an allele with every informative RFLP identified. Patients PN1 and PN6 retained constitutional heterozygosity with YNM67, physical map location 17pl1.2, and lost constitutional heterozygosity with all informative markers distal to this location. Thus, these data indicate that a recessive oncogene may be located on 17p between 17p11.2 and pter (Fig. 2). Of note, the percentage of tumors showing allelic loss on 17p in this report (26.1%) is quite similar to the percentage of tumors showing an iso17q abnormality (33.3%) on cytogenetic analysis of 30 tumors (13). Thus, the

Fig. 1. Reduction to homozygosity on chromosome 17p. Southern blots on normal (A) and tumor (T) DNA from patient PN1 (A) and PN6 (B) probed with YNM67 and YNZ22.1. Left ordinate, alleles identified; right ordinate, corresponding length in kilobases. DNA samples were digested with RsaI (YNM67) or TaqI (YNZ22.1).

Fig. 2. Patterns of 17p marker loss in 6 PNET. Diagram of allele loss patterns among the six tumors with loss of constitutional heterozygosity for loci on 17p. The physical map location of each marker is shown relative to the karyogram (left). Right, hybridization results for each tumor; ordinate, tumor identifiers; black circles, marker not informative (constitutional homozygosity); light gray circles, loss of one marker in tumor DNA (loss of constitutional heterozygosity); dark gray circles, presence of both alleles in tumor DNA (retention of constitutional heterozygosity).

Fig. 3. Gain of heterozygosity on chromosome 6q. Southern blot of normal (N) and (T) DNA from patient PN2 digested with HindIII and probed with phosH16. Ordinate, allele lengths in kilobases. A single 5.4-kilobase allele is present in the normal DNA, whereas the tumor DNA contains the 5.4-kilobase allele and a 5.0-kilobase allele not present in the normal DNA.
tumors with 17p deletions may correspond to those tumors with iso17q chromosomes identified cytogenetically. Unfortunately, this point cannot be proven because karyotypes are unavailable for many of the tumors in this series.

Loss of a portion of 17p has been described for a number of other tumor types, including colorectal carcinoma, breast carcinoma, lung carcinomas, and osteosarcoma (18–20). In addition, loss of 17p has been reported in other central nervous system tumors. In a series of 37 gliomas, James et al. (21) found that 22% of the tumors showed loss of constitutional heterozygosity on 17p. El-Azouzi et al. (22) found that 5 of 10 astrocytomas had lost a portion of chromosome 17p. Similar to our data, the smallest area of common deletion in the 5 tumors was from 17p11.1 to 17pter. In a study of 35 tumors, either anaplastic astrocytoma or glioblastoma multiforme, Fults et al. (17) identified loss of constitutional heterozygosity in 40% of the tumors. The smallest region of common deletion in the tumors of this report was from 17p11.2 to 17pter. Thus, PNETs and gliomas may share a common recessive oncogene important in their development.

The location of the putative recessive oncogene on 17p in PNET derived from the data presented here is large. Possibly with the analysis of more tumors, a smaller segment of 17p will be identified. Alternatively, a large deletion may be occurring because there is more than one tumor suppressor gene important in the development of the tumors located in this chromosomal segment. Of course, not all the tumors in this study show a detectable deletion on 17p. These tumors may have small deletions or point mutations in the region of a recessive oncogene that are beyond the limits of detection of the techniques used here.

The area of interest on 17p in PNET defined in this paper contains the gene for p53, which has been mapped to 17p13.1 (23, 24). Inactivation of both copies of this gene has been demonstrated in colorectal carcinoma and lung carcinoma, suggesting that this gene functions as a recessive oncogene important in the development of these tumors (25, 26). Nigro et al. (27) described the p53 sequences from a number of tumors, including 5 brain tumors. Point mutations in the p53 gene were identified in 4 of the 5 brain tumors. In the tumor without a p53 mutation, no p53 protein could be identified by Western blot. All 5 of the tumors in this report were glioblastoma multiformes.

The p53 gene may be the putative recessive oncogene on 17p in PNET. The role of p53 in PNET can be defined by sequencing the p53 cDNA isolated from PNETs. If point mutations in functionally critical areas are found, a strong argument could be made for a role for p53 in the development of these tumors.

