Oligodendroglioma: Toward Molecular Definitions in Diagnostic Neuro-Oncology

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Abstract. Oligodendroglial tumors have attracted great interest in both basic and clinical neuro-oncology over the past decade. This interest is mainly due to the clinical observation that anaplastic oligodendrogliomas and anaplastic oligoastrocytomas, in contrast to the vast majority of anaplastic astrocytomas and glioblastomas, frequently respond favorably to chemotherapy. In addition, oligodendrogial tumors are associated with longer survival times than the diffuse astrocytic gliomas. These differences in response to therapy and in prognosis have been associated with distinct genetic aberrations, in particular the frequent loss of alleles on chromosome arms 1p and 19q in oligodendroglial tumors. In addition, other genetic changes have been reported as indicators of poor response to therapy and short survival, including homozygous deletion of the CDKN2A gene at 9p21, mutation of the PTEN gene at 10q23, and amplification of the EGFR gene at 7p12. In this review we summarize the current state of the art concerning the molecular genetics of oligodendroglial tumors. A particular focus is placed on the role of molecular genetic findings in the diagnostic and prognostic assessment of these neoplasms. As a result of the recent advances in the field, we propose that clinical decisions in the management of patients with oligodendroglial tumors should be based on the combined assessment of clinical and neuroimaging features, histological classification and grading, as well as molecular genetic characteristics.

Key Words: Brain neoplasms; Chemotherapy; Molecular genetics; Oligodendroglioma; Prognostic factors; Survival.

INTRODUCTION

Until approximately 15 years ago, there was little practical importance in the distinction of oligodendroglial tumors from diffuse astrocytic gliomas. The treatment regimens largely overlapped, leaving prognostic estimation as the sole reason to distinguish oligodendroglial tumors. In general, however, prognostic estimation is not the major interest of either neuro-oncologists or patients once a diagnosis of glioma is established. They are concerned with choosing the most appropriate therapy, and most often will fight hard against the disease regardless of the prognostic estimates.

In 1988, it was first demonstrated that recurrent anaplastic oligodendrogliomas sometimes responded dramatically to the combination chemotherapy of procarbazine, lomustine (CCNU) and vincristine, a regimen subsequently dubbed “PCV” (1). Then, in 1990, it was shown that newly diagnosed anaplastic oligodendrogliomas also frequently responded favorably to PCV treatment (2). These initial findings have been corroborated in a number of independent retrospective studies from different institutions (3–6). Furthermore, there is evidence that anaplastic oligoastrocytomas may also show durable responses to PCV chemotherapy (4, 7). More recent data indicate that the chemosensitivity of oligodendroglial tumors is not restricted to PCV treatment but also seen upon treatment with temozolomide, a new alkylating drug that can be administered orally (5, 6).

Taken together, these clinical observations revolutionized the diagnostic needs in glioma pathology, since they implied that the diagnosis of a malignant oligodendroglial tumor would be acted upon in a different way from the diagnosis of a malignant astrocytic glioma. Unfortunately, the clinical importance of differentiating oligodendroglial from astrocytic tumors highlights the sometimes-difficult histological distinction of these gliomas. Although the diagnosis of classic examples of oligodendroglioma and diffuse astrocytoma is straightforward, a significant fraction of the diffuse gliomas, in particular among the high-grade malignant tumors, shows ambiguous histological features that make their classification as either oligodendroglial or astrocytic glioma both difficult and, to a large degree, subjective. At present, there is no specific marker available that allows a reliable distinction of oligodendrogliomas from astrocytomas by immunohistochemical analysis or in situ hybridization. The oligodendrocyte lineage transcription factors Olig1 and Olig2 have recently been reported as potential markers for oligodendrogliomas (8–10). Although promising, these results were based on the analysis of relatively few tumors using in situ hybridization. Therefore, it remains to be confirmed whether the reported selective expression of Olig1 and Olig2 in oligodendrogliomas holds true when larger series of different glioma types are investigated. In addition, it is unknown whether these antigens...
Oligodendrocytes or glial precursor cells

- 1p loss (1p34-p35, 1p36.2, 1p36.3-pter)
- 19q loss (19q13.3)
- other chromosome losses (rare)
- p14ARF / CDKN2A/B methylation

Oligodendroglioma WHO grade II

- 9p loss (CDKN2A/B / p14ARF homozyg. del. or methylation)
- CDKN2C mutation / homozyg. del.
- RB1 methylation
- 10q loss / PTEN mutation (rare)
- TP53 mutation (rare)
- other chromosome losses

Anaplastic oligodendroglioma WHO grade III

- EGFR overexpression
- PDGF/PDGFR overexpression

- VEGF overexpression
- Proto-oncogene amplification (rare): CDK4, EGFR, PDGFR

Fig. 1. Flow chart showing typical molecular aberrations associated with the initiation and progression of oligodendrogial tumors (modified from ref. 16). The most common early chromosomal changes in WHO grade II oligodendrogliomas are losses on 1p and 19q, which are found in about 80% of the cases. Promoter methylation of the p14ARF, CDKN2A, and/or CDKN2B genes on 9p21 is also frequent in oligodendrogliomas (54). In addition, oligodendrogliomas commonly overexpress the epidermal growth factor receptor (EGFR) as well as platelet-derived growth factors and receptors (PDGF and PDGFR). Malignant progression to anaplastic oligodendroglioma is associated with various chromosomal and genetic alterations, including most notably aberrations in cell cycle regulatory genes, such as CDKN2A, CDKN2B, CDKN2C, and RB1. A minor fraction of anaplastic oligodendrogliomas carries PTEN mutations and/or allelic loss on the long arm of chromosome 10, as well as TP53 mutation. Anaplastic oligodendrogliomas frequently demonstrate overexpression of vascular endothelial growth factor (VEGF). Amplification of proto-oncogenes, such as CDK4, EGF, or PDGFR, is restricted to a small fraction of the cases (<10%).

can be easily demonstrated by immunostaining in the routine diagnostic setting. Another marker, the microtubule-associated protein 2 (Map2), was found to be invariably and strongly expressed in oligodendrogliomas (11, 12). However, Map2 expression is not specific for oligodendrogliomas since it is also found in diffuse astrocytic gliomas and in a fraction of neuronal tumors (11, 12).

