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Morphologic and molecular genetic aspects of oligodendroglial neoplasms¹

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Morphologic criteria for diagnosing oligodendrogliomas and for classifying them as well-differentiated (World Health Organization grade II) and anaplastic (World Health Organization grade III) are well recognized. Nevertheless, applying these guidelines to specific cases often reveals discrepancies among different observers. In addition, whether a given tumor also contains an astrocytic component may be debatable. Loss of heterozygosity studies have demonstrated that oligodendroglial neoplasms have a high incidence of loss of the 1p and 19q chromosomal arms. Although loss of heterozygosity for portions of 19q are sometimes seen in astrocytic neoplasms, these tumors seldom show complete loss of 19q accompanied by loss of 1p. Loss of 9p or homozygous deletion of the *CDKN2* gene or both are associated with anaplastic oligodendrogliomas, whereas loss of 17p or *TP53* gene mutations or both are frequent in astrocytomas, but rare in oligodendrogliomas. These observations suggest that molecular genetic parameters could provide an objective, reproducible framework for classifying oligodendroglial neoplasms. *Neuro-Oncology* 1, 52–60, 1999 (Posted to *Neuro-Oncology* [serial online], Doc. 98-20, January 19, 1999. URL <neuro-oncology.mc.duke.edu>

Oligodendrogliomas were first described as a specific histologic type of glial neoplasm by Bailey and colleagues (Bailey and Bucy, 1929; Bailey and Cushing, 1926). Since the original description,

these tumors have been recognized as often containing a mixture of astrocytic and oligodendroglial elements. In addition, anaplastic oligodendrogliomas, which display histologic features such as cellular atypia, increased cellular density, endothelial proliferation, and necrosis, often behave more aggressively than the slow-growing, well-differentiated tumors. The WHO³ classification scheme for brain tumors sets forth criteria for distinguishing between grades II and III oligodendrogliomas and mixed gliomas. Nevertheless, application of these criteria to individual cases often reveals a lack of consistency and reproducibility in classifying oligodendroglial tumors among observers.

Objective means of determining whether a tumor contains neoplastic oligodendroglial or astrocytic elements or both and distinguishing between grades II and III lesions are critical because the survival and therapy is different for each category of tumor. A subset of oligodendroglial tumors characterized by loss of chromosomal arms 1p and 19q suggests that demonstration of this pattern by LOH, FISH, or CGH studies might provide an objective, reproducible means of identifying this category of tumors. Furthermore, LOH for 9p or deletions of the *CDKN2* gene or both appear to occur more frequently in anaplastic oligodendrogliomas than in well-differentiated, grade II tumors.

Maintz et al. (1997) observed that though oligodendroglial tumors have frequent loss of 1p and 19q and a low incidence of *TP53* gene mutations, astrocytomas have the opposite profile. They evaluated these parameters in a series of gliomas, which included 38 oligoastrocytomas of grades II and III. This study demonstrated that most oligoastrocytomas contained either 1p/19q loss or *TP53* gene mutations and that both abnormalities seldom occur in the same tumor. Furthermore, histologic evaluation of these cases revealed that the lesions with 1p/19q loss often had a predominance of oligodendroglial cells, whereas tumors with *TP53* gene mutations were more likely to show astrocytic differentiation.

We believe that histologic classification of gliomas should remain the gold standard for categorizing these

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³Abbreviations used are as follows: CGH, comparative genomic hybridization; FISH, fluorescence in situ hybridization; GFAP, glial fibrillary acidic protein; LOH, loss of heterozygosity; PCV, procarbazine cytoxan vincristine; WHO, World Health Organization.

