Molecular Diagnostics of Gliomas
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Context.—Gliomas are the most common primary brain tumors of adults and include a variety of histologic types and morphologies. Histologic evaluation remains the gold standard for glioma diagnosis; however, diagnostic difficulty may arise from tumor heterogeneity, overlapping morphologic features, and tumor sampling. Recently, our knowledge about the genetics of these tumors has expanded, and new molecular markers have been developed. Some of these markers have shown diagnostic value, whereas others are useful prognosticators for patient survival and therapeutic response.

Objective.—To review the most clinically useful molecular markers and their detection techniques in gliomas.

Data Sources.—Review of the pertinent literature and personal experience with the molecular testing in gliomas.

Gliomas in adults are the most common primary brain tumors and include a variety of histologic types. Currently, the histologic identification is based on the morphologic resemblance of the tumor cells to nonneoplastic glial cells (ie, astrocytes, oligodendrocytes, and ependymal cells), and, therefore, most malignant gliomas of adults are classified into astrocytic, oligodendrogial, mixed oligoastrocytic, and ependymal tumors, according to the 2007 World Health Organization (WHO) classification.

Molecular testing of ependymomas is of limited value and will not be discussed in this review. In adults, the most important types of gliomas are those that diffusely infiltrate the surrounding brain tissue, making these “diffuse gliomas” resistant to surgical resection. An exception is the pilocytic astrocytoma (WHO grade I), the most common pediatric glioma, which is relatively well demarcated from the surrounding tissues and can be resected. Diffuse gliomas are categorized into low-grade gliomas (WHO grade II), which usually demonstrate relatively slow growth, and high-grade gliomas (WHO grades III and IV), which grow more rapidly. The most malignant (and most common) of the high-grade gliomas is glioblastoma multiforme (GBM). Primary GBMs arise de novo in older patients and have a short duration of clinical symptoms (<3 months). On the other hand, secondary GBMs develop from preceding grade II or III gliomas, have a longer duration of symptoms, and frequently develop in patients younger than 40 years. During the past decade, understanding of gliomagenesis has expanded significantly. Current evidence suggests that the initiation and progression of gliomas may involve the accumulation of multiple genetic alterations. For example, isocitrate dehydrogenase (IDH) 1 and IDH2 mutations are identified in most low-grade gliomas, suggesting that IDH mutations are an early event in gliomagenesis. Other genetic abnormalities may accumulate during tumor progression and include 1p/19q codeletion in oligodendrogial tumors and TP53 mutation or 17p13 loss in astrocytic tumors. Oligoastrocytomas may exhibit any of these genetic abnormalities (Figure 1). Anaplastic gliomas are characterized by loss of 9p and homozygous deletion of the CDKN2A/B gene (p16/p15), and the progression of an anaplastic astrocytoma to a secondary GBM is frequently associated with 10q loss. Primary and secondary GBMs have a different subset of genetic abnormalities. In particular, primary glioblastomas demonstrate frequent epidermal growth factor receptor (EGFR) amplification, 10q loss, and mutations in the phosphatase and tensin homolog (PTEN) gene, but rarely have IDH mutations. On the other hand, secondary glioblastomas lack EGFR amplification but show mutations in the TP53 and IDH genes (Figure 1). Most of pilocytic astrocytomas are characterized by BRAF fusion (BRAF/KIAA1549) but rarely have other genetic abnormalities (Figure 1).

Although histologic evaluation remains the gold standard for glioma diagnosis, diagnostic difficulty may arise from tumor heterogeneity, overlapping morphologic features, and tumor sampling. Malignant gliomas are incurable, and most patients die despite aggressive

Conclusions.—This article provides an overview of the most common molecular markers in neurooncology, including 1p/19q codeletion in oligodendrogial tumors, mutations in the isocitrate dehydrogenase 1 and 2 genes in diffuse gliomas, hypermethylation of the O’-methylguanine-DNA methyltransferase gene promoter in glioblastomas and anaplastic gliomas, alterations in the epidermal growth factor receptor and phosphatase and tensin homolog genes in high-grade gliomas, as well as BRAF alterations in pilocytic astrocytomas. Molecular testing of gliomas is increasingly used in routine clinical practice and requires that neuropathologists be familiar with these genetic markers and the molecular diagnostic techniques for their detection.

