A commentary on the biology and growth kinetics of low-grade and high-grade gliomas

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Cell kinetics studies of patients with various gliomas published in the past decade have shown that the average labeling index (LI) obtained from a pulse of \(^{3}\)H-thymidine is very high in medulloblastomas (12.0% ± 1.3%, standard error of the mean) and glioblastoma multiforme (9.3% ± 1.0%), low in well differentiated gliomas (less than 1%), and intermediate in anaplastic astrocytomas (4.0% ± 0.8%). The higher the LI, the faster the tumor grows, probably reflecting a larger growth fraction. In tumor tissues obtained at autopsy, two glioblastomas diluted out the labeling compound in the 2- to 4-month interval after labeling, whereas three glioblastomas and two anaplastic astrocytomas retained labeled neoplastic cells for 3 weeks to 5 months. Most patients whose tumors contained foci of labeled cells at autopsy survived longer. Well differentiated gliomas harbored labeled cells for 21 to 7 years. These findings indicate that the kinetics of proliferation in well differentiated gliomas are different from those in glioblastomas or anaplastic astrocytomas.

KEY WORDS • cell kinetics • glioma • glioblastoma • anaplastic astrocytoma • low-grade glioma • brain tumor

All intracranial gliomas are clinically malignant, regardless of the degree of histological differentiation or anaplasia, because without appropriate treatment they are fatal to the host. Neoplasms originating in the other tissues of the body—for example, polyps in the digestive system—are not always life-threatening and are clearly different from invasive fast-growing tumors such as adenocarcinoma and scirrhous carcinoma, which are fatal without early appropriate treatment. The histopathological malignancy of such tumors correlates fairly well with the prognosis.

Most gliomas are classified as glioblastoma multiforme; anaplastic astrocytoma of varying degree; or well differentiated tumors, such as fibrillary astrocytoma, oligodendroglioma, or ependymoma. Tumors in this latter group are separated by vague diagnostic border lines. Some clinicians assume that well differentiated or "low-grade" gliomas are "benign" because they usually grow very slowly and patients who have them survive longer than those with anaplastic gliomas or glioblastoma multiforme. Even low-grade gliomas, however, have some malignant characteristics, such as the lack of a clear border between the tumor and surrounding brain and the tumor's invasiveness in normal tissue.

The degree of cellular anaplasia, or magnitude of differentiation, in glial tumors is very important not only for understanding the biology of individual tumors, but also for determining prognosis and evaluating treatment. Cell kinetics studies have been vigorously pursued in the past two decades in an attempt to understand the growth of tumors and to improve therapy. In this present report, we analyze the prognosis, cell kinetics, and proliferation patterns of intracranial gliomas to determine whether a low-grade glioma exists and, if it does, to define its characteristics. The therapeutic implications of these findings are also discussed.

Prognostic Considerations

The most extensive series of glioma cases is that of the Japanese Brain Tumor Registry. Figure 1 shows the survival rates of patients with low-grade, differentiated gliomas, anaplastic gliomas, and glioblastoma multiforme in that series. Approximately 50% of the patients with well differentiated gliomas (excluding pontine gliomas) survived for 5 years, compared with 10% to 15% of those with malignant gliomas or glioblastoma multiforme. The patients who died within 5 years might have included patients who had astrocy-
mas with anaplastic foci that were not detected or astrocytomas transformed to more anaplastic phenotypes after surgery. Exclusion of those cases would further improve the survival statistics of patients with well differentiated gliomas.

Table 1 shows the relationship of the 5-year survival rate to the amount of tumor removed from patients with low-grade gliomas in the same Japanese series. The amount of tumor resected correlated positively with increased survival; gross total removal of tumor resulted in a 75.5% 5-year survival rate. Among patients with malignant glioma or glioblastoma multiforme, the survival rate was increased to a 20% to 30% 5-year survival rate only by gross total resection of the tumor.

These and other observations lead us to believe that well differentiated gliomas grow very slowly at a steady rate, and that their biological behavior differs from that of anaplastic gliomas and glioblastoma multiforme. Because residual tumor size affects the survival time of patients with well differentiated gliomas, we believe that these tumors are primarily space-occupying lesions with limited proliferative capacity. Surgical inaccessibility and, primarily, mass effect from the tumor or associated edema appear to be important prognostic factors.