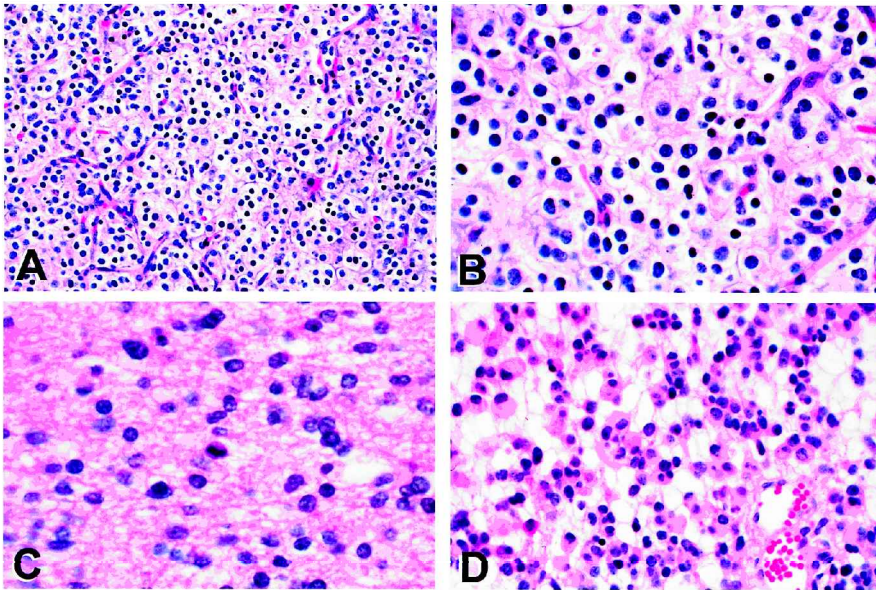


Fig. 1. Histologic features of well-differentiated oligodendrogliomas. A. These tumors are often hypercellular, composed of sheets of cells with a fine vascular network (H&E, $\times 200$). B. The round, regular nuclei are frequently surrounded by clear halos (H&E, $\times 400$). C. Mitotic figures may be seen, even in grade II lesions (H&E, $\times 198$). D. These tumors sometimes contain mini-gemistocytes (H&E, $\times 400$).

tumors. Nevertheless, the addition of molecular approaches can provide a means of arbitrating difficult or borderline cases and establishing objective, reproducible standards, which will be tested prospectively for their ability to predict prognosis and responsiveness to therapy. Here we review the morphologic aspects of oligodendroglial neoplasms and discuss the molecular genetic characteristics, which can be useful in subdividing and further classifying this group of brain tumors.

Clinical and Morphologic Features

Macroscopically, oligodendrogliomas exhibit predominantly cerebral hemispheric locations favoring the frontotemporal region. These tumors are generally identified in adulthood, particularly in the fourth and fifth decades (Kros et al., 1990; Mørk et al., 1985; Shaw et al., 1992). The tumors often exhibit a slow evolution with numerous reports indicating 10- to 15-year prediagnostic symptomatology referable to the neoplasm. Bailey and Bucy (1929) are credited with the original characterization of oligodendrogliomas as a specific histologic type of brain tumor. The presence of reactive astrocytes within these tumors and the identification of transitional cell forms between oligodendroglial cells and astrocytes have also been noted (Bailey and Bucy, 1929).

Oligodendrogliomas are most commonly found in the subcortical white matter with frequent extension into the cerebral cortex. An affinity for the cerebral cortex is often reflected in transcortical penetration of the tumors, with infiltration into the subarachnoid space and the leptomeninges. On cut surface, the tumors are solid and often appear grayish-pink. The subarachnoid accumulations are grossly characterized by surgeons as having a "toothpaste" morphology. Mucinous change is also evident and provides a gelatinous consistency to the tumor. Foci of necrosis and cystic degeneration may be seen in larger specimens and occasionally may be

accompanied by palpable deposits of calcification, particularly near the periphery. Spontaneous hemorrhage is a rare occurrence but may be devastating (Ernest et al., 1950; Mørk et al., 1985).

Well-differentiated Oligodendrogliomas

The oligodendroglioma is characterized histologically by rounded cells with well-defined cytoplasmic membranes and water-clear cytoplasm, which contains small spherical nuclei exhibiting a threadlike chromatin pattern and no nucleoplasm (Fig. 1A). Cytoplasmic clearing is a result of expansion and vacuolization of the cytoplasm with concomitant retraction and ultimate loss of the cell processes (Fig. 1B). This morphologic artifact is aggravated by a prolonged period before fixation and erased by either rapid fixation of small biopsies or freezing fresh material. Overall cellularity of a well-differentiated oligodendroglioma varies considerably from moderate white matter hypercellularity with concomitant increased cortical hypercellularity to sheets of pure tumor cells. The tumor is commonly accompanied by a delicate capillary network, which has been described as netlike and reminiscent of the vascular pattern found in liposarcomas. In contrast to the well-differentiated astrocytoma in which mitotic figures are, by definition, not found (Fig. 1C), mitotic figures are variable but usually easily found. A feature of well-differentiated oligodendrogliomas is satellitosis, the presence of cells accumulating about blood vessels and neurons. Satellitosis is variably found throughout the brain, but most commonly seen in the temporal lobe, also a common site of the oligodendroglioma. Typically, the well-differentiated oligodendroglioma is from moderately to markedly hypercellular and the tissue neoplastic. Furthermore, these tumors tend to be easily recognized as oligodendroglial, although the identification of a possible astrocytic component is a subject of lively debate. Even within typical oligodendrogliomas, variability of histologic features may be found. The most common morphologic variant of oligodendroglial tumor cells is the

so-called “mini-gemistocyte,” a tumor cell characterized by a round regular nucleus with an eccentric droplet of eosinophilic cytoplasm (Fig. 1D). Focally, these cells may fill an entire microscopic field and raise the differential diagnosis of its larger cousin, the astrocytic gemistocyte. In contrast to the gemistocyte, the oligodendroglial mini-gemistocyte has a single nucleus, which is identical to its peers; the astrocytic gemistocyte tends to exhibit nuclear pleomorphism and multinucleation. Calcification, though not a reliable diagnostic point for oligodendrogliomas, is useful in supporting a diagnosis of oligodendroglioma. This calcification tends to be punctate and related to blood vessels.