(Arch Pathol Lab Med. 2011;135:558–568)
therapy. Advances in neurosurgery, radiation, and chemotherapy during the past decade have provided only small improvements in clinical outcome. As with many other tumors, the development of new molecular markers is expected to lead to improved diagnosis and prognosis and to aid in the clinical management of gliomas. Currently, the most common molecular markers in neurooncology are 1p/19q codeletion in oligodendroglial tumors, mutations in the IDH1/2 genes in diffuse gliomas, hypermethylation of the O6-methylguanine-DNA methyltransferase (MGMT) gene promoter in glioblastomas and anaplastic gliomas, alterations in the EGFR and PTEN genes, and 10q deletions in GBMs, as well as BRAF alterations in pilocytic astrocytomas. Some of these markers can be used diagnostically to help the neuropathologist in glioma classification and grading, especially for tumors with ambiguous histologic features. Alternatively, some of them can be used to estimate prognosis for patients and to predict response to certain therapies.

**1p AND 19q CODELETION**

Loss of the short arm of chromosome 1 (1p), along with the long arm of chromosome 19 (19q), is an established genetic marker of oligodendrogial tumors. The frequency of 1p and 19q codeletion has been reported to be 80% to 90% in oligodendrogliomas (WHO grade II), 60% in anaplastic oligodendrogliomas (WHO grade III), and 30% to 50% in oligoastrocytomas (Table 1).

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>WHO Grade</th>
<th>BRAF Fusion, %</th>
<th>1p/19q Deletion, %</th>
<th>IDH Mutations, %</th>
<th>MGMT Methylation, %</th>
<th>10q LOH/PTEN Mutation, %</th>
<th>EGFR/EGFRVIII, %</th>
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<td>II</td>
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Figure 1. Molecular pathways and common genetic alterations in astrocytic, oligodendroglial, and oligoastrocytic neoplasms. Abbreviations: ampl., amplification; BRAF, v-raf murine sarcoma viral oncogene homolog B1 gene; CDKN2A/B, cyclin-dependent kinase inhibitors 2A and 2B genes; EGFR, epidermal growth factor receptor gene; GBM, glioblastoma multiforme; IDH, isocitrate dehydrogenase gene; mut., mutation; PTEN, phosphatase and tensin homolog gene; TP53, tumor protein p53 gene; WHO, World Health Organization.
sitivity. In 1998, Cairncross and colleagues\(^4\) reported that loss of 1p (and combined loss of 1p and 19q) predicts a better response to procarbazine-lomustine-vincristine chemotherapy and a longer survival in patients with anaplastic oligodendroglioma. Since the first report, these findings have been reproduced in subsequent studies, including prospective, randomized phase 3 trials, which confirmed the prognostic and predictive utility of 1p/19q codeletion.\(^{11,12}\) Moreover, oligodendrogial tumors with loss of 1p/19q showed a response to treatment with the alkylating drug temozolomide and radiotherapy, indicating its predictive value for a broader spectrum of therapeutic regimens.\(^{13-15}\) As a result, 1p/19q codeletion has become a useful diagnostic, prognostic, and predictive marker and is widely used in neurooncology practice.

One of the limitations of this marker is its inability to guide the choice of a specific treatment regimen (chemotherapy versus radiotherapy). Also, the concurrent presence of prognostically unfavorable genomic alterations (ie, 9p or 10q loss) may lead to poor outcome independent of the 1p/19q status.\(^{16}\) Therefore, molecular testing for a wider spectrum of genetic abnormalities is needed for a more accurate prognostic assessment. Furthermore, the prognostic significance of the 1p/19q deletion might be affected by the deletion size. The typical 1p/19q codeletion in oligodendrogliomas shows complete loss of 1p and 19q chromosomal arms, in contrast with the partial deletions frequently observed in astrocytic tumors (mostly in GBMs).\(^{17}\) Partial loss of chromosome 1p in oligodendrogliomas has an opposite prognostic significance when compared with tumors that have a complete 1p/19q loss.\(^{37}\) Evaluation of the whole 1p arm is needed to discriminate between a partial and complete loss of 1p and to appreciate the real clinical significance of this marker.

Assessment of the 1p/19q deletion in oligodendrogliomas is most frequently performed by fluorescent in situ hybridization (FISH) and loss of heterozygosity (LOH) analysis (Figure 2, A, C, and D); however, newer techniques, such as array comparative genomic hybridization and single nucleotide polymorphism array, are becoming available for clinical use. The choice of technique depends on laboratory expertise, equipment available for testing, and the pathologist’s preferences.

