Pathology of low-grade gliomas: An update of emerging concepts

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Although the term low-grade glioma (LGG) is useful for its connotation of a slow-growing, better prognosis CNS primary neoplasm typically occurring in a young patient, it also serves as a potential diagnostic wastebasket, occasionally leading to conceptual errors, therapeutic uncertainty, or misinterpretation of clinical data. For example, the LGG designation is occasionally invoked as a justification for lumping together biologically unrelated entities such as pilocytic astrocytoma and diffuse astrocytoma. Whereas the former represents a benign and potentially surgically curable neoplasm that virtually never undergoes malignant transformation, the latter is a surgically incurable low-grade malignancy, prone to further malignant progression and eventual fatality. Therefore, although rare cases lacking a clear distinction may be encountered, the term LGG should be abandoned for a more specific diagnosis whenever possible. The primary goals of this paper are to review practical surgical pathology issues related to the diagnosis of diffuse LGGs and to update the reader on emerging clinicopathologic and molecular genetic concepts. Also discussed are current controversies of classification/grading and the role of ancillary testing via immunohistochemical and genetic techniques.

The topic of low-grade glioma (LGG)2 pathology and molecular classification has recently been reviewed in detail (Fuller and Perry, 2001; Louis et al., 2001; Perry, 2001), and the 2000 WHO “blue book” similarly provides a wealth of useful information (Kleihues and Cavenee, 2000). Therefore, the current review focuses primarily on common and practical issues encountered in surgical neuropathology and provides an update on the most current concepts regarding these challenging neoplasms. Given that the most widely utilized classification and grading scheme today is that of the WHO, it is the one that is emphasized throughout this paper (Kleihues and Cavenee, 2000).

Definition of Low-Grade Glioma

Within a complex multidisciplinary field such as neuro-oncology, a common language is of critical importance in order to maintain meaningful communication among the subspecialists and to insure optimal patient management. It is sometimes surprising, however, to note the spectrum of perceptions and misperceptions associated with useful, albeit imprecise nomenclature such as low-grade gliomas. The definition of LGG can be either extremely broad or narrow. Therefore, this seems the logical place to begin a pathology review.

In its loosest context, LGG refers to any primary CNS neoplasm of presumed glial histogenesis (i.e., showing glial differentiation) for which the expected biologic behavior corresponds to either WHO grade I (roughly equivalent to benign or an expected survival of >10 years) or grade II (roughly equivalent to low-grade malignant or an expected survival of 5–10 years). Defined as such, LGG would encompass roughly a dozen distinct entities (Table 1), each with its own unique clinicopathologic, radiographic, and genetic features (Kleihues and Cavenee, 2000). Although a WHO grade has yet to be assigned to the tumor known as astroblastoma, it is included in this list because of published low-grade...
examples with clinical behavior roughly equivalent to that of other grade II gliomas. The dysembryoplastic neuroepithelial tumor (DNT) is technically classified as a glioneuronal tumor, though its radiographic and histologic overlap with oligodendroglioma commonly places it in the discussion with LGGs. Similarly, ganglioglioma (grade I or II) is the prototype glioneuronal tumor, but is often included in the differential diagnosis of LGG and has similarly been reported in LGG series by some investigators. Obviously, this all-inclusive definition embodies a broad spectrum of tumors, and studies incorporating all of them would be of limited value.

In contrast, the most restrictive definition for low-grade glioma is that of an infiltrating WHO grade II glioma, with the 3 qualifying entities being diffuse astrocytoma, oligodendroglioma, and mixed oligoastrocytoma (MOA). Although this obviously excludes many tumors that are clearly glial and technically “low-grade,” it is probably the most widely applied use of the term low-grade glioma and makes the differential biologic sense, given the substantial clinicopathologic overlap among these glioma subtypes.

The intermediate-type definition of low-grade glioma that has probably generated the greatest confusion and largest number of conceptual inaccuracies is the one that indiscriminately combines grade I neoplasms, such as pilocytic astrocytoma, with grade II gliomas, such as diffuse astrocytoma. Whereas the former is classically a benign and slow-growing or clinically stable glioma with relatively discrete growth and potential cure with surgery alone, the latter is an incurable, eventually fatal, low-grade malignancy with a propensity to undergo further malignant progression to grades III and IV over time. The demographic and radiographic features also differ significantly. Therefore, data derived from series that lump these entities together should be considered suspect unless the results are also stratified for each group separately. Moreover, the pairing of these astrocytomas under the umbrella LGG designation has led to a common misconception that they lie along a single continuum, with grade I examples eventually progressing to grade II and higher. We now know that such a progression almost never occurs, and despite some histopathologic overlap, they should be treated as completely separate entities.