Anaplastic Oligodendrogliomas

A more difficult assessment involves the grading of oligodendroglial neoplasms. Workers have generally concluded that there is no single reliable histologic criteria by which malignant examples can be separated from those that are relatively benign (Burger et al., 1987; Mørk et al., 1986; Shaw et al., 1992; Smith et al., 1983). Most studies have supported a combination of histologic features that tend to identify clinically aggressive tumors. Mørk et al. (1986) indicated that cellular density, necrosis, and absence of microcyst formation are associated with tumors that have significantly reduced lengths of postoperative survivals. Similarly, Burger et al. (1987) found that atypia, mitotic activity, vascular proliferation, vascular hypertrophy, and necrosis were useful histologic markers for identifying the anaplastic tumors (Fig. 2). Although median survival intervals vary among patient groups, generally, tumors that possess many or all of these histologic features are likely to behave aggressively. Necrosis, as a single histologic landmark, seems to be the most common marker found in those tumors called “anaplastic oligodendroglioma.”

Oligoastrocytomas

It is commonly believed that a population of tumors exists in which oligodendroglial cells coexist with astrocytic cells in a neoplastic collision type of tumor. Previous studies by Raff et al. (1985) indicate that the monoclonal antibody A2B5 identifies an antigen on a primitive glial cell called the “O2A cells,” which are capable of producing either astrocytic or oligodendroglial cells in mammalian embryogenesis. Although this finding certainly suggests an origin of some, if not most, oligoastrocytomas, additional molecular and immunohistochemical data are obviously needed.

The most recently revised WHO classification of brain tumors recognizes the existence of gliomas with mixed morphologies (Kleihues et al., 1993). The WHO defines the oligoastrocytoma as a “tumor with a conspicuous mixture of neoplastic oligodendrocytes and astrocytes, either diffusely intermingled or separated into distinct areas” (Kleihues et al., 1993). The authors tacitly accept that oligodendrogliomas have nuclear features that are distinct from astrocytic cells and decline elaboration, only warning that “tumors containing cells with oligodendroglial nuclei and small eccentric GFAP-positive cell bodies are not considered to be mixed gliomas” (Kleihues et al., 1993). Our approach to these

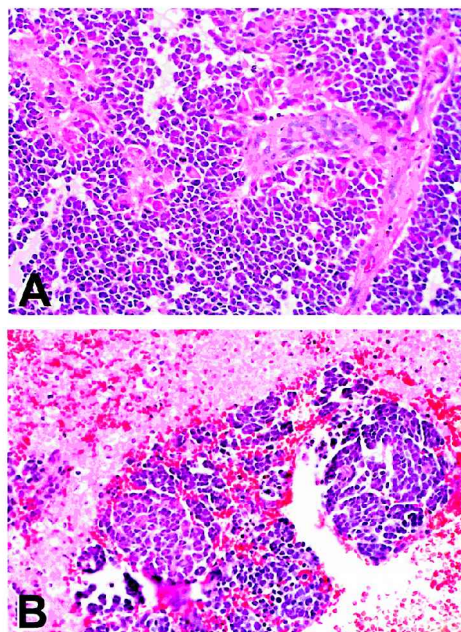


Fig. 2. Histologic features of anaplastic oligodendrogliomas. A. These tumors are hypercellular with anaplastic hyperchromatic nuclei. Endothelial proliferation is typically present (H&E, $\times 200$). B. Necrosis is also seen in these high-grade tumors (H&E, $\times 200$).

tumors is to make the diagnosis of mixed oligoastrocytoma on tissues that have not been previously frozen, recognizing that freezing not only introduces nuclear distortions but also prevents the cytoplasmic changes that otherwise characterize this oligodendroglial cell. Furthermore, we also consider the cytoplasmic and nuclear features of the different populations. Oligodendroglial cells exhibit few short stubby processes, whereas astrocytic populations tend to have elongated fibrillar processes. The two populations may intermingle, but areas in which relatively pure populations of both tumor cells exist should be identifiable in the tissue. Finally, we emphasize that up to 60% of oligodendroglial tumors exhibit a reactive astrocytosis, both along the margin of the tumor and within the tumor, a finding that should not be confused on GFAP staining as revealing an astrocytic component.

