Regional Heterogeneity in the Proliferative Activity of Human Gliomas as Measured by the KI-67 Labeling Index

STEPHEN W. COONS, M.D. AND PETER C. JOHNSON, M.A., M.D.

Abstract. The effects of regional heterogeneity on the accuracy of histological grading of gliomas are well known, but little has been reported about its implications for other diagnostic modalities. This study investigated the relationships of regional heterogeneity in tumor proliferative activity, measured by KI-67 labeling indices (LI), and histological grades for 16 regionally sampled glioma resections. There was a strong correlation between histological grades and KI-67 LI in individual regions (p < 0.001), and both methods demonstrated comparable heterogeneity. Heterogeneity increased with tumor grade, probably as an expression of the increased genetic instability that accompanies tumor progression. Similarly, regions with comparable proliferative activity tended to cluster, paralleling clonal expansion. Thus, both histological grading and KI-67 LI are subject to heterogeneity-induced sampling errors that limit their diagnostic accuracy, particularly in small biopsies. However, fewer grading errors occurred when using both methods together than when using either method alone, suggesting that the use of multiple techniques may reduce the adverse effects of regional heterogeneity on diagnostic accuracy. Regional heterogeneity appears to be a ubiquitous feature of gliomas; it also has been reported in karyotype, p53 oncogene mutations, and PDGF and EGFR expression. The effects of regional heterogeneity on new methods for studying gliomas need to be considered.

Key Words: Gliomas; Histologic grading; KI-67; Proliferation; Regional heterogeneity.

INTRODUCTION

Inaccurate classification and grading of gliomas is a problem both in the management of patients and in the evaluation of the effects of treatment. Some diagnostic errors are inherent to routine microscopic evaluation, which provides indirect and otherwise limited assessment of biologic features that are important for predicting tumor behavior. Furthermore, the subjectivity of many histological criteria results in significant interobserver variation in their application. Incorrect classification also may be caused by sampling problems due to regional heterogeneity in the histological features of tumors, a problem that is exaggerated by small biopsy size (1, 2).

Tumor heterogeneity may be defined as cellular variation in genotype and/or phenotype within a tumor. Brain tumor heterogeneity has been recognized in histological (2–5) and karyotypic (6–8) analyses; by the variable expression of growth factors (9, 10) and their receptors (11); in proliferation markers (12–14); in the expression of oncogenes (15) and a number of glioma-associated antigens (16); and in intrinsic resistance to chemo- and radiotherapy (17, 18). Heterogeneity may occur on a diffuse, cell-to-cell basis or may have a regional distribution. In regional heterogeneity, individual cells tend to be similar to their immediate neighbors, while more distant groups of cells have different characteristics.

Histological regional heterogeneity has been extensively characterized. Autopsy studies of whole brain sections have documented the variability of diagnostically important histological features in astrocytomas, which include cytologic atypia, mitotic activity, microvascular proliferation, and tumor necrosis (2–5). Thus, localized microscopic features that are predictive of tumor behavior may not be found in limited biopsies. Although the diagnostic implications of histological regional heterogeneity have been known for decades, only recently has a systematic study provided quantitative confirmation of the great potential for grading errors (2).

Heterogeneity-induced sampling errors are influenced by biopsy location and size. Modern imaging modalities help in the first regard, and many reports find that image-guided needle biopsies are satisfactory for diagnostic purposes (19–21). However, the small sample size and limited sample site(s) reduce the likelihood of identifying heterogeneously distributed features. A significantly lower rate of diagnosis of glioblastoma multiforme (GBM) in needle biopsies compared to larger, open biopsies has been attributed to non-representative sampling (1).