With these definitional caveats in mind, this review focuses on the low-grade diffuse gliomas highlighted in the restrictive definition above. Some of the remaining entities in Table 1 are mentioned briefly in discussions of differential diagnosis.

### Six Important Concepts of LGG Pathology

#### 1. Location, Location, Location

Neuropathology has been likened to real estate because location is of prime importance in terms of both differential diagnosis and prognosis. This is particularly relevant in the diagnostic workup of LGGs, where there is a remarkably wide morphologic spectrum and, inevitably, some degree of overlap among entities. However, the differential is often limited to only a few common tumors within a given site. Therefore, the same oligodendroglioma-like histology should raise suspicion of central neurocytoma in the lateral ventricle, pilocytic astrocytoma in the cerebellum, and DNT in an intracortical temporal lobe mass. It is precisely for this reason that neuropathology should never be practiced in a “vacuum,” without the appropriate clinical and radiologic data. As such, the specimen slip labeled “brain tumor” does not provide sufficient information. The second caveat regarding tumor location is that there are many regions of high-priced real estate in the CNS. Therefore, even a benign glioma such as pilocytic astrocytoma may generate significant morbidity or mortality when in a functionally critical site.

#### 2. Diffuse Gliomas Are Partially Invisible

Probably the most important concept in the pathology of diffuse LGGs is simply that they are diffuse. In other words, rather than forming a solid mass which destroys or displaces nonneoplastic parenchyma, they are infiltrative, ill-defined tumors (Figs. 1A–1D) that can spread for many centimeters along preexisting white matter tracts, perivascular spaces, and other native elements of the CNS. As such, they contain foci of microscopic disease, which may be grossly and radiologically invisible. It is precisely this infiltrative growth pattern that accounts for the major therapeutic challenges and surgically incurable nature of diffuse gliomas. In fact, this pattern of relatively “peaceful coexistence” between tumor cells and native tissue, along with the low proliferative index, likely explains the typically preserved neurologic function (e.g., high Karnofsky performance score) encountered at presentation and suggests that many, if not all LGG patients have harbored asymptomatic tumors for many years prior to clinical detection. For example, the patient whose LGG is illustrated in Fig. 1A continued to practice law until 3 weeks before his death and was
relatively asymptomatic prior to that time. Given the negligible tumor proliferation seen microscopically, it likely took years to reach this size. At autopsy, there was infiltration well beyond this grossly evident mass, with a focus of malignant transformation in the brainstem. There was uncal herniation, and this was felt to be the mechanism of death.

3. Gliomatosis Cerebri and Multifocal Gliomas: The Ultimate in Diffuse Growth

Despite the emphasis above that all diffuse gliomas are diffuse, our textbooks are somewhat oversimplified, and there remains significant variability in the overall extent of infiltration within this group of neoplasms. Whereas some diffuse LGGs seem to trickle out no more than a few millimeters from the “edge” of the tumor, others involve extensive regions of the brain. The reasons for these differences are not entirely clear, though they have obvious prognostic and therapeutic implications.

Although neither gliomatosis cerebri nor multifocal glioma technically qualifies as LGG, both are commonly characterized by foci resembling LGG and represent the extreme end of the infiltrative spectrum. Gliomatosis cerebri is defined as “a diffuse glial tumour infiltrating the brain extensively, involving more than two lobes, frequently bilaterally and often extending to infratentorial structures and even to the spinal cord” (Kleihues and Cavenee, 2000). As such, it is a clinicopathologic diagnosis that may include any diffuse glia cell type or grade. In reality, though, the prognosis is uniformly poor, it most often resembles fibrillary astrocytoma, and it has been designated as “usually corresponding to WHO grade III” (Kleihues and Cavenee, 2000). The example illustrated in Figs. 1C and 1D involved nearly the entire brain, despite minimal grossly and radiographically detectable abnormalities (i.e., mostly invisible). Most of the tumor resembled diffuse astrocytoma, though there were 2 separate foci of glioblastoma (GBM), each presumably arising from local progression of a single lower-grade progenitor clone. In fact, this scenario likely accounts for the majority of “multifocal gliomas” or “multifocal GBMs.” In other words, there is a microscopic, grossly invisible LGG component in between the foci of more obvious malignant transformation. True multifocal gliomas, which arise independently, are thought to be rare, are difficult to prove, and presumably arise mostly in the setting of hereditary tumor predisposition syndromes such as Li-Fraumeni syndrome.