Anaplastic Mixed Gliomas

Grading mixed oligoastrocytomas is controversial for several reasons. Although the Daumas-Duport guidelines of nuclear pleomorphism, mitotic activity, vascular proliferation, and necrosis are useful, it is unclear whether each population should be graded individually or together (Daumas-Duport et al., 1987; Shaw et al., 1992, 1994). Often the two populations in the biopsy will differ in grade by these guidelines. We grade each component individually, using the same criteria that are applied to astrocytic and oligodendroglial tumors when they occur alone. If the astrocytic component exhibits mitotic activity and nuclear pleomorphism but lacks vascular proliferation and necrosis, we consider the tumor an anaplastic astrocytoma arising in a mixed

glioma. Similarly, if the astrocytic element contains endothelial proliferation and necrosis, we classify the lesion as a glioblastoma originating in a mixed glioma. If the oligodendrogloma component exhibits high cellularity, nuclear pleomorphism, endothelial proliferation, and necrosis, we consider the tumor an anaplastic oligodendrogloma arising in a mixed glioma. Most grading systems accept some mitoses within well-differentiated oligodendroglomas; therefore, if mitoses are confined to the oligodendroglial population, we consider the tumor a WHO grade II. When both components are grade III, we consider the tumor an anaplastic mixed glioma and both components as anaplastic. Although we attempt to follow these guidelines in all mixed gliomas, the classification in individual cases is often not straightforward, particularly since many of the criteria are subjective parameters.

Ultrastructural Features

Ultrastructurally, oligodendroglomas exhibit a moderately high nuclear to cytoplasmic ratio and few short, stubby processes. Mitochondria are often numerous. The cytoplasm typically exhibits few organelles, however, including rare profiles of rough endoplasmic reticulum and polyribosomes. Intermediate filaments are typically lacking, except in the mini-gemistocytes (Bruner, 1987). Microtubular processes are irregularly distributed in small numbers throughout the cytoplasm and may be concentrated in the short stubby processes (Ng et al., 1994). Occasional synapse-like membranous configurations may be encountered on cells otherwise typical for oligodendrocytes (Ng et al., 1994). In contrast, Kros et al. (1992) reported that the mini-gemistocyte exhibits pump cytoplasm filled with intermediate filaments, and tumors that contain these cells often reveal cells with intermediate or transitional features, including small typical cytoplasmic profiles and abundant intermediate filaments. Furthermore, mixed gliomas exhibit cells with features of transitional forms indicative of elements that are ambiguous, between typical oligodendroglial and astrocytic morphologies (Kros et al., 1992).

Immunohistochemical Findings

Normal oligodendroglial cells do not possess cytoplasmic intermediate filaments and do not exhibit GFAP. Nevertheless, immunoreactivity for GFAP may be found in typical oligodendroglomas in which it has been estimated to occur in ~50% of pure tumors. GFAP reactivity in these tumors appears to be related to several factors. Well-differentiated reactive astrocytes may be scattered throughout up to 60% of oligodendroglomas. Alternatively, the tumor may exhibit mini-gemistocytes (Burger et al., 1987; Kros et al., 1990). Although an interesting and confusing observation, the presence of these tumor cells do not adversely affect clinical prognosis (Kros et al., 1990). The origin of the GFAP reactivity appears to have an embryological basis, because human oligodendroglial cells in normal ontogeny may transiently express GFAP

immediately before myelinogenesis (Choi and Kim, 1984; Choi and Lapham, 1978; Ogawa et al., 1985).

Immunoreactivity for the S-100 protein has also been reported in oligodendroglomas irrespective of their positivity for GFAP (Kimura et al., 1986; Nakamura et al., 1983; Nakopoulou et al., 1990). The largest study of presumptive oligodendroglial markers has been performed by Rubinstein and colleagues in which Leu-7 (HNK-1) monoclonal antibody was studied and found to react with >90% of oligodendroglomas and a significant number of astrocytic tumors (Nakagawa et al., 1986; Perentes and Rubinstein, 1986). Immunostaining for the myelin basic protein did not reveal immunoreactivity in oligodendroglomas. Immunoreactivity for the myelin-associated glycoprotein was found by Rubinstein and colleagues to react with only 1 of 30 oligodendroglomas. Carbonic anhydrase, an antigen demonstrable in mammalian oligodendroglial cells including some human oligodendroglial cells, was found to a variable degree in a minority of oligodendroglomas (Nakagawa et al., 1987). Finally, the differential immunohistochemical demonstration of soluble lectins helps to distinguish differentiated from anaplastic oligodendroglomas (Bardosi et al., 1988; Cruz-Sanchez et al., 1991).

A number of histologic and immunohistochemical parameters have been tested for their ability to predict survival among oligodendrogloma patients. Several observers have evaluated nuclear labeling index, as determined by MIB-1 or KI67 staining, to determine its prognostic significance. According to studies from several laboratories (Coons et al., 1997; Deghani et al., 1998; Kros et al., 1996; Schiffer et al., 1997; Wharton et al., 1998), anaplastic oligodendroglomas have higher labeling indices than do well-differentiated oligodendroglomas; thus, MIB-1 labeling index is associated with tumor grade. Whether the labeling index, independent of histologic grade, is an indicator of prognosis, however, remains more debatable. Coons et al. (1997) found a significant association between labeling index and survival in a series of oligodendroglomas that had been stratified for histologic grade and age at diagnosis. Similarly, Deghani et al. (1998) found labeling index to be a strong predictor of survival among oligodendrogloma patients with grade II tumors, and Kros et al. (1996) reported that labeling index predicted survival independent of tumor grade. In a study of 39 patients with anaplastic oligodendroglomas treated with chemotherapy, MIB-1 proliferation index was unassociated with response to PCV, but higher MIB-1 indices were associated with decreased survival (Cairncross et al., 1998). Wharton et al. (1998) showed that although labeling index was significantly higher in grade III than in grade II tumors, stratification by labeling index alone did not predict clinical outcome.