The absence of reliable immunohistochemical markers for oligodendrogliomas and the fact that malignant gliomas may show ambiguous histological features of both astrocytic and oligodendrogial lineage underlie the considerable inter-observer variability in the classification of these tumors, even among experienced neuropathologists (13). Furthermore, it appears that the histologic criteria required for the diagnosis of oligodendroglioma and mixed glioma are increasingly used in a more relaxed way in many institutions, possibly because of the desire not to impede any patient from gaining a possible benefit of chemotherapy (14). A less stringent histological classification carries the danger that a substantial fraction of astrocytic gliomas are misclassified as oligodendrogliomas and oligoastrocytomas. We will place a particular focus on translational aspects and provide a few practical guidelines concerning the use of molecular markers for the diagnostic and prognostic assessment of these tumors. It is our hope that a more widespread application of molecular analyses in the routine neuropathological assessment of oligodendrogliomas will improve diagnostic accuracy and provide objective and reproducible information for better prediction of response to therapy and prognosis in individual patients. With respect to clinical trials for gliomas, we propose that the obligatory central pathology should be supplemented by molecular analyses in order to avoid the possibility of unrecognized genetic heterogeneity obscuring an effect of therapy (22).

CHROMOSOMAL AND GENETIC ABERRATIONS IN OLIGODENDROGLIOMAS

Figure 1 summarizes the current knowledge about the most important chromosomal and genetic aberrations that
are associated with the development and progression of oligodendrogliomas. The most common early alterations are allelic deletions on 1p and 19q, with the majority of oligodendrogliomas showing combined losses on both chromosome arms (23, 24). The frequent coincidence of 1p and 19q deletions suggests a synergistic effect of both alterations in conferring a selective growth advantage to oligodendroglioma cells. However, the precise reason why these 2 chromosome arms are almost always lost together, as well as the underlying molecular mechanism, are as yet unknown. The frequency of 1p and 19q deletions is highest in oligodendrogliomas of World Health Organization (WHO) grade II, which show loss of heterozygosity (LOH) at polymorphic loci on 1p and 19q in 80% to 90% of the cases. Allelic losses on 1p and 19q are less common in anaplastic oligodendrogliomas (approximately 50%–70% of the cases), suggesting that the histologically defined group of anaplastic oligodendrogliomas is genetically more heterogeneous than the group of WHO grade II oligodendrogliomas. In the majority of oligodendrogliarial tumors, allelic losses involve all informative loci on 1p and 19q, indicating that all, or almost all, of these chromosome arms have been deleted. Nevertheless, a minor fraction of tumors carries small terminal or interstitial deletions, which have been instrumental in the identification of candidate regions for oligodendroglia-associated tumor suppressor genes.

Candidate Regions and Genes on 1p

So far, deletion mapping studies have identified 3 distinct candidate regions on 1p, which map to 1p34-p35, 1p36.2, and 1p36.3-p32, respectively (25–28) (Fig. 2). The tumor suppressor genes expected to map within these regions are not yet identified. Occasional anaplastic oligodendrogliomas (<5%) carry mutations or homozygous deletions of the cyclin dependent kinase inhibitor gene CDKN2C (p18INK4c) at 1p32 (26, 29, 30). However, the absence of detectable CDKN2C alterations in the vast majority of oligodendrogliomas with 1p loss clearly indicates that this gene is not the major oligodendroglioma suppressor on 1p. Two other candidate genes from 1p, TP73 (1p36.3) and RAD54 (1p32), have also been studied for mutations in oligodendrogliomas but no alterations have been detected in these genes (31–33). More recently, one study reported on promoter hypermethylation and transcriptional silencing of the TP73 gene in nearly 40% of the oligodendroglial tumors (34). However, other authors have found that TP73 hypermethylation is restricted to a small percentage (15%) of anaplastic oligodendrogliomas and is absent in WHO grade II oligodendrogliomas (35). The TP73 gene product, p73, has significant homology to the tumor suppressor protein p53 and might therefore be expected to act as a tumor suppressor, too. In contrast to p53, however, the role of p73 as a tumor suppressor is far from being established (36). Recent studies demonstrated that 2 different transcripts are generated from this gene, one of which is frequently upregulated in cancer and has oncogenic rather than tumor suppressive properties (37, 38). Thus, the available data do not support a major role for TP73 inactivation in oligodendrogliomas.

Candidate Regions and Genes on 19q

Several deletion mapping studies of gliomas have narrowed down candidate tumor suppressor gene regions on 19q. In contrast to 1p, however, the results of these studies are mainly based on data obtained from the analysis of astrocytic gliomas for the following reasons: 1) unlike 1p, allelic losses on 19q are also common in anaplastic astrocytomas and glioblastomas (39, 40), and 2) partial deletions of 19q appear to be more common in astrocytic than in oligodendrogliarial tumors (41). With respect to mapping the glioma suppressor gene or genes on 19q, Smith et al (27, 42) reported on a candidate region at 19q13.3 that maps between the anonymous markers D19S596 and D19S597. This region shares about 150 kb
candidate region on 19q13 is extremely rich in CpG islands, suggesting that many genes may be transcriptionally silenced by CpG island hypermethylation (47). Interestingly, the alleles lost on 19q, but not on 1p, appear to be preferentially of paternal origin (48). Therefore, it is possible that 19q harbors several imprinted genes whose transcription could be silenced in tumor cells when the nonimprinted allele is deleted. The PEG3 gene on 19q13.4 has been reported as a first candidate for a paternally expressed and imprinted gene whose expression is frequently reduced in glioma cell lines (49, 50). PEG3 maps distal to the glioma candidate region between D19S219 and D19S246 at 19q13.3 (47). Nevertheless, introduction of PEG3 cDNA into a glioma cell line resulted in a loss of tumorigenicity in nude mice, suggesting that the Peg3 gene product, a zinc finger containing protein, has tumor suppressor properties (49). Recent data indicate that Peg3 functions as a mediator between p53 and Bax in a neuronal cell death pathway activated by DNA damage and ischemia (51, 52), suggesting that loss of Peg3 possibly contributes to the escape of glioma cells from apoptosis. However, screening of PEG3 for mutations in gliomas has been negative to date (DNL, unpublished data).