In contrast to well differentiated gliomas, malignant gliomas grow very fast, infiltrate extensively into surrounding tissues, and often fill up the space provided surgically with unexpected rapidity. Analysis of cell kinetics may help to explain this striking difference in the pattern of proliferation of low- and high-grade gliomas.

Analysis of Cell Kinetics

Neoplastic tissue is characterized by an unrestricted increase of the cell population by cell division. There are, however, two classes of cell reproduction: 1) cell renewal; and 2) an expanding cell population. Cell renewal is seen in the cellular regeneration of most adult tissues; the cell population remains stable because the birth of a new cell is balanced by the death and loss of another cell. The expanding cell population system comprises both controlled growth (exemplified by embryonal tissue growth, regeneration of the liver after partial hepatectomy, and wound healing) and uncontrolled growth (as in neoplastic growth).

The proliferative activity of tissue is measured by the frequency of mitosis, or the "mitotic index." However, because the duration of mitosis is short compared with the entire proliferative cycle (cell cycle time), the mitotic index is too small to be a reliable measure. Moreover, the frequency of mitosis in gliomas often does not correlate with malignancy: even glioblastoma multiforme sometimes fails to show mitosis, and oligodendroglioma shows a considerable number of mitoses. Another measure of the proliferative activity of tissue is the labeling index (LI), defined as the proportion of cells labeled shortly after a pulse administration of hydrogen-3 (\(^3\)H)-thymidine, which indicates the percentage of cells in the DNA (deoxyribonucleic acid) synthesis phase. Generally, the higher the LI, the faster the growth of tissue.

Table 2 summarizes the LI's of 28 neuroectodermal tumors studied at the Brain Tumor Research Center of the University of California, San Francisco, in the past decade. The average LI was 12.0% ± 1.3% (standard error of the mean) for medulloblastomas, 9.3% ± 1.0% for glioblastoma multiforme, and less than 1% for well differentiated gliomas (three fibrillary astrocytomas and one ependymoma). The average LI for anaplastic astrocytomas was 4.0% ± 0.8%, and varied from 2.2% to 8.3% according to the extent of anaplasia. Thus, well differentiated gliomas appear to have far less proliferative activity.

Kaplan-Meier analysis of the survival times of these patients showed a significant difference between those who had a tumor LI of 5% or more and those who had a tumor LI of less than 5%, regardless of histopathology. All of the patients with more anaplastic tumors and an LI over 5% died within 6 months after surgery, whereas patients with an LI of less than 5% survived, with few exceptions, more than 1 year and usually 5 years after diagnosis.

Although the LI represents some of the proliferative activity of each tumor, tumor growth is modulated by
the cell cycle time, growth fraction (the ratio of proliferating to nonproliferating cells), and cell loss.\(^6,7\) In our previous studies,\(^6,9\) the cell cycle time of gliomas was fairly constant, and active proliferation in the tumors with a higher LI appeared to be the result of higher growth fraction. Thus, low-grade gliomas, which have low LI's, contain many noncycling cells.

Noncycling cells in low-grade gliomas are biologically different from those in malignant gliomas. The noncycling cells in malignant tumors are not all sterile; they are usually temporarily out of cell cycle, mostly because of overcrowding, low oxygen tension, or insufficient nutrients, and can re-enter the proliferating pool whenever environmental conditions improve. In low-grade gliomas, the noncycling cells are not suffering from overcrowding or nutritional deficiency, and the necrotic foci that are very common in malignant gliomas are absent. Therefore, noncycling cells in low-grade gliomas may be mature cells that have left the cycling pool permanently, resulting in the slow growth and "benign" nature of this tumor. The following cell kinetics study provides further support for this hypothesis.

Of the 28 patients from Table 2 whose tumors were labeled with \(^3\)H-thymidine, 10 (five with glioblastoma multiforme, three with anaplastic astrocytoma, and two with well differentiated gliomas) died at various intervals after biopsy, and underwent autopsy. Tissue from each tumor was studied autoradiographically, and the LI was compared with the LI obtained at biopsy\(^5\) (Table 3). For this study, glioblastoma multiforme was defined as a glial neoplasm with the histological characteristics of marked hypercellularity, extreme nuclear pleomorphism, numerous mitoses, pseudopalisading around areas of tumor necrosis, and marked endothelial hyperplasia of capillaries. Anaplastic astrocytoma was defined as a tumor consisting predominantly of astrocytes with moderate nuclear pleomorphism, a variable degree of endothelial hyperplasia of capillaries, and mitoses in at least some areas.

