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Review

Molecular biology of high-grade gliomas: what should the clinician know?

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Abstract

The current World Health Organization classification system of primary brain tumors is solely based on morphologic criteria. However, there is accumulating evidence that tumors with similar histology have distinct molecular signatures that significantly impact treatment response and survival. Recent practice-changing clinical trials have defined a role for routine assessment of O-6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation in glioblastoma patients, especially in the elderly, and 1p and 19q codeletions in patients with anaplastic glial tumors. Recently discovered molecular alterations including mutations in *IDH-1/2*, epidermal growth factor receptor (*EGFR*), and *BRAF* also have the potential to become targets for future drug development. This article aims to summarize current knowledge on the molecular biology of high-grade gliomas relevant to daily practice.

Key words High-grade glioma, molecular biology, IDH-1/2, MGMT, 1p/19q, EGFRvIII, diagnosis, prognosis, prediction

Gliomas account for approximately 70% of primary brain tumors in adults. The yearly incidence for Caucasians and Asians is about 6 cases per 100,000. Risk factors for gliomas are largely unknown, except for hereditary syndromes such as neurofibromatosis, tuberous sclerosis, Li Fraumeni syndrome, Turcot syndrome, and Cowden syndrome, as well as ionizing radiation to the head. Whether radiofrequency electromagnetic fields emitted by mobile phones induce gliomas remains unclear. Both inherited disorders and irradiation are rare occurrences, accounting for less than 10% of all gliomas and suggesting that complex genetic abnormalities combined with unknown environmental factors predispose individuals to glioma development.

For the last decades, the World Health Organization (WHO) histomorphologic classification of brain tumors together with clinical prognostic factors has guided clinicians in treating patients with high-grade gliomas. Tumor markers have not been readily available and their impact on decision making has not been supported by clinical trials. Patient- and tumor-related prognostic factors are still keys in decision making despite enormous progress in understanding the molecular biology of gliomas. Favorable clinical prognostic factors include young age, macroscopically complete tumor resection, and good Karnofsky performance status. Recursive partitioning analysis of large prospective trials refined clinical prognostic classes in the 1990s, which is still valid today[1,2]. In one of the largest cohorts of Chinese glioma patients (n=1,235), the clinical characteristics and prognostic factors of patients with WHO grade II–IV glioma were similar to those of the Caucasian population[3].

Gliomas are classified using histomorphologic criteria and are designated as WHO grade I through IV according to their degree of malignancy[4]. WHO grade III and IV tumors are commonly lumped together as high-grade gliomas and comprise about 75% of all gliomas.

The WHO classification is based on subjective criteria and is imperfect in predicting patient outcome. Tumors may appear virtually identical by histology, yet still have very different outcomes. This is due, in part, to marked interobserver variability in making a diagnosis. Another contributing factor is whether the surgical specimen is representative of the overall lesion.

Progress in molecular techniques has allowed the identification of a number of markers and genetic profiles that characterize gliomas beyond their histologic criteria. So far, most have not had the awaited clinical impact, as data are not yet robust enough for clinical decision making.

A few molecular markers, however, have been introduced into the clinic in recent years and have been proven useful for identifying glioma subtypes (*diagnosis*), as well as guiding clinicians as to the course of the disease (*prognostication*) and on the choice of treatment (*prediction*). This notably holds true for patients with WHO grade III astrocytic and oligodendroglial gliomas, which may be

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difficult to distinguish on morphological criteria alone.

In 2013, three molecular markers were considered useful tools for the management of high-grade gliomas: 1p/19q chromosomal codeletion, O-6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation, and isocitrate dehydrogenase (*IDH*) 1 and 2 mutations. An additional biomarker, namely a specific mutation of the epidermal growth factor receptor (EGFR) variant III (EGFRvIII), serves as a potential target for yet to be proven experimental therapies (**Tables 1** and **2**).

Molecular Marker, Clinically Useful for High-Grade Gliomas

1p/19q chromosomal codeletion

This codeletion is an unbalanced reciprocal translocation of 19q and 1p. Tumors that contain this translocation have been associated with an oligodendroglial phenotype, a better prognosis, and a better response to postoperative treatment, though the biological role of this marker remains unclear.