Other Aberrations in WHO Grade II Oligodendrogliomas

Studies employing conventional karyotyping, comparative genomic hybridization, and/or loss of heterozygosity (LOH) analysis have revealed that variable fractions of WHO grade II oligodendrogliomas carry numerical or structural aberrations of chromosomes other than 1p and 19q, including deletions affecting chromosomes 4, 6, 11p, 14, and 22q most commonly (16). In contrast to diffuse astrocytomas, LOH on 17p and TP53 gene mutations are rare in oligodendrogliomas (approximately 10%–15% of the cases) (16, 53–56). In fact, combined LOH on 1p and 19q on the one hand and TP53 mutation on the other hand seem to be virtually exclusive alterations (53, 54, 56). In keeping with this finding, WHO grade II oligodendrogliomas with classic histological features and LOH 1p/19q were generally found to lack TP53 mutations (53, 54, 56). Nonetheless, the p53 pathway could be deregulated in oligodendrogliomas, because an important other member of this pathway, p14ARF, is commonly downregulated by epigenetic gene silencing in these tumors (54, 56). The p14ARF protein regulates the activity of p53 by binding to Mdm2 and inhibiting Mdm2-mediated degradation of p53 (57). Thus, the p53 pathway is frequently affected in both diffuse astrocytomas and oligodendrogliomas, with TP53 mutations predominating in astrocytic gliomas and p14ARF hypermethylation in oligodendrogliomas. However, the functional consequences of these distinct alterations are unlikely to be equivalent since p14ARF has functions that are independent from p53 and vice versa (57).

![Diagram of candidate tumor suppressor gene regions on 19q13.3](http://jnen.oxfordjournals.org/)

**Fig. 3.** Candidate tumor suppressor gene regions on 19q13.3 identified in human gliomas (modified from ref. 47). Shown are the commonly deleted regions identified in 5 different studies that performed deletion mappings of 19q in human gliomas: A: Rubio et al (111); B: Yong et al (46); C: Rosenberg et al (41); D: Smith et al (27); E: Smith et al (43). The approximate positions of the 19q markers (D19S219, D19S112, D19S412, D19S606) that were used by Cairncross et al (17) and Ino et al (20) for the correlation of molecular genetic findings with response to therapy and survival time of anaplastic gliomas are indicated in the figure.
Progression-Associated Aberrations in Oligodendrogliomas

The average number of chromosomal and genetic aberrations increases from WHO grade II oligodendrogliomas to anaplastic oligodendrogliomas (23, 58–62). Thus, malignant progression of oligodendrogliomas is associated with the accumulation of multiple genetic abnormalities. The most important progression-associated aberrations identified to date are deletions of chromosome arms 9p and 10q. Such deletions were found in a small percentage of WHO grade II oligodendrogliomas but are much more common in anaplastic tumors. With respect to target genes, the CDKN2A tumor suppressor gene at 9p21 is homozygously deleted in up to one third of the anaplastic oligodendrogliomas (9, 17, 20, 55, 59, 63, 64). Homozygous CDKN2A deletions are particularly common in anaplastic oligodendrogliomas without LOH on 1p and 19q (9, 17, 20), but may also be present in tumors with 1p and 19q loss (55, 59). Most anaplastic oligodendrogliomas with homozygous CDKN2A deletion have additionally lost both copies of the adjacent p14ARF and CDKN2B tumor suppressor genes (64). CDKN2A and CDKN2B encode the p16INK4a and p15INK4b proteins, respectively, which function as regulators of G1/S-phase cell cycle transition by inhibiting the activity of cyclin-dependent kinases Cdk4 and Cdk6 (65, 66). Cdk4 and Cdk6, in turn, phosphorylate the retinoblastoma protein pRB, which is of critical importance for G1/S-phase progression. Similar to findings in malignant astrocytic gliomas (67, 68), the pRB-dependent cell cycle checkpoint is therefore altered in the vast majority of anaplastic oligodendrogliomas, either by homozygous deletion or hypermethylation of CDKN2A and/or CDKN2B, amplification and overexpression of CDK4, or hypermethylation and/or loss of expression of RB1 (54, 64). Thus, loss of the G1/S-phase cell cycle checkpoint is an important step in glioma progression in general, independent of astrocytic or oligodendrogial phenotype.

Less than 10% of anaplastic oligodendrogliomas carry mutations in the PTEN tumor suppressor gene at 10q23.3 (20, 69–71). PTEN mutation and/or 10q loss are preferentially found in anaplastic oligodendrogliomas without LOH on 1p and 19q (9, 17, 20, 70). The observation that LOH on 10q is more frequent in anaplastic oligodendrogliomas than PTEN mutation (71), as well as the finding of 2 oligodendrogliomas with partial deletions on 10q25–q26 that were distal to the PTEN locus (72), suggest that 10q likely carries another, not yet identified oligodendroglioma-related tumor suppressor gene.

In addition to the losses on 1p, 9p, 10q and 19q, several other chromosomes, including chromosomes 4, 6, 7, 11, 13q, 15, 18 and 22q, have been found to be gained or lost at more than random frequency in anaplastic oligodendrogliomas (23, 25, 59–61). In contrast to glioblastomas, only a small subset (<10%) of anaplastic oligodendrogliomas demonstrates amplification of protooncogenes, most commonly affecting the EGFR, PDGFRA, or CDK4 genes (9, 16, 20, 64, 73). Although EGFR gene amplification is rare in anaplastic oligodendrogliomas, EGFR transcripts and protein are overexpressed in more than 50% of WHO grade II and anaplastic oligodendrogliomas (74, 75). Smith et al (73) reported that PDGFRA amplification is specifically associated with highly anaplastic oligodendrogial tumors showing histological features corresponding to WHO grade IV. These authors did not detect any PDGFRA amplification in glioblastomas, which is in contrast to the results of other studies that found PDGFRA amplification in about 8% of glioblastomas as well as in individual gliosarcomas (76, 77). Rare instances of anaplastic oligodendrogliomas with amplification of MDM4, MYC, or MYCN have also been reported (16, 78).