4. Gliomas Are Heterogeneous Tumors

Regional heterogeneity in morphology, proliferative activity, ploidy, and genetic aberrations has been well documented in diffuse gliomas (Coons and Johnson, 1993a, b; Coons et al., 1995; Harada et al., 1998; Jung et al., 1999; Kros et al., 2001; Walker et al., 2001). Therefore, the accuracy of glioma classification and grading is highly dependent on the extent of sampling. Despite their convenience and popularity, it is a reality that small biopsies, such as those obtained by the stereotactic needle approach, are prone to sampling error. Therefore, the pathologist must consider clinical and imaging features in such cases in order to assess specimen adequacy, particularly at the time of frozen section, when additional tissue can still be obtained.

5. Radiology Provides the Gross Pathology for Surgical Specimens

In essence, the neuroimaging represents the “gross pathology” for surgical LGG specimens, particularly for small biopsies. The majority of diffuse LGGs present as ill-defined nonenhancing masses, best seen on T2-weighted or fluid-attenuated inversion recovery MRI sequences. A tumor epicenter in the cortex suggests the possibility of oligodendroglioma, whereas deeper masses more commonly represent astrocytoma. Although T2-weighted radiodensities are often referred to as “edema” because of increased water content, this is not entirely accurate.
given that neoplastic or inflammatory infiltrates, gliosis, or some combination of these may also account for this appearance. Nevertheless, there is no doubt that the imaging provides important clues beyond those obtained by histopathology alone. For example, a ring-enhancing lesion is not compatible with a microscopic diagnosis of diffuse LGG. Typically, these represent the infiltrative edge of an undersampled GBM. A cystic lesion with a mural enhancing nodule is similarly incompatible with diffuse LGG, but suggests instead a more favorable entity such as pilocytic astrocytoma, ganglioglioma, or pleomorphic xanthoastrocytoma.

6. Role of the Intraoperative Frozen Section

There is often a push to get as precise a diagnosis as possible at the time of intraoperative frozen section, mostly out of curiosity and a desire to provide rapid feedback to the patient and family. However, the most important uses of this technique are to (1) insure that diagnostic tissue is obtained for permanent sections and (2) obtain information that might alter the surgical procedure. An example of the former might be providing intraoperative feedback as to the optimal needle placement in a stereotactic biopsy. An example of the latter might be the differential of an enhancing mass in an older patient. A neurosurgeon would likely stop at a biopsy for a frozen section diagnosis of lymphoma, proceed with a resection for a metastasis, or send additional tissue to the microbiology lab for a suspected abscess. It is rarely necessary to specify the exact cell type and grade of a glioma intraoperatively because typically it would not change the surgical approach, and there is a high likelihood that the diagnosis would be modified on review of the permanent sections the next day. Nonetheless, a relative grade determination is occasionally requested, for instance, if there is a plan to place Gliadel (BCNU; Guilford Pharmaceuticals Inc., Baltimore) wafers within the operative cavity for a high-grade glioma.

In the workup of LGG, the intraoperative diagnosis may be obvious or not so obvious, depending on the cellularity of the tumor and the degree of nuclear atypia. Whereas the frozen section is good for demonstrating architecture, the process of tissue freezing leads to poor cytologic preservation. Therefore, in some pathology labs, including ours, we supplement the frozen section with smear preparations. Whereas this disrupts the underlying architecture, it yields excellent cytologic detail and requires only minute quantities of tissue. An example of this technique’s utility is the differential of gliosis versus diffuse astrocytoma, where the primary distinction is nuclear cytology (Fig. 1B). In particularly difficult examples, the diagnosis is often deferred to permanent sections. Another scenario in which cytologic smear preparations are extremely helpful is in the distinction of macrophages encountered in demyelinating/ischemic processes from atypical astrocytes encountered in astrocytomas.

Unlike in other specialties of surgical pathology, we are rarely asked to provide frozen sections for surgical margins in neuropathology since they are irrelevant in diffuse gliomas. Nevertheless, a surgeon attempting to achieve “gross total resection” in an LGG may have difficulty visualizing the grossly abnormal borders and may then appropriately request a frozen section to distinguish fragments of cellular tumor from largely intact brain parenchyma with only scattered “atypical cells.” A final caveat regarding the intraoperative frozen section is that the procedure results in significant tissue loss and artifacts in the residual tissue. Therefore, provisions should always be made to save some nonfrozen, optimally fixed tissue for permanent sections.