The limitations of histological grading have prompted a search for new or complementary means of evaluating gliomas. Many of the new approaches attempt to evaluate specific characteristics that are believed to affect tumor aggressiveness, such as proliferation, invasion and treatment resistance. The Ki-67 monoclonal antibody is among the techniques being applied to gliomas to evaluate the usefulness of quantitative assessment of tumor proliferative activity. This antibody is directed against a non-histone nuclear matrix protein that has an as yet undefined role in the proliferative process (22, 23). It labels proliferating pool cells (cells in G1, S and G2/M phases of the proliferative cell cycle) (24).
Preliminary studies using a variety of methods to quantify proliferative activity of gliomas generally have shown a correlation between histological grade and proliferative activity, but have reached different conclusions regarding the diagnostic usefulness of the tests (25–30). One cause for the different results may have been regional heterogeneity. The only previous study that specifically addresses this issue evaluated serial stereotactic biopsies from five gliomas (31). Of 28 total regions, only 15 contained microscopically identifiable tumor. While this study confirmed the presence of highly variable numbers of Ki-67-reactive cells in gliomas, the small size of the study and small amount of tissue did not allow a thorough evaluation of the spatial relationships of the labeled cells with each other or with the histological features. A clear understanding of the effects of heterogeneity on the measurement of proliferation is essential to the evaluation of the Ki-67 antibody and other proliferation markers as diagnostic tools for predicting glioma behavior. To this end, we analyzed 16 regionally sampled glioma resections and determined the topographical relationships of proliferation and histological grade in each tumor.

MATERIALS AND METHODS

Materials

Fresh tumor tissue was obtained from 16 supratentorial gliomas from 15 patients who had neurosurgical management of their gliomas at Barrow Neurological Institute. The neoplasms included seven primary and two recurrent astrocytomas, six primary oligodendrogliomas or oligoastrocytomas, and one recurrent oligoastrocytoma. For one patient, both the primary anaplastic astrocytoma (AA) and subsequent recurrence as a GBM were obtained. The recurrent oligoastrocytoma was grouped with the primary tumors for discussion purposes because the patient received no post-operative radiation or chemotherapy for the low grade primary tumor.

The tumors were dissected into blocks that were approximately 1 cm3 on a side. Each block (or region) was cut into three sections: the center section was submitted for routine histology and flow cytometry; the remaining sections were rapidly frozen in a –60°C isopentane bath (Neslab, Portsmouth, NH) and stored in liquid nitrogen. Five to 38 regions were sampled from each tumor for a total of 208 samples.

Histopathology

The astrocytomas were graded using the Ringertz/Burger (32, 33) system. Recent reports have indicated that oligodendrogliaomas and oligoastrocytomas have a common lineage (34, 35), and these tumors were graded using the Kernohan system for oligodendrogliomas (36–39). A histological grade was determined for each region. Each tumor was assigned an overall grade that was the highest grade of any of its regions. In each region, mitoses were counted in 50 contiguous microscopic fields examined at 400× magnification (0.2 mm²/400× field, for a total of 10 mm²) starting in the area with greatest pleomorphism and cellularity.

Ki-67 Immunohistochemistry

Ki-67 immunostaining was performed using modifications of the original method of Gerdes (24). Five μm sections were cut from the frozen tissue block, fixed for 10 minutes in cold acetone and air-dried for at least 2 hours. The slides were incubated for 20 minutes at room temperature with a 1:15 to 1:50 dilution (depending on antibody lot) of Ki-67 antibody (Dako, Santa Barbara, CA), and developed using an alkaline phosphatase-anti-alkaline phosphatase kit with a fast red chromogen (Dako). Intrinsic alkaline phosphatase activity was blocked with levamisole (Vector Laboratories, Burlingame, CA). The labeled sections were lightly counterstained with hematoxylin.

The area with the highest number of labeled cells was identified. In this area, the percentage of Ki-67-positive nuclei (labeling index; LI) was determined by counting 1,000 nuclei in contiguous microscopic fields at 400× magnification. Cells recognizable as pertaining to blood vessels (e.g. endothelial cells and pericytes) were not counted. As a general rule, reactive astrocytes and residual, non-neuronal parenchymal cells could not be distinguished from tumor cells on the frozen section slides. A nucleus was considered positive if it demonstrated either a diffuse or punctate (nuclear) distribution of reaction product. The Ki-67 labeling patterns of the infiltrating edges of the tumors were examined also; proliferation estimates were based on counting all non-vascular elements due to the difficulty in distinguishing individual tumor cells from non-neoplastic cells.