CHROMOSOMAL AND GENETIC ABERRATIONS IN OLIGOASTROCYTOMAS

The chromosomal and genetic alterations in oligoastrocytomas (mixed gliomas) are heterogeneous. Given the differences in diagnostic criteria used to diagnose oligoastrocytomas between different institutions, care must be taken in comparing the results of oligoastrocytoma studies from different centers, and some variation in the reported molecular genetic characteristics of oligoastrocytomas may in fact reflect diagnostic, rather than biological, differences. In general, the genetic alterations in oligoastrocytomas may resemble either the aberrations typically seen in oligodendrogliomas (i.e. LOH on 1p and 19q) or the aberrations characteristically associated with diffuse astrocytic gliomas (i.e. LOH 17p and TP53 mutation) (16). Importantly, there seem to be no specific abnormalities that separate the oligoastrocytomas genetically from the oligodendrogliomas on the one side and the diffuse astrocytomas on the other. Collectively, the available data indicate that about half of the oligoastrocytomas are characterized by allelic losses on 1p and 19q, whereas approximately one third of the cases show LOH on 17p and/or TP53 mutations (18, 54, 79–81). Maintz et al (80) reported that oligoastrocytomas with 1p and 19q loss are histologically oligodendroglia-predominant, whereas oligoastrocytomas with TP53 mutations were more often astrocytoma-predominant. More recent data indicate that not only histological appearance but also tumor location may be associated with different genetic subsets of oligoastrocytomas. According to Müller et al (81), oligoastrocytomas in the temporal lobe less frequently showed LOH on 1p and 19q (33%) than TP53 mutations (45%). In contrast, oligoastrocytomas arising outside the temporal lobe demonstrated LOH on 1p and 19q in nearly 75% of the cases while TP53 mutations were found in less than 20% (81). Based on CGH analyses, 4 distinct genetic subtypes of oligoastrocytomas...
have been proposed: a “−1p/−19q” subtype, a “+7/−10” subtype, an “intermediate” (−1p/−19q plus other chromosomal imbalances) subtype, and a subtype characterized by imbalances of other chromosomes than 1p, 19q, 7 and 10 (“other” subtype) (82). The “+7/−10” group of tumors defined by CGH analysis likely overlaps with the “LOH17p/TP53 mutation” subtype defined by molecular genetic analysis. The “intermediate” CGH subtype consisted mainly of anaplastic oligoastrocytomas that were assumed to have developed by progression from “−1p/−19q” oligoastrocytomas (82).

Concerning clonality of oligoastrocytomas, separate molecular analyses of microdissected oligodendrogial and astrocytic tumor parts have revealed common genetic alterations in both components in the vast majority of cases (79, 83). However, individual oligoastrocytomas displaying different aberrations in the astrocytic and oligodendroglial components have been reported (83). This latter finding may be explained by the evolution of subclones at an early stage of tumor development. Alternatively, rare mixed gliomas could represent collision tumors that originated independently from distinct precursor cells (83), but this possibility seems less likely.

Anaplastic oligoastrocytomas demonstrate a higher number of chromosomal aberrations than WHO grade II oligoastrocytomas (82). Similar to other malignant gliomas, progression-associated changes include losses on 9p and homozygous deletion of CDKN2A, losses on 10, 11p and 13q, as well as amplification of proto-oncogenes, such as EGFR or PDGFRA (23, 73, 82).

**ABERRANT CpG ISLAND METHYLATION IN OLIGODENDROGLIAL TUMORS**

Transcriptional silencing of tumor suppressor genes by hypermethylation of CpG islands in their promoter regions is an important epigenetic mechanism that is involved in the pathogenesis of many human cancers (84, 85). Several recent studies have reported on hypermethylation of tumor suppressor genes and certain other genes in oligodendrogial tumors (54, 56, 64, 83, 86, 87). Genes found to be hypermethylated to date in variable fractions of oligodendrogial tumors include CDKN2A, CDKN2B, p14ARF (all on 9p21), RB1 (13q14), TP73 (1p36.3), DAPK1 (9q34.1), ESR1 (6q25.1), and MGMT (10q26). A considerable percentage of oligodendrogliomas have simultaneously hypermethylated 2 or more of these genes (54, 86). Therefore, one may speculate that oligodendrogliomas carry a general defect in the regulation of DNA methylation, reminiscent of the so-called CpG island methylator phenotype (CIMP) observed in subgroups of colorectal carcinomas and in some other types of epithelial and hematopoietic cancers (88). The molecular mechanisms causing CIMP are unknown at present and it remains to be shown if oligodendrogliomas indeed demonstrate similarly widespread changes in DNA methylation as CIMP-positive colon carcinomas. At least the DNA mismatch repair gene hMLH1, which is frequently hypermethylated in CIMP-positive and microsatellite unstable colon carcinomas (89), is not hypermethylated in oligodendrogliomas (86).

As mentioned above, anaplastic oligodendrogliomas with LOH on 1p respond significantly better to chemotherapy than anaplastic oligodendrogliomas without these alterations (17, 20). In this respect, it may be of interest that the transcription of certain drug resistance genes, such as the DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT) and the glutathione-S-transferase pi (GSTP1) gene, may be silenced by promoter methylation (90, 91). In one study of glioblastomas, MGMT hypermethylation was associated with survival and possibly with response to chemotherapy (92). In addition, oligodendrogliomas showed a lower MGMT expression level on average than diffuse astrocytomas (93). According to Dong et al (86), the MGMT promoter is hypermethylated in 60% of oligodendrogliomas and hypermethylation correlates with combined LOH on 1p and 19q. Other authors, however, detected MGMT hypermethylation in equal percentages of low-grade diffuse astrocytomas and oligodendrogliomas, without showing an association with allelic loss on 1p and 19q (56). Taken together, the role of MGMT silencing as a mechanism contributing to the chemoresponsive phenotype of oligodendrogliomas is therefore still uncertain and needs to be addressed in more detail. Transcriptional silencing of the GSTP1 does not seem to play a major role in oligodendrogliomas since the GSTP1 promoter was found consistently unmethylated in these tumors (86).

**MICROARRAY-BASED EXPRESSION PROFILING OF OLIGODENDROGLIAL TUMORS**

Gene expression profiling using microarray-based technologies permits the simultaneous analysis of large numbers of genes and has been successfully used both for the identification of novel candidate genes as well as for the categorization of human cancers into clinically relevant subgroups (94, 95). With respect to primary brain tumors, microarray analyses have revealed distinct gene expression pattern in different types of gliomas (96), as well as in gliomas of the same lineage but of different WHO grade (97–98). Furthermore, gene expression profiles identified by oligonucleotide-based microarray analyses allowed a molecular differential diagnosis of the major types of pediatric central nervous system tumors (99). The latter study additionally identified expression profiles that were highly predictive of the outcome of medulloblastoma patients.