Diffuse Astrocytoma (WHO II)

Pathology

Diffuse astrocytomas may occur anywhere along the neural axis, though the majority present as cerebral hemispheric masses in young adults. The epicenter is typically in the white matter, though cortical involvement is also common and may account for the seizures experienced by some of these patients. They are significantly rarer than their malignant (grade III or IV) counterparts, accounting for only 5% to 15% of the astrocytomas. Overall patient survival averages 5 to 8 years, with most eventually succumbing to malignant progression and/or complications of mass effect. Factors accounting for the variable time to progression have not been entirely worked out, though patient age remains a powerful prognostic parameter, inversely associated with survival time (Shafqat et al., 1999). It is hoped that molecular markers will provide additional clues. (See the Molecular Pathology section on diffuse astrocytoma.)

Diffuse astrocytomas are characterized by cytologically atypical fibrillary astrocytes with hyperchromatic, elongated nuclei that either appear “naked” within a fibrillary background or display discernable eosinophilic cytoplasmic processes (Figs. 1B–1D). Less common variants include gemistocytic and protoplasmic cells (Klei- huys and Cavenee, 2000). Grade II astrocytomas lack mitotic activity, endothelial proliferation, and necrosis.

Diffuse astrocytomas have been diagnosed less often in the last decade, and there are probably 2 primary reasons for this shift. First, the St. Anne-Mayo astrocytoma grading scheme, essentially adopted in the 1993 version of the WHO system, stated that even a single mitotic figure is sufficient to warrant an anaplastic or grade III designation (Daumas-Duport et al., 1988). However, many pathologists have questioned the “one mitosis” rule for large resection specimens, where intuitively the finding of a rare mitotic figure might not have the same dire implications as it does in a small biopsy. In fact, survival times for anaplastic astrocytoma patients with a solitary mitosis were recently found to be similar to those of grade II astrocytoma patients (Giannini et al., 1999; Perry et al., 1999), supporting the notion that the “one mitosis” rule is too restrictive. Proliferative indices, as measured by the MIB-1 (Ki-67) antibody, may provide useful information beyond that of simple mitosis counting, though hard and fast rules are lacking, and there are significant methodological and interpretive differences among labs through-
out the country (Giannini et al., 1999). Unfortunately, this proliferation issue has not been entirely resolved. At Washington University, we generally upgrade for a single mitosis only when encountered in a small biopsy. In borderline examples (informally referred to as grade II and a half), we supplement routine histology with MIB-1 semiquantitative determinations of low, moderate, or high labeling indices, and this may push the final grade determination up or down. This is basically in agreement with the revised WHO philosophy that “mitotic activity is generally absent but a single mitosis does not yet allow the diagnosis of anaplastic astrocytoma” (Kleihues and Cavenee, 2000).

The second reason for the modern decline of the grade II astrocytoma is the heightened awareness of “oligodendroglial features” by pathologists and clinicians alike and the expanded morphologic spectrum of oligodendrogliomas now accepted (Burger, 2002; Perry, 2001). Therefore, whether appropriately or inappropriately, some of the diffuse astrocytomas of the past are now being diagnosed as oligodendrogliomas and MOAs.

Differential Diagnosis

The 3 most important differential considerations for diffuse astrocytoma include reactive gliosis, pilocytic astrocytoma, and oligodendroglioma. As diffuse astrocytomas are primarily recognized by their increased cellularity and nuclear atypia, those that deviate minimally in these attributes may be difficult to distinguish from gliosis. Helpful features in favor of LGG include cellular clustering rather than even spacing, secondary structures of Scherer (perineuronal satellitosis, subpial condensation, and perivascular aggregation) (Fig. 1C), increased MIB-1 labeling indices, and p53 protein immunoreactivity. Cases that still remain unclear are often signed out as “atypical astrocytic proliferation” with a recommendation for close follow-up or rebiopsy. Although pilocytic astrocytomas may have dense regions that resemble fibrillary astrocytoma, the presence of Rosenthal fibers, eosinophilic granular bodies (EGBs), relatively solid components, and characteristic imaging features help to distinguish them (Figs. 1G and 1H). Oligodendrogliomas have the greatest degree of overlap with diffuse astrocytoma, but the former are generally characterized by rounder, more delicate nuclei, often with clear perinuclear haloes.