The FISH technique uses fluorescently labeled DNA probes to provide a targeted approach for detecting (directly on a tissue slide) chromosomal abnormalities in interphase nuclei. The FISH approach does not require corresponding normal tissue or blood as a control and allows for the preservation of tissue architecture. It can be used for assessment of polysomy, which was recently shown to be a predictor of earlier recurrence in anaplastic oligodendrogliomas with 1p/19q loss.\(^{18}\) For accurate FISH diagnostics, appropriate controls and scoring criteria have to be used, especially for testing in formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The threshold value for positive results has to be established to avoid overinterpretation of small cell populations, where signals are lost because of the truncation of nuclei.\(^{19}\) However, FISH may produce a false-negative result if there is uniparental disomy, that is, when cancer cells have lost one chromosome in the presence of duplication of another chromosomal allele. Also, FISH does not allow testing for multiple chromosomal loci. An example of FISH for chromosome 1p is illustrated in Figure 2, C. Commercially available kits consist of one probe complementary to the chromosomal region on 1p36.32 and a differently labeled control probe that targets the 1q25 region on the long arm of chromosome 1. When the 1p deletion is present, there will be only one 1p36 signal but 2 signals corresponding to the control probes.

An alternative to FISH is a polymerase chain reaction (PCR)–based approach that uses the amplification of microsatellite repeats located in the area of the deletion (Figure 2, B). For LOH detection, primers are designed to flank the microsatellite region. The PCR amplification of tumor DNA and normal tissue DNA is performed, and PCR products are subjected to capillary gel electrophoresis (Figure 2, D). The PCR products from normal tissue (usually blood) are used to determine whether a given patient is heterozygous for that locus (ie, has 2 alleles of different size) and is, therefore, informative for LOH analysis. If the locus is informative, the LOH can be determined as either a complete absence or a significant decrease in amplification of 1 of the 2 alleles and calculated based on the difference in ratios of 2 allelic peaks in normal tissue compared with the same peaks in tumor tissue. In most cases, an allele ratio within the range of less than 0.5 and greater than 2.0 is considered evidence of LOH. For 1p and 19q LOH analysis, several microsatellite loci are used to assess the deleted region. Use of multiple markers in the 1p and 19q arms helps avoid false-positive cases that have only partial deletion of 1p or 19q. However, there are limitations to this technique: LOH analysis requires a control (blood or normal tissue), it may produce a “pseudo-LOH” pattern because of chromosomal copy number gain rather than loss, and it has limited use in the detection of homozygous deletions.

Newer techniques, such as comparative genomic hybridization array and single nucleotide polymorphism array, enable evaluation of the whole genome for chromosomal gains, losses, and polysomy in a single run. Currently, they are not yet widely used in clinical testing because of the high cost of equipment and reagents, difficulties in array data analysis, and limitations in the processing of small FFPE tissue samples (these techniques require a large amount of DNA for the analysis, which is difficult to obtain from small brain biopsies). However, with the increased availability of these techniques, decreased costs of testing, and optimization of methodology, they might replace FISH and LOH studies in the future.

**IDH1 AND IDH2 MUTATIONS**

Mutations in the IDH1 gene were discovered in 2008 during a genome-wide analysis of 22 glioblastomas as a part of the Cancer Genome Atlas Project.\(^{20}\) It was shown that the presence of the mutation is associated with young age, a secondary-type GBM, and increased overall survival. Follow-up studies identified IDH1 mutations in 60% to 90% of WHO grades II and III diffuse gliomas, as well as in the secondary glioblastomas that developed from these lower-grade tumors\(^{21-30}\) (Table 1). In contrast, IDH1 mutations are rare in primary GBMs and are completely absent in pilocytic astrocytomas.\(^{3}\) Mutations in the IDH2 gene were detected in a smaller proportion of gliomas, mostly in oligodendrogial tumors.\(^{35}\) The fact that IDH1 mutations were identified in both oligodendrogial and astrocytic tumors suggests they may have a common cell of origin (Figure 1). IDH mutations in oligodendrogliomas frequently coexist with 1p/19q dele-
ition and in astrocytomas with TP53 mutations. Importantly, IDH1 and IDH2 mutations are not found in nonneoplastic conditions that can histologically mimic gliomas (eg, reactive gliosis, radiation changes, viral infections, infarcts, demyelinating conditions, etc), allowing for improved diagnostic accuracy of suboptimal brain biopsies. These mutations are rare in non-central nervous system solid tumors, being found only in acute myeloid leukemias (8%-14%), rare prostate carcinomas, and a few B-cell acute lymphoblastic leukemias.