With respect to oligodendrogial neoplasms, microarray analysis of 4 low-grade oligodendrogliomas of WHO grade II and 3 anaplastic oligodendrogliomas of WHO
grade III revealed that expression information for approximately 1,100 genes allowed the tumors to be exactly divided according to histological grade (98). Statistical analysis further identified a subset of 196 genes that were differentially expressed between the WHO grade II and WHO grade III oligodendrogliomas (98). More recently, Mukasa et al (100) compared the expression profiles of more than 12,000 genes and expressed sequence tags in 6 oligodendrogliomas with LOH on 1p versus 5 oligodendrogliomas without LOH on 1p. The tumors with LOH on 1p displayed expression profiles more closely related to non-neoplastic brain tissue. A total of 209 genes was reported to be differentially expressed between oligodendrogliomas with and without 1p loss, including 86 genes showing higher expression in tumors with 1p loss and 123 genes showing lower expression. Based on the expression of these 209 genes, oligodendrogliomas with and without LOH on 1p could be clearly distinguished from each other, indicating that the 1p status is reflected in distinct expression profiles. Interestingly, from the 123 genes downregulated in tumors with 1p loss, 50% mapped to chromosome 1, and 10% to chromosome 19. This finding would be in line with the hypothesis that haploinsufficiency of multiple genes on 1p and 19q may provide a selective growth advantage to oligodendroglioma cells. Unfortunately, the expression data did not provide a direct hint towards any specific gene or genes whose differential expression is responsible for the chemoresistance of the tumors with intact 1p. Nevertheless, we anticipate that large-scale microarray-based profiling of gliomas will soon result in the identification of expression patterns that provide useful information for routine diagnostic and prognostic purposes. Eventually, these expression profiles will help to identify and verify a set of novel markers, from which specific ones can be selected to solve individual diagnostic problems, ideally by means of conventional immunohistochemical analysis.

CORRELATION OF GENETIC ABERRATIONS WITH TUMOR LOCATION

Two recent studies have addressed the question whether oligodendrogliomas and oligoastrocytomas growing in different regions of the brain might vary with respect to their genetic alterations (81, 101). In a study of 64 patients with anaplastic oligodendrogliomas, Zlatescu et al (101) observed that the tumor genotype, i.e., allelic loss on 1p and 19q versus no allelic loss on 1p, was related to the tumor location and the extent of tumor spread in the brain. Anaplastic oligodendrogliomas located in the frontal, parietal, and occipital lobes demonstrated significantly more frequent LOH on 1p than anaplastic oligodendrogliomas in the temporal lobe, insula, and diencephalon. Loss on 1p and 19q was additionally found to be associated with bilateral tumor growth. These findings help to explain the clinical observation that anaplastic oligodendrogliomas in the temporal lobe, insula, and diencephalon respond less commonly to chemotherapy than anaplastic oligodendrogliomas in the frontal, parietal, and occipital lobes (101). In addition, the high frequency of LOH on 1p and 19q in frontal anaplastic oligodendrogliomas, including all investigated tumors with bifrontal tumor spread, may be related to the reported better outcome of patients with frontal oligodendrogliomas (102).

Müller et al (81) studied a large series of gliomas from 203 patients, including 73 oligodendrogliomas, 68 oligoastrocytomas, and 62 astrocytomas, for LOH on 1p and 19q as well as TP53 mutation. Correlation of the molecular findings with tumor location confirmed the findings reported before for anaplastic oligodendrogliomas (101). However, Müller et al (81) additionally found that not only oligodendrogliomas but also oligoastrocytomas demonstrated significantly less common LOH on 1p and 19q when growing in the temporal lobe as compared to other tumor locations. Furthermore, TP53 mutations were significantly more common in temporal oligoastrocytomas than in oligoastrocytomas in other cerebral lobes. In contrast to the oligodendroglial tumors, the authors detected no association between tumor genotype and location for diffuse astrocytomas.

CORRELATION OF GENETIC ALTERATIONS WITH RESPONSE TO THERAPY AND SURVIVAL

Response to PCV Chemotherapy Correlates with 1p and 1p/19q Status in Patients with Anaplastic Oligodendrogliomas

The frequent response of anaplastic oligodendrogliomas to PCV chemotherapy and the ability to measure such responses by neuroimaging provided a unique setting in which to evaluate the utility of molecular subtyping in neuro-oncology. In 1998, Cairncross et al (17) reported that allelic loss of 1p or combined allelic losses of 1p and 19q, as determined by LOH analyses, were powerful predictors of chemosensitivity. In a series of 39 high-grade oligodendrogliomas, some newly diagnosed and some recurrent, all 24 tumors with 1p loss responded to chemotherapy, and 24 of 27 chemosensitive tumors had 1p loss; conversely, all 9 chemoresistant tumors that could be evaluated for 1p status had retained both copies of 1p (17). In addition, although 19q status alone did not correlate with chemotherapy response, combined 1p/19q LOH was closely associated with chemosensitivity. In 2001, the same group reported further analysis of a larger group of more homogeneous tumors: 50 anaplastic oligodendrogliomas sampled at the time of diagnosis and treated in a relatively uniform manner (20). The strong association between PCV chemosensitivity and 1p or 1p/19q LOH was confirmed; in this series, 21 of 21 newly diagnosed anaplastic oligodendrogliomas with 1p loss...
Fig. 4. Flow chart showing typical molecular aberrations associated with the initiation and progression of oligoastrocytomas (modified from ref. 16). The available data indicate that there are distinct genetic subtypes of oligoastrocytomas (80, 81). Oligoastrocytomas with 1p/19 loss are preferentially found in nontemporal locations and tend to be histologically oligodendroglioma-predominant. In contrast, oligoastrocytomas with 17p loss and/or \( \text{TP53} \) mutation frequently develop in the temporal lobe and tend to be histologically astrocytoma-predominant. Progression to anaplastic oligoastrocytoma is accomplished by similar genetic aberrations as in “pure” astrocytic or oligodendrogial tumor, including most notably allelic loss on 9p and/or homozygous deletion of \( \text{CDKN2A} \), \( \text{p14}^\text{ARF} \), and \( \text{CDKN2B} \), as well as allelic loss on 10q and/or \( \text{PTEN} \) mutation. Rare cases of anaplastic oligoastrocytomas show amplification of proto-oncogenes.