Molecular Pathology

Relatively little is known regarding the earliest genetic changes of diffuse astrocytomas, and although there is ample evidence for astrocytic differentiation, the true cell of origin remains a mystery. Difficulties in deriving this type of information for LGGs in general are that (1) tumors have likely been around for a long time before they are diagnosed, (2) by nature, diffuse glioma specimens are contaminated with nonneoplastic cells, (3) because of a low proliferative index, it is hard to culture LGGs, (4) LGGs are substantially less common than their high-grade counterparts, and (5) the techniques we commonly utilize to detect genetic alterations may be too insensitive to detect subtle changes in well-differentiated neoplasms. Nevertheless, there is evidence for at least 2 common alterations in grade II astrocytomas: p53 mutation and platelet-derived growth factor receptor alpha overexpression. Because these alterations occur frequently in all grades, it is felt that they represent early events. Roughly half of all grade II astrocytomas harbor p53 mutations (Reifenberger et al., 1996; Watanabe et al., 1997). Even more common is p53 protein immunoreactivity, though some of these do not harbor mutations, and the significance of such cases is unknown (Watanabe et al., 1997). Platelet-derived growth factor receptor alpha overexpression is also common in diffuse astrocytoma, where it may be involved in an autocrine loop signaling pathway (Hermanson et al., 1996).

Oligodendroglioma (WHO II)

Pathology

The pathology of oligodendroglial neoplasms and associated controversies of classification were recently reviewed in detail (Perry, 2001). In short, it is important to recognize that there are variable definitions and interpretations for oligodendrogliomas and MOAs in the neuropathology community, with both strict and loose criteria applied (Burger, 2002). As opposed to astrocytomas, where the majority are already high-grade at time of diagnosis, oligodendrogliomas commonly present at the grade II stage. They can occur at any age or location, but are distinctly uncommon in children and are almost never encountered in the brainstem, cerebellum, or spinal cord. The majority present as hemispheric masses in young to middle-aged adults, with the frontal lobe representing a favored location. The prognosis is significantly better than for astrocytomas, with average survival times of 10 years or more and improved chemosensitivity profiles (Fortin et al., 2001; Glass et al., 1992; Olson et al., 2000; Shaw et al., 1992). As with astrocytomas, though, there is a wide range of times to progression and overall survival.

The most classic and uniformly accepted oligodendroglial features include uniformly round nuclei, bland chromatin, clear perinuclear haloes imparting a “fried egg” appearance, and a rich, branching capillary network reminiscent of “chicken wire” (Fig. 1E). Other common, though slightly less specific findings include extensive cortical involvement, microcalcifications, mucin-rich microcystic spaces, and perineuronal satellitosis. It is important to recognize that the “fried egg” appearance represents a formalin fixation artifact that is neither absolutely necessary for diagnosis nor encountered in frozen sections or rapidly fixed specimens. The recently expanded morphologic spectrum includes 2 glial fibrillary acidic protein (GFAP)-expressing cells, previously thought to represent astrocytic differentiation: the mini- or microgemistocytes and gliofibrillary oligodendrocytes. The former are gemistocyte-like cells with small eccentric bellies of eosinophilic cytoplasm; round, bland nuclei resembling those of classic oligodendroglioma nuclei;
and no cytoplasmic processes (Fig. 1F). The latter are histologically identical to ordinary oligodendroglioma cells on H&E stain but exhibit strong GFAP-immunoreactivity, typically as a thin perinuclear rim, occasionally with a thin tadpole-like process. These 2 cell types have been accepted as part of the oligodendroglioma spectrum because (1) they are commonly encountered in otherwise classic-appearing oligodendrogliomas, (2) they are reminiscent of normal GFAP-positive oligodendroglial precursor cells, and (3) they do not appear to impact negatively on prognosis (Jagadha et al., 1986; Kros et al., 1990, 1992).