Figure 2. Detection of 1p deletion in oligodendrogial tumors by fluorescent in situ hybridization (FISH) and loss of heterozygosity (LOH) analysis. A, Oligodendroglioma (World Health Organization grade II; hematoxylin-eosin, original magnification ×100). B, Schematic representation of chromosome 1 and location of FISH probes and LOH microsatellite markers. The most commonly used FISH probe is located at the telomeric part of 1p at 1p36.32, and microsatellite markers are located from 1p22 to 1p36.32, allowing for detailed evaluation of the 1p region. C, Analysis of FISH demonstrates loss of 1p chromosomal arm (one red signal LSI 1p36 SpectrumOrange probe, counterstained with DAPI) and presence of 2 green signals that correspond to the 1q control probe in interphase nuclei (LSI 1p25 SpectrumGreen probe, counterstained with DAPI, original magnification ×400). D, Analysis of LOH using microsatellite markers D1S171 and D1S1172 demonstrates loss of the 1p allele (indicated by arrow) in the oligodendroglioma as compared with normal tissue. Abbreviations: N, normal tissue; T, tumor tissue.
In addition to their diagnostic value, IDH gene mutations are restricted to a highly conserved arginine residue, R132, in the IDH1 gene and R172 in the IDH2 gene. These heterozygous mutations alter the normal enzyme activity in a way that results in either loss of function or gain of function (Figure 3, B). Heterozygous mutation in the IDH1 gene decreases the ability of the IDH1 enzyme to catalyze the conversion of isocitrate to α-ketoglutarate and NADPH, making the cell more susceptible to oxidative stress (Figure 3, B). Furthermore, mutated IDH1 gains a new enzyme activity that promotes the reduction of α-ketoglutarate to R(−)-2-hydroxyglutarate (2HG) (Figure 3, B). Accumulation of 2HG was reported to lead to an elevated rate and NADPH, thus further decreasing the protection from oxidative stress provided by these 2 compounds. Finally, mutant IDH1 is associated with an increased level of hypoxia-inducible factor-1α, which is a transcription factor that modulates processes important in carcinogenesis, including angiogenesis and apoptosis (Figure 3, B). The most common mutation type for the IDH1 gene is R132H (approximately 90%) followed by R132C (4%). Other, less common IDH1 mutations (R132S, R132G, and R132L) are found in approximately 6% of tumors. IDH2 mutations are encountered in roughly 5% of gliomas.

Sanger sequencing analysis is the most commonly used method for detection of IDH1 and IDH2 mutations (Figure 4, A). It allows for detection of all mutational variants and can be performed on FFPE tissue, but its sensitivity is relatively low (approximately 20% of mutant alleles or 40% of tumor cells with heterozygous mutation). Tissue microdissection before molecular analysis is recommended to enrich the tumor cell population and to increase the sensitivity of this assay. Another sequenc- ing strategy is pyrosequencing. It has better sensitivity than Sanger sequencing and allows for the detection of 5% to 10% of mutant alleles or 10% to 20% of tumor cells with heterozygous mutations. Real-time PCR amplification and melting curve analysis was recently reported as another approach for diagnosis of IDH1 and IDH2 mutations (Figure 4, B). LightCycler (Roche Diagnostics Corporation, Indianapolis, Indiana) real-time reverse transcription-PCR uses fluorescence resonance energy transfer probes, which bind to the PCR product in a head-to-tail fashion. Post-PCR fluorescence melting curve analysis exploits the fact that even a single base-pair mismatch between the labeled probe and the sequence of interest will significantly reduce the specific melting temperature. For example, if there is no IDH1 or IDH2 mutation present in the sample DNA, the probes will bind perfectly and melt at a higher temperature (64°C), showing a single peak on post-PCR fluorescence melting curve analysis (Figure 4, B). In contrast, if a heterozygous point mutation is present, probes will bind to the mutant amplicon imperfectly and will melt (dissociate) at a lower temperature, producing 2 melting peaks. Therefore, IDH1 R132H mutation has a Tm of 56°C, IDH1 R132S Tm of 58°C, IDH1 R132L Tm of 55°C, and IDH2 R172M Tm of 55°C. This method is fast, less laborious, and more sensitive than...
Sanger sequencing. It can be performed in 80 minutes and allows detection of as little as 10% mutant alleles or 20% of cells with mutations in a background of normal DNA. As compared with other sequencing techniques, it benefits from being performed in a closed system without post-PCR processing steps, which minimizes the length of procedure, errors in sample handling, and risk of contamination.

