The Chromosomal Complement of Human Solid Tumors*

II. Karyotypes of Glial Tumors

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No information has been available concerning the chromosomal complements of brain tumors or solid tumors in children. The present report is concerned with the karyotypes of three glial tumors, one of which occurred in an 8-year-old girl. Glial tumors were selected for study because of the relative frequency of mitotic figures in routine sections. These tumors demonstrated three markedly different types of abnormal karyotypes.

Technic

The cells were prepared directly from primary lesions as previously reported except that trypsin was not used. One alteration in the manner of analysis was made, however. Only cells with sufficiently good morphology and spread to permit the construction of a karyotype have been included in the analysis. The less reliable cells, if included as counts, give a falsely wide spread of numbers of chromosomes.

The arrangement of the chromosomes into two separate 2n karyotypes (Figs. 1c, 2c and 3c) was made to facilitate visual analysis, and it is not intended to imply that one or the other of the two groups represents the normal or original chromosomes. Chromosomes abnormal in appearance or present in greater than the 4n number for a particular group are also set apart in a box to facilitate further visual analysis.

The karyotypes in Case 1 were generally clear-cut, but occasional uncertain areas in Case 2 and several unclear areas in each cell of Case 3 were present, either because of overlapping or the presence of chromosomes that are intermediate in size between the several groups. Only those chromosomes whose appearance was obviously abnormal were so counted, and it is quite probable that certain chromosomes which appeared of an intermediate size or smaller than other members of a group (such as the last 19–20 in Fig. 3c and several of the small acrocentrics) actually represented a deleted chromosome. Thus the degree of abnormality may be underestimated.

While these limitations prevent as precise an analysis of the abnormalities as desired in most tumors, they do not prevent analysis of the degree and general types of changes that have occurred in neoplastic cells. Distinction between biological variation and loss of chromosomes during spreading was not possible, except when consistent differences in karyotype or marker chromosomes permitted the positive statement of biological variation. It is likely that both occur, but because of the frequent concurrence of good spreading and cellular breakage, as well as the lack of a consistent pattern of loss, most of the variation is felt to be the result of breakage of cells.

Case Reports

Case 1. P.H., an 8-year-old white girl, was admitted in May 1963 to Yale-New Haven Medical Center with headache and diplopia dating from December 1962. Eleven and 7 months prior to these symptoms the patient had unusually severe cases of chicken pox and measles. She was considered a bright child.

Examination revealed bilateral papilledema, paralysis of the right VIth cranial nerve and bilateral nystagmus. The patient could not stand alone. A ventriculogram revealed anterosuperior displacement of the 4th ventricle.

At operation a medulloblastoma (Fig. 1a) of the 4th ventricle was found and biopsied and karyotypes were prepared from this material. A biopsy

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Fig. 1a. Case 1. Medulloblastoma. The tumor is hypercellular with small cells having scanty cytoplasm and hyperchromatic, round to fusiform nuclei. Hematoxylin and eosin, X 150.

of the arachnoid in the area of the foramen magnum revealed neoplastic cells.

Postoperative roentgen-ray therapy, cytoxin and terminally Methotrexate were given, but the patient expired 8 months later. A buccal smear was positive for chromatin, and no double masses of chromatin were seen.

At autopsy tumor was found throughout the

Fig. 1b. Case 1. Cell #7.
meninges and marrow, and in the parietal pleura.

Case 2. H.R., a 40-year-old white male, was admitted to Yale-New Haven Medical Center in July 1959. An astrocytoma diffusum was excised incompletely. In November 1962 he was admitted to the West Haven Veterans Administration Hospital with a moderate expressive aphasia and forgetfulness. A left carotid arteriogram demonstrated a tumor stain in the frontoparietal area.

A large mass of tumor was removed partially and was classified as oligodendroglioma (Fig. 2a).

Karyotypes were prepared from this material. On review, the histological sections from the initial excision revealed some areas suggestive of oligodendroglioma.

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Fig. 1c. Case 1. Karyotype of Cell #7, showing normal complement on the left with 2 extra 6-13, X chromosomes, a round fragment, and 92 double fragments to the right.

Fig. 2a. Case 2. Oligodendroglioma. Section showing classical enlarged rounded cells with well-defined outer membrane and rather empty-looking cytoplasm in which the small spherical nucleus appears to float. Hematoxylin and eosin, X150.
Radiation therapy (4475r tumor dose) was given postoperatively, and the patient is alive and well 17 months later.