By definition, grade II oligodendrogliomas cannot have “significant mitotic activity, endothelial hyperplasia, or necrosis” (Kleihues and Cavenee, 2000). Of course, the term significant mitotic activity is fairly vague and subject to variable interpretations. While it is generally agreed that a greater number of mitoses are required for an anaplastic oligodendroglioma (grade III) designation than for astrocytoma, the exact number has not been defined. In a recent large series, a cutoff of 6 per 10 high-powered fields was found to correlate with decreased survival (Giannini et al., 2001). Unfortunately, most oligodendrogliomas eventually undergo malignant progression to anaplastic oligodendroglioma. Such foci are typically characterized by hypercellularity, numerous mitoses, and endothelial hyperplasia. Although the WHO does not further recognize a grade IV oligodendroglioma or MOA, at Washington University, we currently apply the grade IV designation to examples with foci of pseudopalisading necrosis in addition to the features described above. In other words, these are tumors that otherwise harbor all the criteria for GBM, but still show convincing oligodendrogial features. Such cases have been referred to as “markedly anaplastic oligodendroglioma,” “markedly anaplastic MOA,” or “GBM with oligodendrogial features,” and there is evidence to suggest that they behave more favorably than conventional GBMs (Donahue et al., 1997; He et al., 2001; Kraus et al., 2001). Nevertheless, this is a grading issue that remains unresolved and deserves further study with larger cohorts.

**Differential Diagnosis**

Many tumors may have round nuclei with clear haloes, and therefore, the differential diagnosis for oligodendroglioma is relatively broad (Table 2). The greatest overlap is with astrocytoma, which generally shares its clinical presentation and infiltrative growth pattern. The current distinction rests primarily on nuclear cytology (Figs. 1B–1E), which unfortunately is far from perfect (Burger, 2002; Perry, 2001). GFAP-immunoreactive processes support astrocytic over oligodendroglial derivation, though even this is fairly unreliable since one may be fooled by processes belonging to entrapped reactive astrocytes or by nonspecific background staining of axons. Likewise, there are many oligodendrogliomas with GFAP-positive minigemistocytes and gliofibrillary oligodendrocytes. (See the Pathology section on oligodendroglioma.) Thus, much of the remaining diagnostic difficulties rest on the fact that there are currently no specific oligodendroglioma markers available. Though some would argue that 1p/19q testing now fulfills that role, the sensitivities and specificities are still far from ideal. (See the Molecular Pathology section on oligodendroglioma.)

Other entities are more readily distinguishable from oligodendroglioma by their specific clinical, gross/radiologic, and histopathologic features (Table 2). Although it is not widely appreciated, pilocytic astrocytomas may display regions that resemble not only diffuse astrocytoma (Fig. 1G) but oligodendroglioma as well (Fig. 1H). Fortunately, the clinical features are usually distinctive, and a pediatric tumor in the cerebellum, optic pathway, hypothalamus/third ventricle, thalamus, dorsal brainstem, or spinal cord is far more likely to represent pilocytic astrocytoma than oligodendroglioma. Furthermore,
the majority of pilocytic astrocytomas harbor at least a few Rosenthal fibers and/or EGBs, though neither is absolutely necessary or specific for the diagnosis (Figs. 1G and 1H). An “intraventricular oligodendroglioma” is a central neurocytoma until proven otherwise, and its neuronal differentiation is easily verified with a synaptophysin immunostain. A DNT may be impossible to separate from oligodendroglioma on a needle biopsy, but larger specimens typically reveal the characteristic intracortical localization, nodular growth pattern, and “floating neurons.” Clear cell ependymoma may look remarkably similar at first glance, but careful examination will reveal its pushing, rather than infiltrative, border and occasional perivascular pseudorosettes characteristic of ependymal neoplasms. Electron microscopy can resolve the rare cases that remain uncertain.

**Molecular Pathology**

The genetic characterization of oligodendrogliomas has provided some of our most important clues in the battle against diffuse gliomas (Perry, 2001). Though little was found in early oligodendroglioma karyotypes (lack of appropriate cells being cultured), subsequent loss of heterozygosity, fluorescence in situ hybridization (FISH), comparative genomic hybridization, and quantitative microsatellite analyses have all revealed a characteristic codeletion or loss of one entire 1p and one entire 19q chromosomal arm in 50% to 80% of cases (Bello et al., 1995; Kraus et al., 1995; Nigro et al., 2001; Perry et al., 2003; Reifenberger et al., 1994; Smith et al., 1999). More exciting, however, were the observations that this genetic signature is associated with both prolonged survival and an almost uniformly favorable response to PCV (procarbazine, CCNU, vincristine) chemotherapy and/or radiation therapy (Bauman et al., 2000; Cairncross et al., 1998; Smith et al., 2000). Understandably, these studies have generated a great deal of interest in ancillary genetic testing for 1p and 19q status. Of the 2 markers, 1p has greater specificity, since 19q losses have also been reported in high-grade astrocytomas and MOAs (Smith et al., 2000). Also of practical value, oligodendroglioma mimics that we tested (DNTs, central neurocytomas, clear cell ependymomas) had no deletions, with the exception of occasional “extraventricular neurocytomas.” The recent finding of rare oligodendrogliomas with neurocytic differentiation suggests that there may actually be greater overlap between these 2 diagnostic entities than previously appreciated (Perry et al., 2002).