The LI from biopsied material represents the average of all viable areas counted. In the autopsy specimens, only heavily labeled areas were used to determine the LI, because actively dividing areas in malignant gliomas very quickly dilutes tagged DNA. The LI's are lower in LI, because actively dividing areas in malignant gliomas may be mature cells that have left the cycling pool permanently, resulting in the slow growth and "benign" nature of this tumor. The following cell kinetics study provides further support for this hypothesis.

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In two glioblastomas (Cases 1 and 2, Table 3), there were no labeled tumor cells in tissue obtained at autopsy 2 to 4 months after the \(^3\)H-thymidine pulse. The three other glioblastomas and two of the anaplastic astrocytomas contained no labeled neoplastic cells in most areas, but retained some labeled cells in both the differentiated and the anaplastic areas as long as 5 months after the pulse of \(^3\)H-thymidine. Surprisingly, two well differentiated gliomas retained the labeled tumor cells 2½ years and 7 years after the pulse.

Cells labeled shortly after administration of \(^3\)H-thymidine are assumed to be capable of proliferation, and the number of labeled cells will therefore reflect the proliferative activity of the tissue.\(^5,6,7\) Conversely, the presence of labeled cells in autopsy material obtained long after administration of \(^3\)H-thymidine implies retardation of proliferative activity in the labeled cells within a few mitoses.\(^5,8\) Therefore, these tumors contained cells that have the potential to differentiate or limit their own proliferation. Three patients with glioblastoma (Cases 3, 4, and 5) not only had less dilution

\(\text{Table 2}\)

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>No. of Cases</th>
<th>Labeling Index*</th>
<th>Mean Survival Time (mos)$\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>medulloblastoma</td>
<td>4</td>
<td>12.0% ± 1.3%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7.0%-14.4%)</td>
<td></td>
</tr>
<tr>
<td>glioblastoma</td>
<td>13</td>
<td>9.3% ± 1.0%</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.5%-15.9%)</td>
<td></td>
</tr>
<tr>
<td>anaplastic astrocytoma</td>
<td>7</td>
<td>4.0% ± 0.8%</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.2%-8.3%)</td>
<td></td>
</tr>
<tr>
<td>fibrillary astrocytoma</td>
<td>3</td>
<td>0.8%</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.3-0.9%)</td>
<td></td>
</tr>
<tr>
<td>ependymoma</td>
<td>1</td>
<td>1.9%</td>
<td>156</td>
</tr>
</tbody>
</table>

*Values are means ± standard error of the means. Ranges are given in parentheses.

$\dagger$ From time of onset. Survival data for the medulloblastoma patients are not yet available because follow-up studies are incomplete.

\(\text{Table 3}\)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Pathology</th>
<th>Survival Time</th>
<th>Biopsy LI (%)$\dagger$</th>
<th>Interval: Biopsy to Death</th>
<th>Autopsy LI (%)$\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>glioblastoma</td>
<td>10 mos</td>
<td>8.6</td>
<td>4 mos</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>glioblastoma</td>
<td>7 mos</td>
<td>15.9</td>
<td>2 mos</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>glioblastoma</td>
<td>18 mos</td>
<td>11.3</td>
<td>5 mos</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>glioblastoma</td>
<td>4 yrs</td>
<td>11.0</td>
<td>2 mos</td>
<td>7.1</td>
</tr>
<tr>
<td>5</td>
<td>glioblastoma</td>
<td>20 mos</td>
<td>NA</td>
<td>1 mo</td>
<td>7.5</td>
</tr>
<tr>
<td>6</td>
<td>anaplastic astrocytoma</td>
<td>7 mos</td>
<td>5.1</td>
<td>2 mos</td>
<td>4.8</td>
</tr>
<tr>
<td>7</td>
<td>anaplastic astrocytoma</td>
<td>4 mos</td>
<td>2.2</td>
<td>3 wks</td>
<td>1.6</td>
</tr>
<tr>
<td>8</td>
<td>anaplastic astrocytoma</td>
<td>7 yrs</td>
<td>8.3</td>
<td>6 mos</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>astrocytoma</td>
<td>14 yrs</td>
<td>0.9</td>
<td>7 yrs</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>ependymoma</td>
<td>13 yrs</td>
<td>1.9</td>
<td>2½ yrs</td>
<td></td>
</tr>
</tbody>
</table>

* NA = not applicable (no biopsy was performed).