Case 3. R.S., a 43-year-old white male, underwent an incomplete excision of an astrocytoma of the right frontal lobe in April 1959 at the West Haven Veterans Administration Hospital.

In May 1961 the patient had evidence of recurrence and an extensive right frontal lobectomy was performed. The tumor was more cellular at this time but was again interpreted as an astrocytoma.

He received a course of radiation therapy (4500r tumor dose) to the entire head in August 1961. Symptoms and signs of increased intracranial pressure led to his readmission in October 1962.

Craniotomy revealed an extensive tumor which was partially resected. The histological diagnosis was glioblastoma multiforme (Fig. 3a). Karyotypes were prepared from this tissue. The patient improved for only a short time and expired 2 months after operation.

Slides were prepared from four additional glial tumors but did not yield a sufficient number of well spread mitoses for analysis.
Results and Interpretation

Case 1 (Medulloblastoma). Nine cells were karyotyped (Table 1). The most striking abnormality was the presence of 1 to 32 small double fragments of chromosomes in each dividing cell. These were not seen in a nondividing cell and were present in every mitosis examined. Their texture and staining were identical to the chromosomes including yellow fluorescence with acridine orange under ultraviolet illumination. There was no obvious loss of chromosomes in cells with many fragments, so that it seems unlikely that they resulted from repeated fragmentation of chromosomes. It is felt, therefore, that they represent a small fragment of a chromosome which underwent random segregation between daughter cells over a series of cellular divisions. Their persistence as well as the proximity of the duplicated fragments suggests that they might contain a portion of a centromere. No comparable deletion was evident, although a deletion of this size in one of the larger chromosomes or 6-12 group could have escaped detection. A slightly larger round fragment, which was not duplicated obviously, was present in 8 of 9 cells.
This fragment may represent a small ring chromosome. The variation in size of ring chromosomes from cell to cell is well known, and it is possible that the small fragments were also tiny ring chromosomes, in view of the subsequent finding of definite ring chromosomes in tumor cells in marrow.

Chromosomes were present in greater than the diploid number only in the 6–12, X or 4–5 group but these were arbitrarily placed in the 6–12, X group. The karyotype was otherwise normal except for some loss in the 16–18 and 19–20 groups. Thus both unequal segregation of chromosomes and fragments and one or two instances of breakage of chromosomes were the major mechanism in the evolution of the abnormal karyotype.

During the month prior to death a number of special studies, which will be reported in detail elsewhere, were carried out on the pa-

![Fig. 3c. Case 3. Karyotype of Cell #5. Box to right includes 4 abnormal acrocentric chromosomes and 3 acentric fragments. Box to left contains submetacentric chromosomes (6–12, X and 16–18-like) present in greater than tetraploid number.](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Chromosome Group</th>
<th>( n^* )</th>
<th>Deviation from Normal Karotype (9 Cells, Case 1)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1–3</td>
<td>6</td>
<td>+5</td>
</tr>
<tr>
<td>4–5</td>
<td>4</td>
<td>+5</td>
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<tr>
<td>6–12, X</td>
<td>16</td>
<td>+1</td>
</tr>
<tr>
<td>13–15</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>16–18</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>19–20</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>21–22</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>51</td>
</tr>
</tbody>
</table>

Abnormal chromosomes
Double fragments 1 9 4 5 10 5 32 18 7
Round fragments 1 1 1 1 1 0 1 1 1

* This column lists the number of chromosomes normally present in a group or present in a tetraploid cell (4n)
tient’s marrow and peripheral blood. Approximately 30 per cent of the cells in the peripheral blood and 98 per cent of the dividing cells in the marrow showed similar double fragments, with some cells in the marrow containing over a hundred fragments. Definite larger ring chromosomes were present variably. Most of these cells were interpreted as metastic cells. A few normal female karyotypes were found among a series of 400 mitotic cells in the marrow and in the peripheral blood. Although mosaicism can not be ruled out, it is most likely that the patient’s karyotype was originally normal. The karyotypes of the patient’s father and mother were normal.