**Ancillary 1p/19q Genetic Testing**

At Washington University, we have been routinely performing 1p/19q FISH analysis on all in-house and consultation oligodendroglioma or suspected oligodendroglial tumors for the past 4 years (approximately 300 cases). Although several techniques are suitable, we chose FISH because of its basis in morphology, easy applicability to paraffin sections, and minimal tissue requirements without the necessity for matched nonneoplastic patient DNA (Fuller and Perry, 2002). We have found 1p/19q codeletion in 70% of oligodendrogliomas (Figs. 1I and 1J), 17% of MOAs, and 4% of astrocytomas with “vague oligodendrogial features” (Table 3). Thus, there is a tight relationship between histopathology and genotype. Similarly to findings of Zlatescu and colleagues (2001), we found some site-related associations as well, with temporal lobe tumors less likely to harbor the codeletion pattern (Table 4) (Perry et al., 2003). Additionally, a solitary 19q deletion pattern was encountered most often in parietal lobe tumors, usually high-grade MOAs (Perry et al., 2003). Also of practical value, oligodendroglioma mimics that we tested (DNTs, central neurocytomas, clear cell ependymomas) had no deletions, with the exception of occasional “extraventricular neurocytomas.”

<table>
<thead>
<tr>
<th>Location</th>
<th>1p/19q Deleted</th>
<th>-19q Only</th>
<th>Polysomies</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal (N=115)</td>
<td>50%</td>
<td>7%</td>
<td>1%</td>
<td>15%</td>
</tr>
<tr>
<td>Temporal (N=48)</td>
<td>25%</td>
<td>8%</td>
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<td>35%</td>
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<td>Parietal (N=22)</td>
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<td>23%</td>
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<tr>
<td>Occipital (N=5)</td>
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<td>40%</td>
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<td>Thalamus (N=5)</td>
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<td>0</td>
<td>40%</td>
<td>60%</td>
</tr>
<tr>
<td>Other* (N=21)</td>
<td>29%</td>
<td>10%</td>
<td>19%</td>
<td>43%</td>
</tr>
<tr>
<td>P-value**</td>
<td>0.001</td>
<td>0.042</td>
<td>0.046</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Other = multiple lobes or cerebral hemisphere, not otherwise specified.
** P-value = frontal lobe versus all other locations, P-value = parietal lobe versus all other locations, P-value = temporal lobe versus all other locations.

Table 3. FISH patterns versus glioma type at Washington University

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>1p/19q Deleted</th>
<th>-19q only</th>
<th>Polysomies</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>O (N=109)</td>
<td>70%</td>
<td>2%</td>
<td>16%</td>
<td>16%</td>
</tr>
<tr>
<td>MOA (N=109)</td>
<td>17%</td>
<td>21%</td>
<td>47%</td>
<td>23%</td>
</tr>
<tr>
<td>A (N=28)</td>
<td>4%</td>
<td>8%</td>
<td>53%</td>
<td>33%</td>
</tr>
<tr>
<td>P-value*</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.122</td>
</tr>
</tbody>
</table>

Abbreviations: A, astrocytoma; MOA, mixed oligoastrocytoma; O, oligodendroglioma.
*P-value is for O versus MOA and A.

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Table 4. FISH patterns versus tumor location

<table>
<thead>
<tr>
<th>Location</th>
<th>1p/19q Deleted</th>
<th>-19q Only</th>
<th>Polysomies</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other*</td>
<td>29%</td>
<td>10%</td>
<td>19%</td>
<td>43%</td>
</tr>
<tr>
<td>P-value**</td>
<td>0.001</td>
<td>0.042</td>
<td>0.046</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Other = multiple lobes or cerebral hemisphere, not otherwise specified.
** P-value = frontal lobe versus all other locations, P-value = parietal lobe versus all other locations, P-value = temporal lobe versus all other locations.