The relationships between Ki-67 LI and histological grades and mitotic counts were evaluated using the Mann-Whitney U-test.

RESULTS

Table 1 shows the histological features and growth patterns of the tumors. Table 2 summarizes the regional variability by tumor grade. Table 3 summarizes the Ki-67 LI and mitotic count data.

Histological Features

Microscopic examination identified tumor in 195 of 208 regions; solid foci suitable for evaluation of Ki-67 LI were present in 154 regions. Only individual tumor cells (ITC) were present in 41 regions. No neoplasm was identified in 13 regions.

The (low grade) astrocytoma (LGA) and grades 1 and 2 oligodendrogloma/oligoastrocytomas demonstrated little heterogeneity. Among the 63 regions from these six tumors, the histological grades of all the regions in each tumor were the same, except for case 1427 which had eight grade 1 regions and a single grade 2 region.

In contrast, pronounced histological heterogeneity was seen in the seven high grade primary gliomas and the recurrent non-irradiated oligoastrocytoma. Variation in specific histological features, including hypercellularity, pleomorphism, the presence of mitotic figures, microvascular proliferation and necrosis, frequently produced different histological grades in individual regions within tumors. Among the anaplastic astrocytomas, 76% (16/
TABLE 1
Summary of Histopathologic and Ki-67 LI Data

<table>
<thead>
<tr>
<th>Case #</th>
<th>Grade</th>
<th>Total</th>
<th>Normal/ Reactive</th>
<th>ITC Only</th>
<th>Solid Tumor</th>
<th>Grade by Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LGA: 8</td>
</tr>
<tr>
<td>1346</td>
<td>LGA</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>LGA: 8</td>
</tr>
<tr>
<td>1167</td>
<td>AA</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>AA: 4</td>
</tr>
<tr>
<td>1039</td>
<td>AA</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>AA: 7</td>
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<tr>
<td>1226</td>
<td>AA</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>AA: 7; GBM: 1</td>
</tr>
<tr>
<td>1215</td>
<td>GBM</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>AA: 4; GBM: 1</td>
</tr>
<tr>
<td>335</td>
<td>GBM</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>AA: 1; GBM: 5</td>
</tr>
<tr>
<td>708</td>
<td>GBM</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>53</td>
<td>0</td>
<td>5</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

Recurrent Astrocytomas

|       |       |       |                  |          |             |                |
| 195   | P/R: AA | 12    | 0                | 0        | 12          | AA: 12         |
| 217*  | P:AA/R;GBM | 18    | 0                | 0        | 18          | GBM: 18        |
| Total |       | 30    | 0                | 0        | 30          |                |

Oligodendroglia/ Oligoastrocytomas

|        |       |       |                  |          |             |                |
| 486    | Oligo 1 | 12    | 0                | 4        | 8           | Oligo 1: 8     |
| 1427   | Oligo 2 | 28    | 8                | 11       | 9           | Oligo 1: 8; Oligo 2: 1 |
| 291    | Oligo 2 | 8     | 0                | 1        | 7           | Oligo 2: 7     |
| 712    | Oligo 3 | 17    | 0                | 4        | 13          | Oligo 2: 8; Oligo 3: 3 |
| 399    | O-A 1   | 12    | 0                | 3        | 9           | O-A 1: 9       |
| 1316   | O-A 2   | 38    | 5                | 11       | 22          | O-A 2: 22      |
| 1429   | P-O-A 2/R;O-A 3 | 10    | 0                | 2        | 8           | O-A 2: 4; O-A 3: 4 |
| Total  |       | 125   | 13               | 36       | 76          |                |

LGA = (Low grade) Astrocytoma; AA = Anaplastic Astrocytoma; GBM = Glioblastoma Multiforme; Oligo 1–3 = Oligodendroglia grade 1–3; O-A 1–3 = Oligoastrocytoma grade 1–3; P = Primary tumor; R = Recurrent tumor.

* Primary tumor was Case 1059 (anaplastic astrocytoma).