In 2012, follow-up results of more than 11–12 years in the Radiation Therapy Oncology Group (RTOG) 9402 and European Organization for Research and Treatment of Cancer (EORTC) 26951 trials demonstrated that an overall survival benefit from the addition of chemotherapy to radiotherapy was confined to patients with anaplastic oligodendroglial tumors with (versus without) 1p/19q co-deletion[5,6]. The complete 1p/19q codeletion must be distinguished from partial 1p or 19q loss that, so far, lacks prognostic significance. Evidence suggests that 1p/19q codeletion is homogeneous within a tumor and does not change during disease evolution[7].

Currently, two international randomized trials are investigating sequence and the combination of radiotherapy and chemotherapy in WHO grade III tumors stratified according to 1p/19q status[8,9].

MGMT promoter methylation

Methylation of the *MGMT* gene promoter results in epigenetic silencing of the methyltransferase, which loses its gene repair activity. *MGMT* methylation seems to be a prognostic factor prevalent throughout WHO grades II–IV gliomas, though with decreasing frequency as the malignant potential rises[10].

More than 15 years ago, reports indicated that high activity of the MGMT protein in glioma tissue was associated with resistance to alkylating agents, which, at that time, were largely nitrosoureas. In 2000, methylation of the promoter region of the *MGMT* gene was linked to improved outcomes. In 2005, *MGMT* promoter methylation assessed by a methylation-specific polymerase chain reaction was able to predict benefit from the addition of temozolomide (TMZ) chemotherapy to radiotherapy in the treatment of newly diagnosed glioblastoma multiforme (GBM)[11]. However, standardizing the *MGMT* assay for widespread clinical use was challenging, and treatment decisions continued to be performed without knowledge of the *MGMT*

Table 1. Role of glioma markers in clinical practice

Marker	Diagnostic	Prognostic	Predictive
1p/19q	Oligodendroglial tumors[1]	WHO II–III	WHO III[2]
IDH	WHO > I; 2° GBM; exclusive for some glioma entities[3]	WHO II–IV	No predictive role
MGMT	No diagnostic role	WHO III–IV	WHO IV; alkylating agents, especially in elderly
EGFRvIII	1° GBM[4]	Not clearly defined	WHO IV; vaccine or targeted therapies (experimental)

[1]Almost all oligodendroglial tumors have loss of heterozygosity 1p/19q. [2]Predicitve for the treatment with radiotherapy and/or alkylating agents. [3]Ependymoma and pilocytic astrocytoma do not have *IDH* mutation. [4]EGFRvIII in ~33% of primary GBM.
IDH, isocitrate dehydrogenase; MGMT, O-6-methylguanine-DNA methyltransferase; EGFRvIII, epidermal growth factor receptor variant III; GBM, gliblastoma multiforme. 2° GBM, secondary GBM progress from low-grade diffuse astrocytoma or anaplastic astrocytoma. 1° GBM, 90% of GBM develop rapidly *de novo* and are termed primary GBM.

Table 2. Overview of suitable methods for assessment of glioma markers in clinical practice

Marker	IHC	FISH	PCR/ Sequencing
1p/19q	No	Yes	Yes
IDH/*IDH*	Yes	No	Yes
MGMT	No	No	Yes
EGFRvIII/*EGFRvIII*	Yes	No	Yes

IDH, isocitrate dehydrogenase; MGMT, O-6-methylguanine methyltransferase; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; PCR, polymerase chain reaction; FISH, fluoresence *in situ* hybridization.

status. In 2012, two randomized trials performed in the growing population of elderly GBM patients demonstrated consistently that a methylated *MGMT* promoter is a powerful predictive biomarker for benefit from TMZ alone. In the German NOA-08 trial, patients older than 65 years were treated with either standard 6-week, fractionated (1.8–2.0 Gy) radiotherapy or dose-dense TMZ chemotherapy (week on/week off). Patients with tumors exhibiting methylated *MGMT* fared better if they were treated with TMZ alone than those treated with radiotherapy alone[12]. Similarly, the Nordic trial found standard-dose TMZ (5 out of 28 days) to be superior to radiotherapy in patients older than 60 years with methylated *MGMT* promoter[13]. Thus, at least in the elderly population, *MGMT* testing should become a standard procedure for decision making (chemotherapy vs. radiotherapy), though the test is not yet widely available.