Molecular Genetics

Oligodendroglomas: WHO grades II and III

Reports by Ransom et al. (1991) and von Deimling et al. (1992) described an association between LOH for

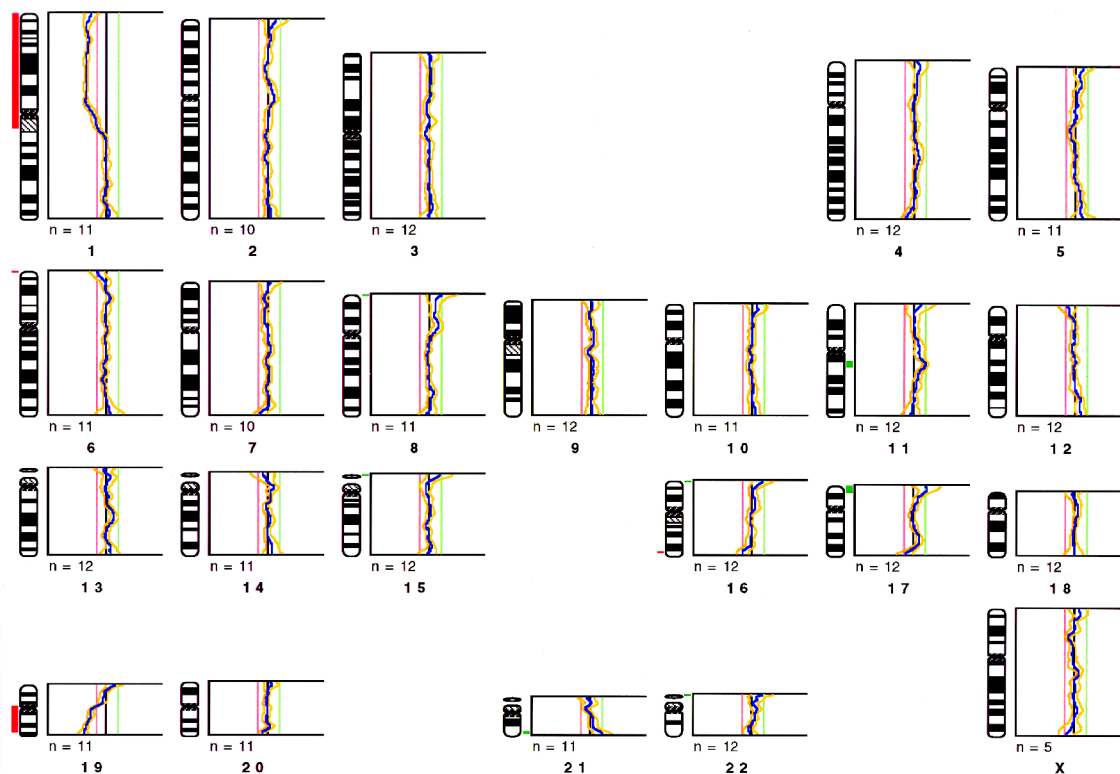


Fig. 3. Comparative genomic hybridization profile from the same well differentiated oligodendroglioma (case 988) as that in Fig. 1A and B. The only deviations are loss of one copy of 1p and 19q.

regions on 19q and glial neoplasms, including tumors that contain an oligodendroglial component. Bello et al. (1994, 1995) and Reifenberger et al. (1994) noted that oligodendroglial tumors also contain a high incidence of 1p deletions. Further studies have shown that oligodendroglial tumors display a distinctive genetic profile that consists of loss of the entire 1p and 19q chromosomal arms with retention of 1q and 19p (Kraus et al., 1995; Ritland et al., 1995). Although the smallest region of overlapping deletion on 19q has been narrowed to a 900-kb region (Rosenberg et al., 1996; Yong et al., 1995), the small deletions have generally been in astrocytic lesions, not oligodendroglial ones (Ritland et al., 1995; von Deimling et al., 1994). The incidence of 19q loss for oligodendroglial tumors is as high as 81% in some studies (Reifenberger et al., 1994), while LOH for 1p has been reported in up to 94% of these tumors (Bello et al., 1995).

Reifenberger et al. (1994) performed an allelotypic analysis using markers from all chromosomal arms on a series of 37 oligodendroglial tumors. More than one-half of the cases with loss of 1p and 19q showed additional losses on other chromosomes. Regions most frequently lost were 9p and 14q, but 9p LOH occurred only in anaplastic grade III tumors. LOH for 17p and chromosome 10 were also seen, but usually in cases without 1p and 19q LOH. Zhu et al. (1998) studied 25 grades II and III oligodendroglomas. They described, in addition to loss of 1p and 19q, loss of regions on chromosomes 4, 6, and 11 in tumors of both grades. Loss of 17p was restricted to grade III lesions. Weber et al.