$\dagger$ Values for the most labeled area. In Cases 9 and 10 there were too few labeled cells to measure.
The proliferative pattern of anaplastic astrocytomas can proliferate whenever conditions are favorable. Blastoma cells can keep dividing, and the increase in the noncycling pool. Most cells in the noncycling proliferating cells as well, unless some of them move into the noncycling pool. In contrast, glioblastoma cells can stop temporarily or permanently (Fig. 2). If a tumor follows the former pattern, the number of cells after "n" cell cycle times is N₀ (n + 1), where N₀ is the number of tumor cells at time "0"; a tumor proliferating in the latter pattern will have N₀ × 2ⁿ cells, and therefore grows much faster. Proliferation in the former pattern indicates a higher rate of cell loss), and frequent movement between noncycling and cycling cell populations (Fig. 3). It would not be wise to concentrate only on removing as much tumor as possible, because growth in glioblastomas seems to be depressed by crowding; partial removal might stimulate cells in the nonproliferating pool to move into the proliferating pool, causing more rapid filling of the former tumor site. Thus, resection would only temporarily reduce the proliferating population. We must be prepared not only to kill the proliferating cells in the residual mass but also to attack the rest of the cells that may enter the proliferating pool. The chloroethylnitrosoureas and perhaps other cell cycle-nonspecific drugs, in addition to their lipophilic nature and rapid distribution through the blood-brain barrier, probably is a mixture of these two extremes. In anaplastic areas, proliferation follows a pattern similar to that of glioblastoma multiforme, while in differentiated areas, proliferation resembles that in well differentiated gliomas. This theory cannot be substantiated on the basis of 10 cases. It may, however, afford a working hypothesis with which to approach the biological behavior of tumor groups whose proliferative activities are morphologically similar but whose responses to a particular treatment modality may vary enormously. Unfortunately, lack of a suitable animal model prevents us from proving this hypothesis experimentally.

**Therapeutic Considerations**

Although various chemotherapeutic and radiation approaches have been vigorously pursued to treat malignant gliomas, including glioblastoma multiforme, therapy for well differentiated gliomas has been limited to surgery and irradiation. Because well differentiated gliomas are seldom treated as aggressively as malignant gliomas, data on therapy are limited, making it difficult to determine the best approach to treatment. Previous cell kinetics studies have shown that glioblastomas have a higher growth fraction than astrocytomas, numerous foci of necrosis (a fact that probably indicates a higher rate of cell loss), and frequent movement between noncycling and cycling cell populations (Fig. 3). It would not be wise to concentrate only on removing as much tumor as possible, because growth in glioblastomas seems to be depressed by crowding; partial removal might stimulate cells in the nonproliferating pool to move into the proliferating pool, causing more rapid filling of the former tumor site. Thus, resection would only temporarily reduce the proliferating population. We must be prepared not only to kill the proliferating cells in the residual mass but also to attack the rest of the cells that may enter the proliferating pool. The chloroethylnitrosoureas and perhaps other cell cycle-nonspecific drugs, in addition to their lipophilic nature and rapid distribution through the blood-brain barrier, probably is a mixture of these two extremes. In anaplastic areas, proliferation follows a pattern similar to that of glioblastoma multiforme, while in differentiated areas, proliferation resembles that in well differentiated gliomas. This theory cannot be substantiated on the basis of 10 cases. It may, however, afford a working hypothesis with which to approach the biological behavior of tumor groups whose proliferative activities are morphologically similar but whose responses to a particular treatment modality may vary enormously. Unfortunately, lack of a suitable animal model prevents us from proving this hypothesis experimentally.

**Proliferation Patterns**

Generally, proliferation follows one of two patterns: 1) one daughter cell retains mitotic activity, while the other stops dividing either immediately or after a few cell divisions, as in cell renewal; or 2) both daughter cells are capable of cell division for an indefinite period unless unfavorable environmental factors force them to stop temporarily or permanently (Fig. 2). If a tumor follows the former pattern, the number of cells after "n" cell cycle times is N₀ (n + 1), where N₀ is the number of tumor cells at time "0"; a tumor proliferating in the latter pattern will have N₀ × 2ⁿ cells, and therefore grows much faster. Proliferation in the former pattern allows labeled cells to be retained long after the administration of ³H-thymidine, because one of the daughter cells losses mitotic ability and will hold tritiated DNA as long as it survives.