Whether patients with *MGMT* promoter methylation of other age groups or other WHO grades should be treated with TMZ alone rather than chemoradiotherapy is an important question for future studies.

IDH1 and *IDH2* mutations

Point mutations in the *IDH1* and *IDH2* genes, originally discovered in 2008, occur in the vast majority of low-grade gliomas (>80%) and secondary high-grade gliomas. The frequency of these mutations does not change during the progression from WHO grade II to WHO grades III or IV (so-called secondary GBM). Evidence has accumulated that primary and secondary GBM develop through different genetic pathways, though they remain largely histomorphologically indistinguishable at diagnosis.

IDH1/2 mutations, which occur early in gliomagenesis, change the function of the enzymes, causing them to produce 2-hydroxyglutarate, a possible oncometabolite, instead of α-ketoglutarate. The mutations are able to drive increased methylation in gliomas. Gliomas with a mutated *IDH1* or, less frequently, mutated *IDH2* are associated with better prognosis compared to their wild-type counterparts[14]. As with loss of heterozygosity 1p/19q, a given IDH status seems to be homogeneous within a tumor and does not change during disease evolution. Mutated *IDH* can easily be detected by immunohistochemistry and potentially even non-invasively by magnetic resonance spectroscopy. Non-tumoral glial cells (i.e. those involved in gliosis) never express mutated *IDH*, a fact that can be used to separate reactive gliosis from gliomas. Pilocytic astrocytoma (WHO grade I), ependymoma, and primary GBM (but not secondary GBM) do not harbor *IDH* mutations[15]. Of note, *IDH* mutations are not glioma-specific alterations. Furthermore, there is currently no drug that targets mutated IDH, although this remains an area of active research.

EGFRvIII

A tumor-specific mutant of the *EGFR, EFGR* variant III (*EGFRvIII*), causes constitutive activation of the receptor's tyrosine kinase activity and is frequently expressed in primary GBM (~33%). This mutation confers enhanced tumorigenic behavior, at least in preclinical experiments[16]. Because it is localized solely on tumor tissue, EGFRvIII presents an ideal target for immunotherapy, reducing the risk of autoimmune toxicity. Immunohistochemical testing for EGFRvIII may be implemented if randomized trials demonstrate activity of EGFRvIII-targeted vaccination[17,18].

Interrelations of various molecular markers

Among low-grade and anaplastic gliomas, nearly all with 1p/19q codeletion also harbor *IDH1/2* mutations. However, some genetic markers, such as *EGFR* and *IDH1*, *EGFR* and *TP53, TP53* and 1p/19q, are mutually exclusive. Molecularly, *IDH1* and *IDH2* mutations are heterozygous, affect only a single codon, and rarely occur together. Although *TP53* mutations and 1p/19q codeletions are mutually exclusive, *IDH1* mutations are common in both of these genotypes[19,20].

Conclusions

The most recent clinical data from randomized phase III trials call for routine testing of 1p/19q for patients with WHO grade III gliomas and for assessing the *MGMT* methylation status, especially in elderly GBM patients too frail to undergo postoperative concomitant radiochemotherapy followed by chemotherapy—the standard treatment for GBM. Molecular marker determination, however, is technically demanding and requires reproducible and validated test procedures. This holds especially true for *MGMT* testing, where results sometimes may fall into a "gray zone."

Outlook

BRAF mutations have been found in a fraction of high-grade glioma patients (e.g. epitheloid GBM) and may present a druggable treatment target for specific inhibitors such as vemurafenib or dabrafenib[21, 22]. Another focus of interest is immunosuppressive molecules (e.g., B7H1 and B7H4); further research is warranted to define the role of immunomodulatory drugs in high-grade glioma[23]. Moreover, we still lack biomarkers with predictive properties to select anti-angiogenic agents for treating gliomas.

Circulating microRNA (miRNA), small non-coding regulatory RNAs that modulate the expression of specific target genes, might be relevant in the future for diagnosis, prognosis, and therapy of gliomas[24]. For example, evaluating the circulating DNA of *EGFRvIII* in plasma may represent a strategy to screen patients for an anti-EGFRvIII therapy and monitor response to treatment[25].

The availability of high-throughput methods will most likely enrich the histomorphological WHO classification with a comprehensive molecular characterization of gliomas.

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