21) of the regions with solid tumor foci had AA histology, while 24% were low grade. Similarly, only 42% (8/19) of the regions from the GBM had the features necessary for the diagnosis of GBM, with the other 58% classified as AA. In the grade 3 oligodendroglia and oligoastrocytoma, only 43% (9/22) of the regions were grade 3, whereas 57% were grade 2. Overall, 28% of the regions from high grade tumors had only low grade or grade 2 features.

The two recurrent radiation-treated astrocytomas were highly homogeneous, with all 30 regions demonstrating high grade histological features.

**Ki-67 Labeling**

A single LI was assigned to each region, but many of the tumors exhibited variability in LI within regions. Several patterns were seen: (1) diffuse, relatively homogeneous labeling; (2) a single area, large or small, with distinctly increased labeling; (3) a scattered, multifocal distribution of the more proliferative areas. The patterns found within regions match those seen among regions. Typical intra- and inter-regional heterogeneity is seen in Figure 1.

Case 217 is the recurrence of case 1059, an AA. The recurrent tumor has the features of a small anaplastic cell GBM. The extremely high cellularity and high percentage of labeled nuclei made exact determination of the LI difficult, and the LI in each region was estimated to be greater than 25%.

The expression of heterogeneity in the Ki-67 LI increased with tumor grade. The LI of the LGA, grade 1
### TABLE 3
Summary of Ki-67 LI and Mitotic Count Data

<table>
<thead>
<tr>
<th>Case #</th>
<th>Diagnosis</th>
<th>Ki-67 LI (%)</th>
<th>Mitoses/50 400× Fields*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (range)</td>
<td>Mean (range)</td>
</tr>
<tr>
<td><strong>Primary Astrocytomas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1346</td>
<td>LGA</td>
<td>0.1 (0.1-0.6)</td>
<td>0.1 (0-1)</td>
</tr>
<tr>
<td>1167</td>
<td>AA</td>
<td>7.8 (4.9-10.5)</td>
<td>10.7 (5-30)</td>
</tr>
<tr>
<td>1059</td>
<td>AA</td>
<td>2.6 (1.0-5.9)</td>
<td>2.8 (0-12)</td>
</tr>
<tr>
<td>1226</td>
<td>AA</td>
<td>4.8 (2.0-7.3)</td>
<td>18.3 (12-32)</td>
</tr>
<tr>
<td>1215</td>
<td>GBM</td>
<td>13.4 (4.8-20.7)</td>
<td>42.0 (15-77)</td>
</tr>
<tr>
<td>335</td>
<td>GBM</td>
<td>7.2 (4.2-12.7)</td>
<td>7.0 (0-9)</td>
</tr>
<tr>
<td>708</td>
<td>GBM</td>
<td>18.8 (10.0-29.0)</td>
<td>93.0 (45-165)</td>
</tr>
<tr>
<td><strong>Recurrent Astrocytomas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>195</td>
<td>P/R; AA</td>
<td>3.4 (0.2-5.4)</td>
<td>0.1 (0-1)</td>
</tr>
<tr>
<td>217</td>
<td>P:AA/R:GBM</td>
<td>&gt;25% (25%)</td>
<td>Numerous</td>
</tr>
<tr>
<td></td>
<td>(P = Case 1059)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oligodendrogliomas/Oligoastrocytomas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>486</td>
<td>Oligo 1</td>
<td>0.4 (0-0.8)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>1427</td>
<td>Oligo 2</td>
<td>2.7 (0.1-4.8)</td>
<td>0.1 (0-1)</td>
</tr>
<tr>
<td>291</td>
<td>Oligo 2</td>
<td>4.2 (0-6.3)</td>
<td>3.0 (0-6)</td>
</tr>
<tr>
<td>712</td>
<td>Oligo 3</td>
<td>4.3 (0.2-14.3)</td>
<td>60.1 (0-306)</td>
</tr>
<tr>
<td>399</td>
<td>O-A 1</td>
<td>0.2 (0-0.8)</td>
<td>0.1 (0-1)</td>
</tr>
<tr>
<td>1316</td>
<td>O-A 2</td>
<td>0.9 (0-2.9)</td>
<td>0.2 (0-4)</td>
</tr>
<tr>
<td>1429</td>
<td>P:O-A 2/R:O-A 3</td>
<td>4.7 (1.5-7.7)</td>
<td>3.8 (0-19)</td>
</tr>
</tbody>
</table>