We postulate that well differentiated gliomas proliferate in the former pattern and grow through increases in the population of noncycling cells. In contrast, glioblastoma cells can keep dividing, and the increase in cell number reflects an increase in the population of proliferating cells as well, unless some of them move into the noncycling pool. (Most cells in the noncycling pool can proliferate whenever conditions are favorable.) The proliferative pattern of anaplastic astrocytomas possibly is a mixture of these two extremes. In anaplastic areas, proliferation follows a pattern similar to that of glioblastoma multiforme, while in differentiated areas, proliferation resembles that in well differentiated gliomas. This theory cannot be substantiated on the basis of 10 cases. It may, however, afford a working hypothesis with which to approach the biological behavior of tumor groups whose proliferative activities are morphologically similar but whose responses to a particular treatment modality may vary enormously. Unfortunately, lack of a suitable animal model prevents us from proving this hypothesis experimentally.

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barrier, are more desirable agents than cell cycle-specific drugs.\textsuperscript{11-14} Cell cycle-specific and phase-specific drugs, such as antimetabolites and vinca alkaloids, are also effective but are mostly limited in action to the fraction of cells in the cycling pool—estimated by growth fraction to be as high as 30\% of tumor cells\textsuperscript{6,9}—and are therefore most effective when given with cell cycle-nonspecific agents.

Astrocytomas are characterized by a lower LI than in glioblastomas,\textsuperscript{4,6} which indicates a very low growth fraction, a cell cycle time that is presumably similar to that of other glial tumors, and very limited proliferating capacity. The most important cell kinetic characteristics of well differentiated gliomas may be the absence of traffic from the noncycling pool to the cycling pool, in addition to the maturation of one progeny without mitotic ability, leaving the other in the cycling pool (Fig. 3). Histological studies of astrocytomas show a fairly homogeneous distribution of mature astrocytes. Necrosis and mitosis are rare, and microcysts, believed by some to represent one form of cell loss, are present. Nevertheless, this small cell loss appears to compensate, in part, for the proliferation of tumor cells, and retards tumor growth.

In well differentiated gliomas, it is important to remove as much tumor as possible.\textsuperscript{1,2,10,16,21} Many patients with low-grade glioma survive for several years after incomplete surgical resection, because even partial removal gives the residual tumor space to grow and reduces the number of cells in the proliferating pool. A more radical treatment would be to attempt to kill the cells in the cycling pool of the remaining tumor. If the noncycling cells do not return to the cycling pool, intensive use of cell cycle-specific or cell cycle phase-specific drugs to kill cells in the small cycling pool might be preferable to use of cytotoxic cell cycle-nonspecific agents. So far, there are no data comparing the benefit of radiotherapy to chemotherapy alone in the treatment of patients with well differentiated gliomas. In an uncontrolled study from the Japanese Brain Tumor Registry,\textsuperscript{2} there was no difference in 5-year survival rates of patients who received radiation therapy and those who did not, although some series do show such benefit.\textsuperscript{1,10,16,19,21}

Malignant astrocytomas or anaplastic astrocytomas, consisting basically of astrocytes with various anaplastic features, had LI's that ranged from as high as those of glioblastoma multiforme to almost as low as those of astrocytomas.\textsuperscript{4,6} They appear to carry both types of proliferation pattern, as demonstrated by the foci with a considerably higher LI in autopsied material taken long after the administration of $^3$H-thymidine. The survival time of patients depends on the timing of anaplastic changes in preexisting, well differentiated astrocytomas and the extent of anaplastic foci in the tumor. The correlation between LI at biopsy and postoperative survival time\textsuperscript{14} shows that once anaplastic transformation begins, the growth rate of the tumor follows that of glioblastoma, and the anaplastic foci overwhelm the growth of the tumor as a whole. Therefore, the therapeutic approach to the tumor should be the same as for glioblastoma multiforme. However, if the extent of anaplasia is limited, as in anaplastic astrocytoma with a low LI, surgical removal probably would have the same beneficial effect as it would on a well differentiated astrocytoma. Thus, verification of the cell kinetics of these tumors is very important.

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