Several studies have reported the use of monoclonal antibodies for detection of \textit{IDH1} R132H mutation by immunohistochemical (IHC) or Western blot analysis. Recently, an \textit{IDH1} R132H mutation-specific antibody became available and allows convenient detection of mutations in tissue sections by IHC. However, IHC will miss approximately 10% of gliomas carrying less-common mutations of \textit{IDH1} and all of the \textit{IDH2} mutations. Also, the specificity of the detection, as well as the limitations of the testing, remains to be fully characterized.

\textbf{MGMT METHYLATION}

The \textit{MGMT} gene (\textit{O\textsuperscript{6}}-methylguanine-DNA methyltransferase) is located at chromosome 10q26 and encodes for a DNA repair protein. Epigenetic silencing of this gene by promoter hypermethylation leads to reduced expression of the MGMT protein. In 2005, Hegi et al\cite{48} reported the results of a collaborative phase 3 trial showing that MGMT gene silencing improves survival in patients with glioblastoma who are treated concurrently with alkylating drug temozolomide and radiation therapy. Alkylating chemotherapeutic drugs have been used in the treatment of patients with malignant gliomas for many years. Temozolomide adds a methyl group to the \textit{O\textsuperscript{6}}-position of nucleotide guanine, which results in DNA damage and cell death. Active MGMT is ensuring repair of damaged DNA by irreversibly transferring a methyl group from the \textit{O\textsuperscript{6}}-position of a modified guanine to its own cysteine residue and, therefore, decreasing the cytotoxic effects of temozolomide. However, inactivation of \textit{MGMT} by promoter hypermethylation leads to decreased MGMT protein expression, which inhibits the cell’s ability to repair alkylated DNA and thus allows alkylating drugs to work more effectively.

\textit{MGMT} promoter methylation is found among different grades of glioma (Table 1) and is considered to be a
prognostic and predictive marker. Hegi and colleagues reported that 49% of patients with glioblastoma and methylated MGMT were alive at 2 years after treatment with temozolomide and radiotherapy, as compared with 15% of patients with unmethylated MGMT. Correlation with prognosis was also reported in pediatric glioblastomas, low-grade gliomas, and more recently, in anaplastic gliomas (WHO grade III). Although the methylation status of MGMT may change with disease progression, the prognostic significance can only be applied to primary diagnosed tumors. The study by Brandes and colleagues reported that recurrent tumors with MGMT methylation did not show prognostic benefits. In addition to prognostic benefit, several studies have demonstrated the predictive value of methylated MGMT in response to temozolomide chemotherapy in patients with glioblastoma. However, for anaplastic gliomas only the prognostic benefit, and not the predictive value of MGMT methylation, was reported.