(1996) evaluated by CGH initially resected tumors from and recurrences by 15 patients, including 5 patients with oligodendroglial neoplasms. In addition to losses of 1p and 19q, they most often experienced losses of chromosomes 4 and 9. Changes seen only in the recurrent lesions, however, included losses of 3p, 14q, and 15q. Cairncross et al. (1998) evaluated LOH for 1p, 10q, and 19q; *TP53* gene mutations; and *CDKN2* gene deletions in 39 anaplastic oligodendroglomas treated with chemotherapy. They found LOH for 1p in 24 (67%) of 36 cases, for 19q in 29 (82%) of 34, and for both 1p and 19q in 22 (65%) of 34 cases. Eight (21%) of 38 tumors had *CDKN2* gene deletions and 6 (15%) of 39 cases had *TP53* gene mutations. Loss of 1p either alone or associated with 19q loss strongly predicted a radiographic response to PCV. In contrast, *CDKN2* gene deletions were associated with a significantly worse prognosis.

A series of oligodendroglial neoplasms evaluated by our laboratory using molecular techniques such as LOH and CGH included 23 well-differentiated oligodendroglomas (WHO grade II) and 24 anaplastic oligodendroglomas (WHO grade III). Sixteen grade II tumors had complete loss of 1p and 19q by CGH, which was seen alone in six of these tumors (Fig. 3), whereas a variety of gains and losses were seen in the remaining 10 cases. The most frequent changes were loss of all or part of chromosome 4 and the Y chromosome. Two of the 16 cases with 1p/19q loss had LOH for 9p, but none had homozygous deletion of the *CDKN2* gene, LOH for 17p, or mutations of the *TP53* gene. By CGH, 20 of the

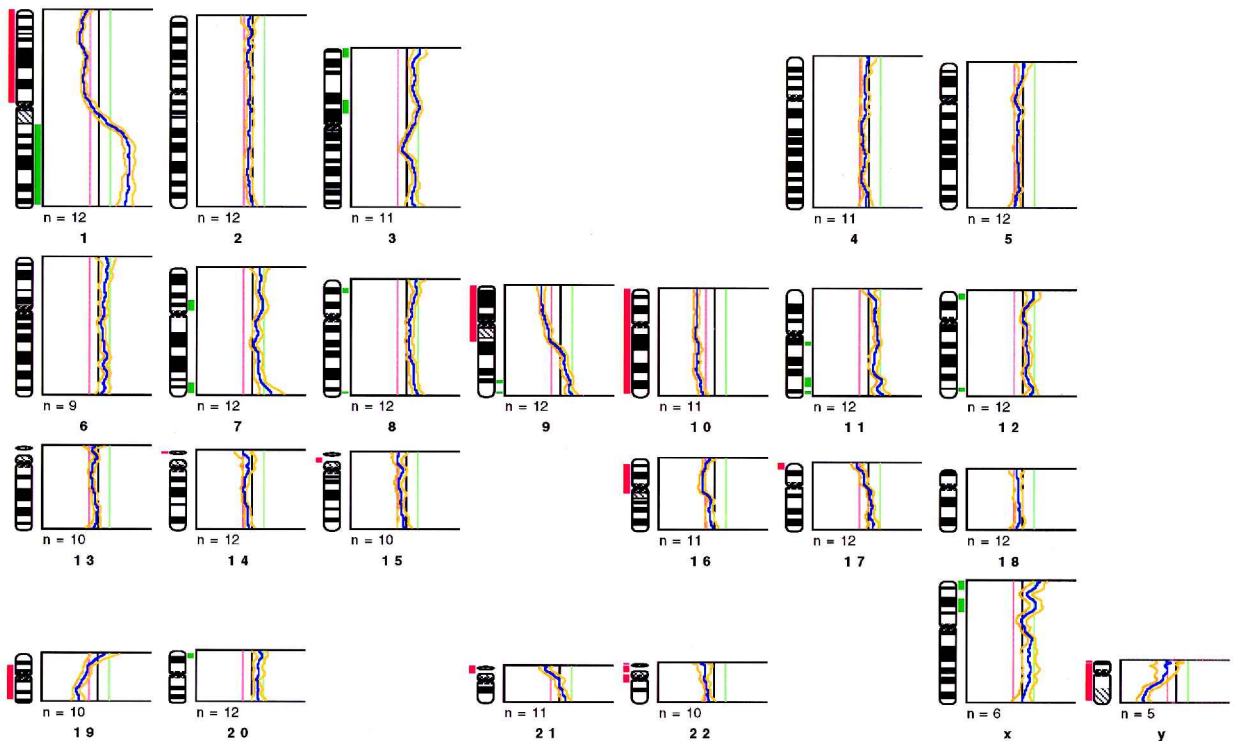


Fig. 4. Comparative genomic hybridization profile from the anaplastic oligodendroglioma (case 745) shown in Fig. 2. This tumor shows loss of 1p and 19q. Additional gains and losses are also present, including loss of 9p. This case also contained homozygous deletion of the *CDKN2* gene.