LGA = (Low grade) Astrocytoma; AA = Anaplastic Astrocytoma; GBM = Glioblastoma Multiforme; Oligo 1–3 = Oligodendroglioma grade 1–3; O-A 2–3 = Oligoastrocytoma grade 2–3; P = Primary tumor diagnosis; R = Recurrent tumor diagnosis.

* 50 400× fields = 10 mm².

### oligodendroglioma and grade 1 oligoastrocytoma were uniformly less than 1%. The grade 2 tumors had LI in the low to moderate range, with LI <4% in 87% (34/39) of the regions. The high grade tumors had LI that ranged from low to markedly elevated in some tumors (cases 1059, 1226, 1429, 712). In others, the LI was consistently moderately to markedly elevated (cases 1167, 1215, 335, 708).

### Ki-67 and Histological Grade

When the grades of the individual regions were considered, the median LI of the low grade regions (LGA and grade 1 or 2 oligodendroglioma/oligoastrocytoma) was 0.8%; for the high grade regions (AA, GBM, grade 3 oligodendroglioma/oligoastrocytoma), it was 7.2%. The difference between the median LI of low and high grade regions was extremely significant (p < 0.0001). When the LI was compared to the overall tumor grades, the difference between the median LI of the low grade tumors (0.6%) and the median LI of the high grade tumors (6.0%) was also extremely significant (p < 0.0001).

### Topographic Features

As noted previously, all but the LGA and grade 1 tumors had potential significant regional variation in LI. The LI tended to be similar in adjacent regions, and in many of the tumors the regions with the highest LI were clustered together. Often there was a decreasing gradient of proliferative activity away from the more proliferative areas. Several of the tumors had similarly high LI in all regions, and in a few cases, the highest LI were more randomly distributed. Topographic representations of tumors that illustrate these features are shown in Figure 2.

### Ki-67 and Mitotic Count

Mitoses were counted in 127 of the 154 regions with Ki-67 LI. In a number of regions, the foci of solid tumor were large enough for Ki-67 evaluation (2-10 400× fields required), but not for mitotic counts (50 400× fields required). Mitoses were not counted for case 217 because the Ki-67 LI were estimates; however, numerous mitoses were seen in all regions. As was seen for the Ki-67 LI,
the high grade tumors demonstrated much greater intratumor heterogeneity in mitotic counts than did the low grade tumors. The distribution of mitotic counts and Ki-67 LI is shown in Figure 3. Whereas there was no significant correlation between mitotic count and Ki-67 LI in individual regions, a strong correlation was present between the mean mitotic count and mean Ki-67 LI of each tumor (p < 0.001). Despite the lack of overall correlation, the presence of frequent mitotic figures in a region was associated with a higher LI (p < 0.0001). Among the regions with three or more mitoses per 50 400 × fields, 85% (33/39) had Ki-67 LI greater than 4%, compared to 18% (16/88) of the regions with fewer than three mitoses in 50 400 × fields. The latter group is highlighted in Figure 4 which shows that there is significant variability in LI even when mitoses are rare.

Infiltrating Tumor Edge

The infiltrating edges of tumors had unpredictable labeling. In highly proliferative tumors, areas with numerous ITC usually had labeled cells. Among moderately proliferative tumors, these areas ranged from no labeled cells to surprisingly high numbers, occasionally with a greater concentration than was seen in solid tumor foci. As the density of ITC and the corresponding ease of their recognition decreased, the number of labeled cells also decreased. In microscopic fields in which the distinction between isolated ITC and reactive astrocytes blurred, labeled cells were rare and never in sufficient numbers to aid in diagnosis.