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As in retrospective studies, many of our currently known long-term survivors are patients with 1p/19q-deleted oligodendrogliomas, and this is obviously useful. However, there is no indication thus far that it is time to retire our microscopes. This genetic pattern identifies only one glioma subset, and we have seen several “genetic exceptions,” including long-term survivors without 1p/19q deletion and short-term survivors despite these deletions. Many of the former were classified as MOAs, suggesting that the histologic recognition of oligodendroglial features, despite its imperfections, still impacts favorably on survival, even in the absence of the “genetically favorable” pattern. In terms of our 3 short-term-surviving patients with 1p/19q deletions, they were older patients with large, bilateral, and/or ring-enhancing tumors that appeared high-grade (III or IV) on histology. It is possible that these patients had more advanced tumors with additional progression-associated alterations. Again, though, this implies that we should not ignore time-honored, well-established clinicopathologic prognosticators and rely solely on genetic results. It is almost certain that the most accurate methods for stratifying glioma patients in the future will be those that take into account clinical, pathological, and genetic data.

Many important issues regarding genetic testing remain, including quality control, standardization of testing, measurements of interlaboratory variability/concordance, reimbursement, etc. Nevertheless, there are at least half a dozen labs around the country currently performing 1p and/or 19q testing on clinical material. With a growing interest and an increasingly savvy patient population, this number will surely increase.

MOAs and Morphologically Ambiguous Diffuse Gliomas (WHO II)

Pathology

As a group, MOA patients have survival rates intermediate between those of pure astrocytomas and oligodendrogliomas, though there is marked individual variability (Perry, 2001; Shaw et al., 1994). Of all the gliomas, MOAs remain the most difficult to define, least reproducible, and most likely to receive discordant diagnoses from expert neuropathologists around the country. Nevertheless, the WHO recognizes 2 basic patterns, (1) a biphasic (“compact”) variant, where the 2 elements are spatially distinct, and (2) an intermingled (“diffuse”) variant, where the cell types are interspersed. Although the former is more often illustrated and discussed, it is actually far less common than the latter. The intermingled variant is usually characterized by some round, delicate nuclei; some irregular, hyperchromatic nuclei; and many difficult-to-characterize cells with intermediate or indeterminate features (i.e., morphologically ambiguous) (Fig. 1F). Therefore, the diagnosis of “diffuse glioma, not otherwise specified” is appropriately rendered for some of these cases. In a recent review of roughly 900 consecutive cases (grades II–IV), it was estimated that 10% of diffuse gliomas fell into this category (Fuller et al., 2002).

Molecular Pathology

Generally, MOAs and morphologically ambiguous diffuse gliomas have been understudied, precisely because the typical study design is to exclude cases with diagnostic uncertainty. A few have understandably focused on the biphasic variant, where microdissection is feasible. Generally, the same mutations have been found in both components, consistent with a monoclonal process (Dong et al., 2002; Jeuken et al., 2001; Maintz et al., 1997; Mueller et al., 2002). Most biphasic examples have been genetically similar to either pure oligodendroglioma or astrocytoma, with only a few cases showing mixed patterns. In a large series focusing on the intermingled MOA and utilizing probes that localize to 1p, 19q, PTEN, DMBT1, p16, EGFR, CEP7, CEP9, and CEP10, we similarly found oligodendroglioma-like and astrocytoma-like genetic subsets, though the 1p/19q codeletion pattern was much less common in this group (8%) (Fuller et al., 2002). Unfortunately, this suggests that 1p/19q testing is diagnostically helpful in only a small subset of the most challenging or morphologically ambiguous diffuse gliomas. Of interest is the fact that our largest subset of grade II MOAs harbored no detectable alterations and was associated with a favorable median survival of >100 months. This suggests that there are probably other “genetically favorable” alterations yet to be identified in this group of tumors.

Future Directions

The last decade has brought about exciting advances in our understanding of LGG pathology and biology, though we have barely begun to scratch the surface. Many unanswered questions remain regarding tumor histogenesis, etiology, precursor pathology, and the earliest events in neoplastic transformation. Newly developed high-throughput technologies such as cDNA, oligonucleotide, and SAGE (serial analysis of gene expression) profiling; tissue microarrays; comparative genomic hybridization microarrays; and proteomics promise to dramatically increase our pace of discovery. These will likely translate into a host of clinicopathologic experiments that will provide the desired candidates for improved classification, prognosis, and most importantly, targeted forms of therapy. The current aspiration of the neuro-oncology community is to administer highly individualized and effective forms of therapy directed at a tumor’s unique molecular profile. This goal will certainly take some time to achieve, though the next few years should be exciting to watch.

Acknowledgement

The author is grateful to Dr. Robert Schmidt, Neuropathology Division at Washington University, for his critical reading of this manuscript.