Fig. 5. Molecular subgroups of anaplastic oligodendrogliomas: correlation with patient age at diagnosis, tumor location, neuroimaging characteristics, frequency, and duration of response to chemotherapy, and survival time after diagnosis (adapted from refs. 20 and 103).
1p/19q loss were alive, whereas 27% of the remaining patients had died. In combination, these 3 studies, reported from 2 different groups and using 2 different detection methods, have clearly illustrated a powerful association between 1p/19q status and survival in high-grade, and possibly low-grade (see below), oligodendrogliomas.

Response to Other Therapies and 1p/19q Status in Patients with Oligodendrogliomas

Preliminary evidence suggests that 1p/19q loss may indicate a greater likelihood of benefit from a variety of therapies, rather than a specific sensitivity to the PCV regimen. Recent data suggest that oligodendrogliomas may respond to temozolomide, a new oral alkylating agent (5, 6), and anecdotal reports have raised the possibility that 1p/19q status could correlate with sensitivity to this agent (unpublished data). With respect to radiation treatment, Bauman et al (19) evaluated 1p LOH status in 55 patients with WHO grade II (n = 19) and grade III (n = 36) oligodendrogliomas who were treated with radiotherapy; notably, some patients had received only radiation therapy at diagnosis, with chemotherapy given at recurrence. The median progression-free survival was 55 months for patients whose tumors had 1p loss and only 6 months for those patients whose tumors had both copies of 1p. These data raise the possibility that tumors with 1p loss may have more durable responses to radiation therapy. Alternatively, the longer time to recurrence could represent a more indolent growth potential of 1p-deleted oligodendrogliomas. In this regard, it would be of considerable interest to evaluate 1p status and time to recurrence in a series of WHO grade II oligodendrogliomas treated with surgery and close follow-up alone.

WHO Grade II Oligodendroglioma Behavior and 1p/19q Status

Grade is an important determinant of survival in oligodendrogial tumors. Within anaplastic oligodendroglialomas, as discussed above, molecular subtyping is a powerful predictor of therapeutic response and survival. For WHO grade II oligodendroglialomas, the data are less clear, largely because fewer studies have specifically focused on lower grade tumors. Furthermore, such tumors are more difficult to study, since they rarely show dramatic radiological responses to therapy and require long follow-up times. In addition, LOH on 1p and 19q is so common in WHO grade II oligodendrogliomas that it may be difficult to recruit a larger number of patients with pure WHO grade II oligodendroglioma that lacks 1p/19q loss. Smith et al (18) included 26 low-grade oligodendrogliomas in their combined group of 36 primary tumors. Although their results did not reach statistical significance when analyzing the low-grade tumors alone for survival differences based on 1p/19q loss, the overall results, given the preponderance of low-grade lesions in the group, suggest that 1p/19q status could correlate with survival if larger groups of WHO grade II oligodendrogliomas were available for study. Sasaki et al (15) evaluated 1p status in a series of 44 WHO grade II gliomas that had been diagnosed as “oligodendrogliomas” by referral pathologists. Fourteen of the 44 cases had been treated with chemotherapy at the time of clinical or radiological tumor progression, with 13 being evaluable for radiological response. Of these, 10 of 11 cases with 1p LOH had responses to PCV whereas neither case that maintained both copies of 1p had responses. Although based on small numbers of cases, these results raise the hope that tumor genotype could predict chemosensitivity in the setting of recurrent tumors that were initially diagnosed as low-grade lesions. Thus, 1p and 1p/19q status could be relevant determinants of behavior in WHO grade II oligodendrogliomas as well.

Possible Genetic Markers of Poor Prognosis in Oligodendrogliomas

A number of genetic alterations have been correlated with poorer response to chemotherapy or worse overall survival in anaplastic oligodendroglialomas. For most of these markers, the associations are less robust and based on far fewer cases than the positive associations between 1p or 1p/19q loss and therapeutic response and survival. Cairncross et al (17) had initially shown an inverse association between patient survival and the presence of homozygous CDKN2A deletions. The subsequent studies by Ino et al (20) suggested that a variety of relatively infrequent genetic alterations (EGFR gene amplification, 10q loss, CDKN2A homozygous deletion, PTEN mutation, and TP53 mutation) were associated with worse prognosis. Interestingly, TP53 mutation was associated with an improved likelihood of chemotherapeutic response but with a poor overall prognosis, since responses were not durable in the setting of TP53 mutation. Sasaki et al (71) subsequently demonstrated that PTEN mutation, although uncommon, was an independent predictor of poor overall prognosis in anaplastic oligodendrogliomas. Recently, Iuchi et al (28) studied 46 oligodendrogial tumors for LOH at 18 1p markers. Six tumors showed LOH patterns suggestive of interstitial deletions affecting the more proximal 1p34 region. The few patients whose tumors had such interstitial deletion patterns had short survivals. At the present time, however, the data on these individual markers of poor prognosis must be viewed as preliminary and determination of their clinical utility awaits further study of larger numbers of cases.

The Biological Basis for the Differential Behavior of 1p- and 1p/19q-Deleted Oligodendrogliomas

The associations outlined above have raised numerous questions about the mechanisms by which such allelic
losses might underlie chemotherapeutic sensitivity. As discussed above, 1p and 19q loss correlates with other clinical and biological parameters, such as tumor location and spread, recurrence-free survival and overall survival, and possibly response to other therapies. The many associations strongly suggest that those oligodendrogliomas with 1p or 1p/19q loss are distinct entities that are different in most ways from histologically similar oligodendroglial tumors, and raise the intriguing hypothesis that these distinct lesions arise in different cellular precursors that are restricted in their genotypic repertoire (101, 103). Unfortunately, the specific genes unmasked by 1p and 19q loss in oligodendrogliomas remain unknown. The identification of the 1p and 19q glioma genes will be the first step toward understanding the biological basis for the markedly different biological behavior of oligodendrogliomas with 1p and 19q loss. For now, it is sufficient to postulate that 1p/19q-deleted tumors are simply “different beasts”; besides microscopic similarities, they share little in common with other histologically defined oligodendrogliomas.