Case 2 (Oligodendroglioma). Six of the 11 cells were either tetraploid or lacked only 1 chromosome of tetraploid karyotype (Table 2). No abnormal chromosomes were seen, and in no instance was there a greater than 4n number of chromosomes in any group (Figs. 2b and 2c). The remaining cells showed no regular pattern and are consistent with random loss from a tetraploid cell during spreading. It is most likely that the tumor was tetraploid, although small deletions and biological variation can not be ruled out.

Case 3 (Glioblastoma multiforme.) Two lines of cells were present in this tumor with 42–47 centromeres in the low-count line and 86–89 in the second line (Table 3). Identical acrocentric marker chromosomes were present in both lines as were 5–10 apparently acentric fragments of chromosomes (Figs. 3b and 3c). The “very long acrocentric” was as long as #2, the “long acrocentric” equivalent to #6 in length and the “short acrocentric” was intermediate in size between the #13 and #21. On two occasions the very long acrocentric was dicentric. A chromosome in the 19–20 group and several in the 21–22 group were suggestively smaller than others in the group, but considered as normal in the analysis. The variation in the number of abnormal chromosomes from cell to cell within each line suggests that at least some additional biological variation occurred in this tumor.

Since the independent production of three identical marker chromosomes in two lines is unlikely, it is probable that the high-count line evolved in some way from the low-count line. A simple doubling of the low-count karyotype would not explain the high-count karyotype since the number of marker chromosomes was not generally doubled and the distribution of chromosomes among the various groups is altered. It is necessary, therefore, to postulate that many changes took place subsequent to doubling in order to explain the relative loss both of large chromosomes and acrocentrics (including some marker acrocentrics) and relative gains in the 6–12, X and 16–18 groups. To increase the number of chromosomes in 6–12 groups by one and decrease group 1–8 by one, for example, require two events of nondisjunction or other unequal segregation, but only one deletion of a 1–5 sized chromosome is necessary to produce a 6–12-like chromosome, so that the latter more direct process is postulated as the predominant one. Simi-
larly, translocations in the acrocentric groups may add to the number of apparent 6–12- and 16–18-like chromosomes. This mode of evolution requires many of the normal-appearing chromosomes to be, in fact, either translocation or deleted chromosomes from other groups.

A second mode of evolution merits discussion, however. The fusion in vitro of neoplastic cellular lines with different marker chromosomes to form hybrid lines with both markers and a roughly doubled number of chromosomes has been demonstrated in several laboratories. Fusion of a cell such as #7 with a cell not containing any markers (either with or without subsequent changes, depending on the composition of the second cell) offers an equally good explanation of the two lines. It is necessary to postulate, however, that the second line either did not persist as an entity or was not found in the cells examined.

**Discussion**

A consistently normal karyotype in a primary human malignant solid tumor has not yet been demonstrated when the tissue was studied without the potentially selective factor of tissue culture. The present study contributes additional evidence for the presence of abnormal karyotypes in tumors from humans. Whether the abnormal chromosomal complements of these cells are a primary or secondary change remains unclear, but there is no à prior reason why histologically similar tumors must have identical karyotypes even if the changes are primary. Each tumor may represent a unique biological experiment with a unique karyotype. Spriggs et al. found a modal number of 80 in a malignant glioma in a 14-year-old girl, but did not give karyotypes.

Several types of karyotypic variation warrant comment. Variation of the karyotype in time may result in histological changes such as those reported in Cases 2 and 3 in contrast to variation of observer or of sampling which is often invoked as an explanation. Since glioblastomas rarely permit survival for longer than a year, it is likely that the histological changes in Case 3, at least, represent real changes in the appearance and behavior of the tumor. No direct correlation of serial histological and karyotypic changes has been presented in tumors from humans, but the necessity for repeated resection in