24 anaplastic oligodendrogliomas had complete loss of 1p and 19q either alone, in one case, or combined with other abnormalities. The most prevalent deviations were losses of chromosomes 4, 15, and 18 and 9p (Fig. 4). LOH for 9p or homozygous deletions of the *CDKN2* gene or both were seen in 11 (42%) of 24 of these tumors (Matthews et al., 1998). In contrast to the studies of Cairncross et al. (1998) in which *CDKN2* gene deletions occurred preferentially in tumors with intact 1p and 19q, all 7 anaplastic oligodendrogliomas with *CDKN2* gene deletions in our series had loss of 1p, and 6 of the 7 cases also had loss of 19q. LOH for 17p or *TP53* gene mutations or both occurred in five grade II and four grade III oligodendrogliomas. Although one of these eight cases had a 19q deletion and two had losses on 1p, none had loss of both 1p and 19q. Gene amplification and *PTEN* gene mutations were not detected in any of the grade II or III oligodendrogliomas.

Mixed Oligoastrocytomas: WHO grades II and III

To date, molecular genetic analyses fail to support the concept that the oligoastrocytoma is truly a collision tumor in which distinct populations of neoplastic astrocytes and oligodendroglia coexist. Maintz et al. (1997) evaluated a series of 38 grades II and III oligoastrocytomas. They found that loss of 1p/19q and *TP53* gene mutations were inversely related, seldom occurring in the same neoplasm. Furthermore, histologic evaluation revealed that lesions with the 1p/19q loss pattern often had a predominantly oligodendroglial morphology, whereas cases with *TP53* gene mutations were more

likely to be astrocytic. Kraus et al. (1995) applied LOH analysis of 1p and 19q to histologically-separable oligodendroglial and astrocytic regions in three oligoastrocytomas. They found that both types of areas within all three cases shared the 1p/19q LOH pattern.

In evaluating these findings, one must return to the basis on which the diagnosis of a mixed oligoastrocytoma is made. Our laboratory takes a conservative approach, separating most gliomas into either oligodendrogliomas or astrocytomas, according to the predominant component, and reserving the designation of oligoastrocytoma for the few cases in which the two elements are equally represented. Using molecular techniques, we studied 24 grade II gliomas with oligodendroglial features, including 23 well-differentiated oligodendrogliomas and one oligoastrocytoma. The oligoastrocytoma had the 1p/19q loss pattern characteristic of oligodendrogliomas. Although the majority of lesions classified as oligodendrogliomas also showed this pattern, four cases lacked complete 1p/19q loss and demonstrated LOH for 17p or *TP53* gene mutations or both. In retrospective histologic evaluation, these four cases had conspicuous astrocytic elements and might well have been designated as oligoastrocytomas in other laboratories. We also evaluated seven anaplastic mixed gliomas. Two cases had loss of 1p and 19q by CGH and LOH analyses; one of these cases also had LOH for 17p and a *TP53* gene mutation. Four cases had LOH for 17p or *TP53* gene mutations or both, and one case lacked any of these abnormalities but showed gain of chromosome 7, loss of chromosome 10, and contained

EGFR gene amplification. Thus, five of the seven anaplastic mixed gliomas that we studied had molecular genetic profiles consistent with astrocytic tumors, one case had an oligodendroglial pattern, and one case showed a *TP53* gene mutation with loss of 1p and 19q. These findings suggest that even when astrocytic and oligodendroglial elements are identified histologically within the same neoplasm, most gliomas display the molecular profile and predominant histology of either an astrocytoma or oligodendroglioma, but rarely both components. Because patients with oligodendrogliomas generally have a better prognosis than do patients with astrocytomas of the same grade, it is possible that long-term follow-up studies will reveal that the molecular profile of an oligoastrocytoma is predictive of its clinical behavior. If so, an argument can be made for including molecular analysis of 1p/19q loss, LOH for 17p, and *TP53* gene mutation in the initial evaluation of neoplasms with oligodendroglial elements.