1p and 19q Loss in Oligoastrocytomas and Diffuse Astrocytomas

As noted above, 1p and 19q loss occurs in a considerable percentage of oligoastrocytomas and in occasional “pure” astrocytic tumors. The clinical significance of 1p/19q status in astrocytomas and astrocytomas, however, remains uncertain. Smith et al (18) examined the prognostic relevance of 1p and 19q loss in a series of 162 diffuse gliomas that included 31 oligoastrocytomas and 79 astrocytomas. While this study confirmed the correlation between 1p/19q loss and survival in oligodendrogliomas, 1p/19q loss did not correlate with survival in oligoastrocytomas or astrocytomas. On the other hand, Schmidt et al (104) found that combined 1p/19q losses were associated with modestly improved prognosis in 5 tumors with combined 1p/19q loss in a series of 73 glioblastomas; patients whose tumors had these alterations had a median survival of 22 months, compared with about 11 months in other patients. Furthermore, Ino et al (105) showed that occasional patients with 1p-deleted astrocytic tumors had good responses to therapy or long survivals. The last 2 studies, however, were based on few cases with 1p alterations, and have to be considered preliminary. For all of these studies, the significant variability in neuropathological diagnosis of oligoastrocytoma must also be taken into account, and complicates comparing reports and generalizing results. Conclusions about the clinical relevance of 1p/19q status in astrocytomas and oligoastrocytomas thus await further study and, if present, will clearly not be as robust as those already demonstrated for pure oligodendroglial tumors.

RECOMMENDATIONS FOR THE MOLECULAR DIAGNOSTIC TESTING OF OLIGODENDROGLIAL TUMORS

Clear distinctions must be drawn between research laboratory analyses performed for investigational purposes and clinical laboratory testing that reports genetic results back to patients and their treating oncologists. While 1p/19q analysis may be of interest in many research situations, we currently recommend diagnostic testing for 1p and 19q loss in only 3 clinical settings: 1) after a diagnosis of anaplastic oligodendroglioma; 2) for a small cell malignant glioma in which the differential diagnosis is anaplastic oligodendroglioma versus small cell glioblastoma; and 3) after a diagnosis of WHO grade II oligodendroglioma. Significantly, we do not believe that, at the present time, 1p/19q loss can be used as an absolute diagnostic criterion for the diagnosis of oligodendroglioma; that is, we do not recommend 1p/19q testing to “rule in” or “rule out” a diagnosis of oligodendroglioma.

Anaplastic Oligodendroglioma

The data indicate strong relationships between 1p/19q status and both chemosensitivity and survival in anaplastic oligodendrogliomas, as discussed above. The information relating to therapeutic decisions at the time of diagnosis is primarily derived from the study of Ino et al (20). It should be borne in mind that this was a single retrospective study of 50 patients, and that larger, prospective studies are clearly needed. The authors found that newly diagnosed anaplastic oligodendrogliomas could be divided into 4 genetic subgroups, based on the analysis of 1p, 19q, 10q, EGFR, CDKN2A, PTEN, and TP53 (Fig. 5). Groups 1 and 4 comprise markedly different patient groups, and therapeutic recommendations for these 2 subgroups may differ from the standard therapeutic combination of PCV and radiation. For example, tumors in group 1, whose genotype is combined 1p/19q loss without the other genetic changes, always respond to PCV, often have extensive diminution of tumor burden, frequently have durable responses, and have median survivals of over 10 years. Group 1 patients should therefore be treated with PCV at the time of diagnosis. A cogent argument could also be made to spare initial radiation therapy in patients with extensive cerebral disease, and reserve radiation until the time of recurrence. Given the potential neurotoxicity of cerebral radiation, deferring such therapy in these patients has the potential to improve quality of life; group 1 patients are expected to have long survivals and the presence of extensive disease would necessitate large radiation fields—both features that would increase the likelihood of developing problems relating to radiation toxicity. On the other side of the spectrum are group 4 tumors that have multiple genetic...
malignant glioma would alert the clinician to a potentially ominous prognosis.

WHO Grade II Oligodendrogliomas

The clinical implications of 1p/19q loss in WHO grade II oligodendrogliomas are based on few studies and few cases, but hint that tumors with 1p/19q loss are associated with longer survival times and greater likelihood of response to chemotherapy at the time of recurrence. Patients have begun to request testing for 1p/19q loss on WHO grade II oligodendrogliomas for a number of reasons. One, it is hoped that the presence of 1p/19q loss will provide additional reassurance that a particular tumor can be closely followed rather than treated with either chemotherapy or radiation at the time of presentation. Two, patients may wish to know the 1p/19q status of their tumors in order to make therapeutic decisions at a future date, but suspect that it will be easier to get such testing done while the blocks are readily available. These assumptions are reasonable and we have therefore agreed to test for 1p/19q status in WHO grade II oligodendrogliomas.

PRACTICAL ISSUES FOR THE MOLECULAR DIAGNOSTIC TESTING OF OLIGODENDROGLIAL TUMORS

Testing for 1p and 19q loss has been established at approximately 10 academic centers across the United States, Canada, and at a handful of institutions in Europe. The spread of diagnostic testing has raised many practical issues regarding these assays, and some of these problems are discussed below.