Interestingly, Rivera and colleagues found that methylated MGMT may predict response to radiotherapy alone even in the absence of adjuvant alkylating chemotherapy. Most of the methods for MGMT analysis are based on evaluation of the methylation status of the CpG island of the MGMT gene. The CpG island is a region of DNA that contains a high frequency of CG nucleotides and is located at the gene promoter region. The CpG island of the MGMT gene has 97 CpGs, which are usually unmethylated in normal brain tissue. In tumors, a cytosine residue is frequently methylated, leading to changes in chromatin structure and transcriptional silencing of the gene. It is technically difficult to evaluate the methylation status of the whole CpG island, and each method focuses on only several CpGs, assuming that their methylation status reflects methylation of the whole promoter region. However, methylation of some CpGs correlates better with MGMT expression than others, which should be taken into consideration in designing a molecular assay. Currently, the 3 most commonly used methods are methylation-specific PCR (MSP), real-time PCR or MethyLight PCR, and methylation-specific pyrosequencing (Figure 5). Each can be used for the analysis of either FFPE tissue samples or snap-frozen tissue. All of these methods require initial treatment of the DNA with bisulfite to convert unmethylated cytosine to uracil. The MSP is the most commonly used method and allows evaluation of methylation status at 6 to 9 CpGs. It is typically performed with 2 primer sets: one pair for detection of unmethylated MGMT and a second pair for detection of methylated MGMT followed by post-PCR gel electrophoresis (bottom left). MethyLight PCR is performed using one set of primers and fluorescently labeled probe complementary to the methylated MGMT sequence. Both methods demonstrate absence of methylation in tumor 1 (only unmethylated band is present in the gel and only housekeeping control COL2A1 is amplified by real-time PCR) and presence of methylation in tumor 2 (strong methylated band is present in the gel and MGMT is amplified by real-time PCR). Abbreviations: COL2A1, collagen type II α1 gene; M, methylated; MGMT, O’-methylguanine-DNA methyltransferase gene; U, unmethylated.
methylation; is frequently used with fusion variants have been methylation remains controversial. First of all, amplification in high-grade gliomas by loss of heterozygosity (LOH) and fluorescent in situ EGFR genes. The fusion FUSION This method is methylation by IHC has failed to This lack of correlation A low level of methylation and genes, or In Molecular Diagnostics of Gliomas—MGMT (LSI 7p12 EGFR SpectrumOrange probe counterstained with DAPI) and 2 normal green signals and BRAF fusion was described. BRAF These genetic abnormalities are amplification by FISH (C). B, The LOH analysis of chromosome 10q at the MGMT 565 BRAF Detection of 10q (EGFR methylation. This assay is more specific than MSP methylation analysis This assay is more specific than MSP because of the addition of an oligonucleotide probe and the increased numbers of studied CpGs (12 to 14 CpGs). Real-time PCR is performed with a regular number of cycles of amplification and, therefore, rarely produces false-positive results. However, use of fluorescently labeled probes makes the assay more susceptible to PCR inhibition that might be due to inadequate DNA purification after bisulfite treatment or some other factors.

Another method used for MGMT methylation analysis is methylation-specific pyrosequencing. This method is based on PCR amplification, followed by pyrosequencing of short DNA fragments, and allows for detection (depending on the assay) of 4 to 12 CpGs. The advantage of pyrosequencing is an ability to quantify methylation at each CpG site within the amplicon. Recently, the extent of MGMT methylation was proposed as a prognostic factor in patients with glioblastoma treated with temozolomide and radiotherapy. A low level of methylation could signify the presence of only a few methylated CpGs and might not necessarily reflect the dense methylation that is required for silencing of the gene.

Because hypermethylation of the promoter region leads to silencing of gene transcription and inhibition of protein synthesis, an immunohistochemical approach for detection of MGMT protein expression would seem to be the most convenient way to perform MGMT analysis. However, the clinical value of immunohistochemical detection of MGMT methylation remains controversial. First of all, assessment of MGMT methylation by IHC has failed to correlate with disease outcome. This lack of correlation is likely due to a number of factors, including interobserver variability, heterogeneity of the glioma sample, contamination with nonneoplastic cells that express MGMT, and differences in tumor immunoreactivity. In addition, many studies have failed to identify a correlation between IHC results and MGMT methylation status detected by MSP. Therefore, at present, IHC is not the method of choice for the detection of MGMT methylation.

**BRAF/KIAA1549 FUSION**

BRAF, which is a part of the mitogen-activated protein kinase (MARK) pathway, is usually activated by the point mutation BRAF V600E. A different mechanism of BRAF activation was first reported in papillary thyroid carcinomas, where an AKAP9/BRAF fusion was described. In gliomas, BRAF activation by gene duplication, which leads to fusion between the KIAA1549 and BRAF genes, or by point mutation has been identified in 60% to 80% of pilocytic astrocytomas. These genetic abnormalities are rare in diffuse astrocytic gliomas and, therefore, may allow the differentiation of pilocytic astrocytomas from low-grade astrocytomas, especially if combined with IDH mutational analysis. The IDH mutations are almost never found in pilocytic astrocytomas, in contrast with their high prevalence in low-grade diffuse astrocytomas (Table 1). Several BRAF/KIAA1549 fusion variants have been reported, making analysis by the reverse transcription-PCR method difficult. Interphase FISH is currently the best method for testing for this fusion and is performed using fluorescently labeled probes corresponding to the KIAA1549 and BRAF genes. The BRAF/KIAA1549 fusion
is diagnosed when one fused signal (red/green or yellow) and one normal pair of red and green signals are found in the interphase nuclei. Similar to any FISH assay, appropriate cutoff levels for fusion detection should be established, especially when analysis is performed in FFPE tissue.