In addition to the deletion on 17p, 22% (5/23) of tumors in this study showed a deletion on 6q, and 14% (3 of 22 informative) of tumors showed loss on 16q. Loss of alleles on 6q has been reported in almost 40% of malignant melanomas and in 3 of 5 ovarian carcinomas (28, 29). A small area of common deletion on chromosome 6 in our 5 tumors cannot be defined despite the use of 6 different probes. One tumor gave especially interesting results on 6q. The constitutional DNA from patient PN2 was homozygous when probed with the c-ros complementary DNA probe, phrosH16. The tumor DNA from this patient was heterozygous with this probe, indicating that a mutation had occurred on 1 chromosome in the tumor near the polymorphic HindIII restriction site detected by this probe. Thus, a putative tumor suppressor gene may be located in 6q near the c-ros gene, although the change seen with c-ros may be coincidental and not related to such a gene. A similar change from constitutional homozygosity to heterozygosity in a tumor has been used to identify a possible tumor suppressor gene on 18q important in the development of colorectal carcinoma (30).

The data presented here suggest that 2, possibly 3, distinct areas of the genome may be involved in the development of PNET. Thus, a series of genomic changes may be necessary for the development of a PNET. The concept of tumor development occurring as a progressive accumulation of genetic changes has been best characterized in colorectal carcinoma (see Ref. 31 for review). In this tumor, a series of changes including chromosome 5 alterations, ras gene activation, chromosome 18q loss (DCC gene), and chromosome 17p loss (p53 gene) leads from a normal colonic epithelial cell through colonic adenoma to colorectal carcinoma. A similar scheme has been developed for the glioma series of brain tumors, in which changes in 17p (p53 gene), epidermal growth factor receptor mutation and/or amplification, and chromosome 10 deletion lead from an astrocyte to glioblastoma multiforme (21, 32).

If a series of genetic changes is required for the development of PNET, oncogene changes, either gene amplification or rearrangement might be expected, in addition to changes in tumor suppressor genes. A candidate oncogene in PNET is c-myc. In our previous report, we demonstrated c-myc amplification in 1 of 10 tumors (16). Elevated levels of c-myc mRNA have been reported in 3 of 6 PNET; 1 of these showed evidence of a c-myc mutation (33). Bigner et al (34) found c-myc amplification in cell lines or xenografts derived from 4 of 7 tumors; 1 cell line without amplification showed evidence of gene rearrangement. Interestingly, this same group reported that c-myc amplification was not present in a series of 20 primary tumor samples, suggesting that c-myc amplification may be present in a small subpopulation of tumors cells that become dominant during in vitro handling (35).

Only one tumor in this series had more than one chromosomal deletion, and no tumor had both a 6q and a 17p deletion. Interestingly, the ages of patients whose tumors harbor a 6q deletion differ dramatically from those of patients whose tumors harbor 17p deletions. The average age of patients in the first group is 4.1 years; the oldest patient is 6 years. The average age of patients in the second group is 9.2 years; only one patient is less than 9 years. Thus, both age and RFLP analysis divide patients with PNET into two groups. This suggests that the group of tumors called PNETs may be heterogeneous and different genetic mechanisms may be responsible for the development of the various members of the group. The answer to whether several genomic changes are necessary for the development of a PNET or whether the group of tumors is heterogeneous with respect to mechanisms leading to a tumor awaits the identification of the recessive oncogene on 6q and the sequencing of both this gene and the p53 gene in a number of PNETs.

In summary, we have performed an RFLP analysis on 23 PNETs. Our data demonstrate that PNETs have a loss of genetic material from chromosomes 17p, 6q, and possibly 16q. The role of the p53 gene in the development of PNETs is being explored.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Raymond F. Gesteland for support and guidance (to G. A. T.), Drs. Donald Kohn, Leonard Sender, and Kenneth Weinberg for reviewing the manuscript, and Denise Petersen for technical support.
REFERENCES


Loss of Heterozygosity on 6q, 16q, and 17p in Human Central Nervous System Primitive Neuroectodermal Tumors

Gregory A. Thomas and Corey Raffel


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/51/2/639

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.