Glioblastomas: WHO grade IV

The glioblastoma is a highly malignant neoplasm with cellular anaplasia, mitotic activity, endothelial proliferation, and necrosis. Astrocytic morphology is required by many observers for a lesion to be given this diagnosis. When a purely oligodendroglial tumor contains these malignant histologic parameters, particularly if the necrosis is accompanied by pseudopalisading nuclei, the question arises whether the lesion should be classified as an anaplastic oligodendroglioma (WHO grade III) or a glioblastoma (WHO grade IV). In support of the latter approach, Burger et al. (1987) found that none of his patients with oligodendroglial tumors exhibiting necrosis with pseudopalisading of tumor cells lived more than 3 years, a survival equivalent to that of glioblastoma. Note, however, that these studies were performed before the advent of PCV therapy. Therefore, these observations concerning the relationship between pseudopalisading necrosis and survival may be entirely inapplicable to currently managed high-grade oligodendrogliomas. Tumors with pseudopalisading necrosis that are composed of mixed oligodendroglial and astrocytic populations could also be assigned to the glioblastoma category. Finally, tumors with the typical morphology of glioblastomas are sometimes considered recurrences of oligodendrogliomas or oligoastrocytomas.

Our laboratory evaluated four glioblastomas with oligodendroglial features and one glioblastoma that was the recurrence of an oligodendroglioma (Matthews et al., 1998). One tumor in this group had the typical morphology of an anaplastic oligodendroglioma, but was classified as a glioblastoma based on the presence of necrosis with pseudopalisading. This case had complete loss of 1p and 19q, LOH for 9p, and lacked 17p LOH, *TP53* gene mutation, gene amplification, and the typical +7, -10 pattern of glioblastoma, a molecular profile supporting the diagnosis of anaplastic oligodendroglioma rather than glioblastoma. The other four cases did not show complete loss of 1p and 19q, but three showed loss of all or part of chromosome 10 by CGH and LOH studies and gain of chromosome 7.

Three of these four cases had gene amplification and two had *TP53* gene mutation. Molecular profiles supported the classification of these four cases as glioblastomas, including three cases of the progressive type (two with *TP53* gene mutation and one with *CDK4* gene amplification) and one case of the de novo type with *EGFR* gene amplification.

LOH studies by Kraus et al. (1995) and Ritland et al. (1995) suggest that glioblastomas differ from oligodendroglial tumors because losses of 19q are more likely to be partial than complete and loss of 1p is uncommon. A CGH analysis that included 14 glioblastomas described in one case complete loss of 19q without loss of 19p, partial deletion of 1p and 10q, but no gain of chromosome 7 (Nishizaki et al., 1998). Finally, Rosso et al. (1997) included two oligodendrogliomas that recurred as glioblastomas in their FISH analyses of chromosomes 1, 7, 10, 17, X, and Y. Loss of 1p36 was seen in both glioblastomas, but accompanied by +7, -10 in only one of the two cases and evaluation of chromosome 19 was not included. These findings suggest that lesions with typical morphology and molecular profiles of oligodendroglioma seldom, if ever, progress to true glioblastomas. Alternately, glioblastomas exist that exhibit a predominate oligodendroglial morphology, but do not possess the complete loss of 1p and 19q, which is typical of oligodendroglioma. This observation agrees with findings discussed above, which support separate progression pathways for gliomas along astrocytic and oligodendroglial lines.

Summary

Histologic evaluation should remain the chief support of brain tumor classification. Nevertheless, molecular genetic studies can play an important part in standardizing tumor categorization and resolving difficult diagnostic problems. Loss of one complete copy of 1p and 19q is characteristic of oligodendroglial neoplasms and is seldom, if ever, seen in any other lesion. This pattern has been shown by LOH and CGH studies. In addition, it should be possible to use interphase FISH to demonstrate this specific pattern on a cell by cell basis and in paraffin-embedded sections. Astrocytic tumors of grades II and III, in contrast, frequently have LOH for 17p or *TP53* gene mutations or both. By using a combination of evaluation of 1p/19q loss and *TP53* gene mutations, it is possible to divide grades II and III gliomas, including cases classified histologically as oligoastrocytomas, into tumors with oligodendroglial versus astrocytic profiles. Long-term follow-up studies will be necessary to determine if cases with oligodendroglial molecular profiles have a better prognosis than those with astrocytic patterns. In addition to the 1p/19q loss pattern, most oligodendroglial tumors show additional aberrations. Although loss of chromosome 4 and 9p are among the most common of these changes, only 9p loss, particularly if accompanied by homozygous deletion of the *CDKN2* gene, is associated with anaplastic histologic features. Finally, progression of lesions with oligodendroglial components seems to develop along two sepa-

rate pathways. Cases with 1p/19q loss show additional abnormalities, including chromosome 4 and 9p loss and *CDKN2* gene deletions, but seldom have 17p loss, *TP53* gene mutations, the typical +7, -10 chromosome pattern of glioblastoma, or gene amplification. In contrast, astrocytic tumors, which sometimes show losses of all or part of chromosome 19, lack complete 19q loss accompanied by loss of 1p. These tumors frequently contain

17p loss or *TP53* gene mutations or both. Progression is associated with additional abnormalities, including loss of all or part of chromosome 10, gain of chromosome 7, and gene amplification, especially of genes other than the *EGFR* gene. Thus, high-grade astrocytomas, including glioblastomas arising from histologically mixed oligoastrocytomas, resemble glioblastomas of the progressive type.

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