**EGFR AND PTEN ALTERATIONS**

EGFR affects cell proliferation and growth through the activation of downstream effector molecules in the MARK and PI3K-Akt pathways. The *EGFR* gene is located on chromosome 7p12. Activation of EGFR signaling through gene amplification or mutations is found in about 30% to 40% of primary glioblastomas (Table 1).\(^2\)\(^{,}\)\(^2\) In addition, approximately one-half of GBMs with *EGFR* amplifications contain a mutant variant of *EGFR* (EGFvIII).\(^2\) This unique genetic variant is characterized by an in-frame deletion of 267 amino acids in the extracellular domain of the *EGFR* gene (exons 2-7) that leads to a truncated protein. Therefore, EGFvIII receptor is lacking an extracellular domain and is unable to bind a ligand; however, it remains constitutively active.\(^7\) Detection of either *EGFR* amplification or EGFvIII is indicative of high-grade glioma and can be used diagnostically. In neuropathology practice, identification of *EGFR* amplification in neoplastic astrocytes strongly supports diagnosis of GBM even if the histologic criteria are not met (Figure 6, A and C). The prognostic role of *EGFR*/EGFvIII in the glioblastoma setting is not clear. Some studies showed no effect of *EGFR* amplification on patient survival, and some studies have reported a poor prognosis in younger patients and in those with anaplastic gliomas.\(^9\)\(^{,}\)\(^2\)\(^9\)

The *EGFR* signaling pathway is an attractive target for new therapies in gliomas. Initial studies failed to document therapeutic benefits of anti-EGFR tyrosine kinase inhibitors in patients with glioblastoma.\(^6\)\(^{,}\)\(^8\) However, subsequent studies suggest that *EGFR* amplification or EGFvIII expression can predict responsiveness to tyrosine kinase inhibitors, especially when *PTEN* expression is preserved.\(^9\)\(^{,}\)\(^2\)\(^9\)\(^{,}\)\(^3\)\(^9\) Recently, results of a new treatment with an anti-EGFvIII vaccine were reported. The addition of a vaccine to radiation and chemotherapy resulted in increased overall survival in patients with glioblastoma carrying the EGFvIII variant.\(^3\)\(^9\) Therefore, the predictive role of *EGFR*/EGFvIII abnormalities in treatment response is not fully understood and remains to be elucidated in upcoming clinical trials. *EGFR* amplification can be easily detected by FISH (Figure 6, C), and EGFvIII analysis can be performed by reverse transcription-PCR amplification using primers flanking the deletion region.\(^8\)\(^9\)

Phosphatase and tensin homolog (PTEN) is a tumor-suppressor gene located on the long arm of chromosome 10 at 10q23. PTEN is counteracting one of the most critical cancer-promoting pathways, the PI3K-Akt signaling pathway. Genetic alterations of the *PTEN* gene at the level of mutations or chromosomal deletions are frequently found in high-grade gliomas (Table 1). The LOH at 10q is common in primary and secondary GBMs and anaplastic astrocytomas and is found at lower rates in anaplastic oligodendrogliomas (Table 1).\(^8\) Mutations at the *PTEN* gene are found in 15% to 40% of primary glioblastomas, but they are practically absent in secondary glioblastomas and other gliomas.\(^8\) The LOH is most frequently detected at 10q23-24 (PTEN), at 10q25-pter, or the entire long arm of chromosome 10 is lost. Most studies to date have identified 10q LOH and PTEN mutations as poor prognostic markers for anaplastic astrocytomas and glioblastomas, and the loss of 10q is associated with tumor progression (Figure 1).\(^8\)\(^{,}\)\(^9\)\(^{,}\)\(^2\)\(^9\)

Loss at chromosome 10q can be reliably detected in FFPE tissue by LOH analysis or FISH (Figure 6, B). At least 3 markers located at the most commonly deleted regions of 10q should be used for LOH analysis to provide reliable evaluation of the 10q region and to avoid noninformative results.

In conclusion, recent years have been characterized by a rapid expansion of our knowledge about the biology and genetics of gliomas and by the development of new molecular markers. Some of these markers have shown diagnostic value, whereas others are useful for patient prognostication and predicting response to therapies. Genetic testing of gliomas is increasingly used in routine clinical practice and requires neuropathologists to be familiar with all aspects of molecular analysis.

**References**