DISCUSSION

Diagnostic Implications

The diagnostic impact of tumor heterogeneity is affected by the actual expression of heterogeneity by a tumor and by the relationship of the heterogeneity observed by one method to the heterogeneity observed by another. The expression of tumor heterogeneity is determined by genotypic and phenotypic factors, and by the interaction of the tumor cell with its microenvironment. Thus, heterogeneity in proliferative activity may reflect such diverse influences as clonal differences and local tissue...
hypoxia. The observed heterogeneity in proliferative activity is also a function of the methods used to measure it. Because mitotic figures are present during only a small period of the proliferative cell cycle, their histological identification may be particularly susceptible to sampling error. Also, because the mitotic activity was measured in relation to area (50 400× fields = 10 mm²), some of the variability in mitotic counts may have been due to differences in cell density. Immunohistochemical methods such as Ki-67, bromodeoxyuridine (BrdU) and proliferating cell nuclear antigen (PCNA) labeling measure a far greater proportion of proliferating cells but are subject to a number of technical problems that relate to tissue processing, staining and counting of labeled cells.

We observed significant regional heterogeneity in the histological features used for tumor grading. The variability of the mitotic counts was of particular interest. Mitotic activity is a major diagnostic criterion for most glioma grading systems (32, 33, 36, 38–42). The presence of frequent mitoses, or, in the cases of the St. Anne/Mayo (41) and revised World Health Organization (WHO) (42) grading systems for astrocytomas, a single mitosis is considered indicative of an aggressive tumor. Overall, the degree of histological heterogeneity observed in this series of tumors was similar to that of previous reports. We found that 29% of the regions from high grade tumors were histologically low grade, compared to 22% in a study by Paulus and Peiffer (2). Similarly, Glantz and Burger (1) found that 49% of needle biopsies were diagnosed as GBM, compared to 82% of larger resections. In our study, only 42% of the regions from tumors with an overall grade of GBM were histologically GBM.

The degree of heterogeneity in Ki-67 LI measurements appeared to be comparable to that seen in the histological grading, but the lack of established reference ranges precludes an exact assessment. The potential diagnostic significance of LI heterogeneity is affected by the range of the LI in a tumor. In a number of tumors, the LI exhibited a wide range from low to high. Limited biopsies of similar cases would yield diagnostically significant differences in the estimates of proliferative activity, depending on sample site. In contrast, several of the high grade astrocytomas had highly variable but consistently high LI. In such tumors, the proliferative activity would be recognized as high irrespective of sample site, and it is doubtful that the LI heterogeneity would have diagnostic significance.

The features that exhibit heterogeneity may vary in concert with one another or may vary independently. This relationship has a profound influence on the diagnostic usefulness of the methods used to measure the features. For example, the LI will be of limited value if low LI are always seen with low grade histological features and high LI with high grade histological features.

Instead, we found that the expression of histological and Ki-67 LI heterogeneity varied somewhat independently, despite the strong correlation between LI and histological grade. The inconsistencies occurred when the LI did not match the degree of cytologic atypia and/or the mitotic count. The poor correlation between the LI and mitotic counts in individual regions was not always diagnostically important. The LI was usually high in regions with frequent mitoses. In these regions, the lack of correlation between the exact LI and mitotic counts was of little significance because both methods recognized the regions as highly proliferative. Diagnostically important differences occurred when mitoses were infrequent. Among high grade tumors, six of 21 regions with no mitoses and three of seven regions with a single mitosis had clearly elevated LI (LI > 4%), and six additional regions had LI > 3%. Similarly, low LI were seen in some regions with frequent mitoses. Other regions had sufficient cytologic atypia to be classified as anaplastic in the absence of mitoses, while a few regions were low grade by all criteria.
Histological analysis and the Ki-67 LI were complementary and together allowed more accurate assessments of proliferative activity and probable behavior than did either method alone. In some cases, the regional LI more consistently matched the overall grade than did the regional histological grades: all five histologically low grade regions of case 1167 had high LI that were comparable to the LI of the anaplastic regions. Similarly, two of four grade 2 regions of case 1429 and high LI comparable to those of the grade 3 regions. In other cases, the regional histological grades were more consistent than the LI. In cases 1059 and 1226, all of the regions were AA, but four of five regions from case 1059 and three of eight regions from case 1226 had low LI (LI < 3%), compared to a median of 7.2% for all AA and GBM.