Recommended Methods for 1p/19q Testing

The 2 major techniques for assessing 1p and 19q status are LOH and FISH assays. LOH testing has some advantages: it is easy to interpret and it detects all allelic losses, including mitotic recombination events leading to uniparental disomy. On the other hand, LOH has the disadvantages of requiring many specialized steps (DNA extraction, PCR, electrophoretic separation) and requiring blood DNA. In a practical sense, this latter requirement is quite problematic, since it necessitates separate acquisition of blood from the patient and therefore requires coordination by a clinician, rather than having a pathologist being able to initiate the testing. FISH has the advantages of involving few steps, of providing in situ information, and of not requiring a separate blood specimen. But FISH has disadvantages as well: it is more difficult than PCR to optimize new assays; it is more time-consuming to score the results; and it fails to detect allelic losses that involve mitotic recombination. Fortunately, as opposed to LOH of 17p in astrocytomas (107), mitotic recombination events affecting 1p and 19q have
not been reported, to our knowledge, in oligodendrogliomas. Smith et al (27) compared the sensitivity of LOH and FISH to detect 1p and 19q loss in gliomas and demonstrated close correlations between LOH and FISH detection of losses, particularly for 1p. In general, preferences for using LOH or FISH are based more on the availability of particular assays at individual institutions than clear superiority of either modality. Some molecular diagnostic facilities are more comfortable with PCR-based approaches, while others have advanced FISH laboratories. Concerning the use of LOH analysis, we would recommend that clinical testing and future research studies of 1p/19q loss should include the same polymorphic microsatellite markers on 1p that were used in the 2 papers demonstrating correlations with chemoresponse and survival: D1S508 (1p36.23), D1S199 (1p36.13), and D1S2734 (1p36.11) (17, 20). The experience at Massachusetts General Hospital shows that use of these 3 polymorphic markers provides informative results in essentially all studied patients. At this point, these markers have shown robust associations with clinical parameters. However, as the definition of candidate gene regions on 1p proceeds, it may become necessary to supplement these markers with additional loci specifically mapping to the region(s) of interest. The choice of 19q markers should target the minimally deleted region on 19q, using the markers previously studied, i.e. D19S219, D19S112, D19S412, and D19S596 (17, 20), or novel, practical polymorphisms such as PLA2G4C (108). Similarly, clinical testing and future research studies of 1p/19q loss that employ FISH should use probes that map to the commonly deleted regions assessed in the studies demonstrating correlations with survival and the close correlations between FISH and LOH (18, 27).

Other approaches have also been suggested as alternatives to LOH and FISH. Comparative genomic hybridization (CGH) is a less widely available technique, with relatively few centers having this expertise, and may have less sensitivity for detecting 1p and 19q loss (27). On the other hand, the advent of array-CGH may make comparative hybridization approaches more practical and more widely available. Quantitative PCR approaches, most notably quantitative microsatellite analysis (QuMA), have been proposed as a powerful alternative to FISH or LOH since they offer higher throughput than standard LOH assays and eliminate the need for polymorphic markers and for normal blood DNA (109). However, these assays may be difficult to optimize and are dependent on the availability of rather expensive quantitative PCR machines.

Centers for 1p/19q Testing

Currently, clinical 1p/19q evaluations are being performed at relatively few institutions worldwide. For the next decade, it is unlikely that routine molecular testing will become available in community pathology laboratories. As a result, there is a need for such testing to be offered at various large, well-equipped laboratories to a wide variety of outside hospitals. This necessitates a uniform approach to tissue preservation and shipments. Ideally, frozen tumor tissue should be used for molecular analysis because this allows for the extraction of high-quality DNA that will work well with all available techniques. Such frozen tissue should be mailed on dry ice by express courier. However, we realize that in many, if not most, instances frozen tumor material is not available and that use of unstained slides of formalin-fixed, paraffin-embedded tissues has the advantages of universal availability and ease of transport. Fortunately, LOH and FISH can be readily performed on archival tissues. In our experience using LOH as a primary diagnostic modality, we have encountered only rare cases from which PCR-quality DNA could be extracted from fixed, embedded tissues; these rare tumors have then been amenable to FISH analysis. A recent retrospective FISH study of archival specimens collected as part of a large national trial showed failure of FISH in only 2 of 52 hybridizations, both with a 19q probe (110). Thus, the available approaches do not necessarily require special processing of tissues and can be applied to small quantities of routinely prepared pathological materials. For standard LOH analyses, the requirement for constitutional DNA also requires a blood sample. We have found that prompt extraction of DNA enhances yield and therefore encourage transport of blood by express courier on Monday through Wednesday, to avoid allowing the blood to sit for longer periods of time such as weekends, with blood packed in a cold pack. We request a single 10 cc blood sample in a test tube containing anticoagulant, e.g. a yellow-topped tube which contains the anticoagulant labeled “ACD.”

For clinical 1p and 19q LOH testing at Massachusetts General Hospital—3 polymorphic markers on each chromosomal arm—the charge is $800. It is estimated that the cost of FISH analysis using probes to 1p and 19q, coupled with control probes, would be similar. At Massachusetts General Hospital, we request payment in advance from the patient. In our experience, this cost has not dissuaded patients from testing and we have performed testing gratis for patients who have lacked the necessary financial resources. Patients in both the United States and Canada have reportedly been reimbursed for the cost of the testing, but we do not have data on the percentage of patients who have received reimbursement from their insurance carriers. It is likely that centralization of the molecular testing could facilitate interactions with insurance companies and thereby increase patient reimbursement for these increasingly requested clinical assays.
CONCLUSIONS AND PERSPECTIVES

The essential goal of oligodendroglioma classification is to distinguish 3 groups of patients: 1) patients whose tumors will follow an indolent course and therefore do not yet require any adjuvant treatment; 2) patients with anaplastic tumors that will respond favorably to chemotherapy; and 3) patients with anaplastic tumors that will not respond well to the currently available therapies. In addition to conventional histological and immunohistochecmical analyses, molecular genetic analysis may provide useful information for guiding clinical decisions, primarily by evaluation of 1p and 19q allelic status. As a result of these advances, clinical decisions in the management of oligodendrogial tumors are now influenced by a combined armamentarium that includes histological findings, proliferation indices, neuroimaging features, patient symptoms, and genetic characteristics.

Thus, oligodendrogliomas represent a “test case” in neuro-oncology that challenges our notion of how neuropathologists classify tumors. Pathology has remained strongly anchored in microscopy-based classification for much of the past century. Over the past decade, many studies in different human tumor types have shown that molecular analyses can augment standard pathology, with oligodendroglioma being just one example. The next question is whether molecular analyses will eventually supplant or override standard pathological definitions, as in the case of specific, defining translocations in certain hematological malignancies and soft tissue sarcomas. For diffuse gliomas comprised of rounded cells, the issue will be whether 1p/19q loss will be required for the diagnosis of “oligodendroglioma.” Given the almost certain reliance of pathology on standard microscopic examination for the next decade, it seems unlikely that molecular definitions will soon replace histological glioma classification. The emerging oligodendroglioma story nonetheless opens the real possibility that the traditional, subjective microscopic approach will yield ground to objective genotyping in the not-too-distant future.

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