### TABLE 3

<table>
<thead>
<tr>
<th>Chromosome Group</th>
<th>4n c</th>
<th>1 2 3 4 5 6 2n c</th>
<th>7 8 9 10 11 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3</td>
<td>12</td>
<td>-6 -6 -6 -6 -6 -6 6</td>
<td>-1 -1 -1 -1 -1 -1 -2</td>
</tr>
<tr>
<td>4–5</td>
<td>8</td>
<td>-1 -1 -1 -1 -1 -1 4</td>
<td></td>
</tr>
<tr>
<td>6–18, X</td>
<td>30</td>
<td>+6 +4 +3 +6 +5 +5 15</td>
<td>+2 +1 -1 +1</td>
</tr>
<tr>
<td>18–15</td>
<td>12</td>
<td>-7 -7 -7 -7 -7 -7 6</td>
<td>-3 -3 -3 -3 -5 -3</td>
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<tr>
<td>16–18</td>
<td>12</td>
<td>+5 +5 +5 +2 +3 +3 6</td>
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<tr>
<td>19–20</td>
<td>8</td>
<td>-1 -1 -1 -1 -1 -1 4</td>
<td></td>
</tr>
<tr>
<td>21–22, Y</td>
<td>10</td>
<td>-3 -3 -3 -3 -3 5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>85 83 82 82 82 82 46</td>
<td>45 43 41 41 41 41 40</td>
</tr>
</tbody>
</table>

Abnormal chromosomes

| Very long acrocentric | 1* 1 1 1 1 2 1 | 1 1 1* 1 1 1 |
| Long acrocentric      | 2 2 2 2 1 2 | 1 2 2 1 1 1 |
| Short acrocentric     | 1 1 1 1 1 1 |                |
| Large acentric fragment | 2 5 3 3 2 2 | 1 2 3 2 5 |
| Small acentric fragment | 1 1 1 1 1 | 3 1 |
| Round fragment        | 1 1 1 1 | 1 |
| Total                | 7 10 9 8 7 6 | 5 5 5 6 6 9 |
| Total centromeres     | 92 93 91 90 89 88 | 50 48 46 47 47 49 |

* Dicentric

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Chromosomal Complement of Human Solid Tumors
many glial tumors offers a good opportunity for such a study. In Case 3 it is also of interest to speculate both on the role of roentgen-ray therapy in producing either part of the abnormal karyotype or the change in histology as well as the remote possibility that the two predominant cellular types, tumor cells and proliferated vascular endothelium, might be correlated with the two lines of cells. The remaining two tumors were untreated at the time of study. In reference to variation from cell to cell within a given tumor, it is likely that the relative contribution of loss of chromosomes during spreading and real biological variation may vary from tumor to tumor.

The three tumors displayed widely different types of abnormalities: simple tetraploidy (Case 2), several extra normal-appearing chromosomes with a varying number of fragments (Case 1), and a highly complex abnormality with two lines of cells and many instances of breakage and structural rearrangement (Case 3). These varying types of abnormalities may ultimately prove of great significance in terms of etiology, but since chemicals, viruses and radiation have all been implicated in producing a wide variety of karyotypic abnormalities, speculation seems premature.

The presence of tetraploidy in a relatively benign tumor (oligodendrogloma) is of particular interest since tetraploidy represents a minimal departure from normal, and indeed occurs in a small portion of normal cells. In contrast to this observation, however, is the apparently identical finding of tetraploidy in a tumor classified as an adenocarcinoma of the bowel (Case 2). This patient expired with widespread metastases 14 months after resection. The relatively mild chromosomal change in Case 2 reported herein is, however, particularly noteworthy when contrasted with Case 3, in which the tumor was also studied about 3 years after initial diagnosis and the karyotypic changes were highly abnormal. It is evident that karyotypes on many similar tumors must be studied before the various questions raised here can be answered.

Summary

The chromosomal complements of three glial tumors were determined directly from the primary tumor without intervening tissue culture. Cells from a medulloblastoma in an 8-year-old girl revealed the consistent presence of 2–5 extra chromosomes in the 6–12, X or 4–5 groups with a variable number of small duplicated fragments of chromosomes. Similar karyotypes and fragments were seen subsequently in many cells in both the peripheral blood and marrow, and these cells were therefore interpreted as metastatic neoplastic cells. Cells from the most benign tumor, an oligodendrogloma, were consistent with a tetraploid karyotype with the lower counts being secondary to cellular breakage or, less likely, biological variation. This patient is well currently. In the third tumor, termed first an astrocytoma and 2½ years later glioblastoma multiforme, two distinct lines of cells were present. Although three nearly identical abnormal acrocentric chromosomes (or marker chromosomes) were usually present in the same number in each line, there was otherwise a nearly two-fold difference in number of chromosomes as well as many other differences in the karyotypes. Fusion of two different lines of cells is one possible explanation for the evolution of the high-count line from the low-count line.

References