We reported similar findings in a systematic study of glioma regional heterogeneity in flow cytometry measurements of ploidy and S-phase fraction (SPF) (43). Ploidy, SPF and histological heterogeneity varied somewhat independently and the use of both modalities provided the best characterization of the tumor. The results of these studies suggest that a multimodal approach may improve diagnostic accuracy by reducing heterogeneity-induced errors.

Regional heterogeneity appears to be a ubiquitous feature of gliomas. There are isolated reports of regional heterogeneity in karyotype (18, 44), p53 oncogene mutations (15), and in platelet-derived growth factor (PDGF) (10) and epidermal growth factor receptor (EGFR) (11) expression and distribution. The expression of regional heterogeneity is less thoroughly characterized in these modalities; however, there is no evidence that heterogeneity-induced sampling errors will be less frequent.

Infiltrating Tumor Edge

The regional model employed in this study also allowed the evaluation of the infiltrating edges of the tumors. It is sometimes impossible to distinguish ITC from reactive or normal glia. It was hoped that the Ki-67 antibody would help in this regard by marking neoplastic proliferative activity. However, the number of labeled cells in the infiltrating edges varied so widely that their presence was not useful in predicting overall tumor grade. Labeled cells were not present in regions where tumor cells could not be identified morphologically, confirming the observations of the earlier study of Ki-67 heterogeneity (31). Overall, routine microscopic evaluation was clearly superior to the Ki-67 LI in the evaluation of the infiltrating edges of tumors.

Biologic Implications

Tumor heterogeneity appears to reflect the genetic instability that is a fundamental property of gliomas and is central to the process of tumor progression. Malignant evolution involves mutation to produce clones that compete more successfully for resources in a hostile environment that may include both natural defenses and therapeutic factors such as radiation and chemotherapy. Because most mutations are unsuccessful, a high rate of mutation presumably increases the likelihood of producing successful clones. Thus, it is not surprising that tumor heterogeneity consistently increases with grade. In this study, the high grade tumors demonstrated greater heterogeneity in histological features and in Ki-67 LI. We reported similar findings regarding flow cytometry DNA measurements (43) and others have noted this in cyogenetic studies (18, 44, 45). We also observed that the regions with the highest LI tend to cluster, with a decreasing gradient of proliferative activity away from this area. Flow cytometry measurements of regional differences in the percentages of aneuploid cell lines (43) demonstrated a similar pattern. These findings are indicative of expandse infiltration of a more aggressive cell line and support Nowell’s hypothesis that tumor progression is the result of local mutation and clonal expansion (46).

Summary

Heterogeneity appears to be a fundamental feature of gliomas. Regional heterogeneity exists in the proliferative activity of gliomas as measured by the Ki-67 LI and this heterogeneity has significant diagnostic and biologic implications. The degree of heterogeneity in Ki-67 measurements is comparable to that reported for histological grading and imposes similar limitations on its clinical usefulness. Regional heterogeneity appears to be a ubiquitous feature of astrocytomas and oligodendrogliomas, and must be addressed as new methods of evaluating these tumors are developed. The diagnostic effects of regional heterogeneity are dependent on biopsy size; this study reinforces the need for extensive sampling to minimize diagnostic errors. However, the somewhat independent expression of heterogeneity in histological grade and Ki-67 LI suggests that a multimodal approach to classification and grading may reduce diagnostic sampling errors.

The clustering of regions with similar proliferative activity supports the hypothesis that tumor progression is the result of local mutation followed by expansion of successful clones. This model for tumor progression actually predicts the juxtaposition and overlap of competing clones and admixed unsuccessful mutations that combine to produce the observed patterns of regional and cell-to-cell heterogeneity. The complexity of these tumors must be considered in the design of new treatment strategies, particularly as agents that target specific antigens are developed.

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