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REVIEW

MGMT promoter methylation in malignant gliomas

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Abstract The O^6-methylguanine-DNA methyltransferase (*MGMT*) gene is located at chromosome 10q26 and codes for a DNA repair enzyme that—if active—can counteract the effects of alkylating chemotherapy. Malignant gliomas often have the *MGMT* gene inactivated due to aberrant methylation of its promoter region. The assessment of the *MGMT* promoter methylation status has become of clinical relevance as a molecular marker associated with response to alkylating chemotherapy and prolonged survival of glioblastoma patients. *MGMT* promoter methylation testing is also on the merge of being used as a marker for patient selection within clinical trials, e.g., the current CENTRIC trial that is specifically focusing on patients with *MGMT* promoter-methylated glioblastomas. In anaplastic gliomas, *MGMT* promoter methylation is a favorable prognostic marker independent of the type of therapy, i.e., radio- or chemotherapy. This occurrence might be associated with the high incidence of other prognostically favorable molecular markers in these tumors, such as *IDH1* mutation, 1p/19q deletion or yet to be identified novel aberrations. A variety of different methods are being used to assess *MGMT* promoter methylation in clinical samples, which may give rise to inter-laboratory variations in test results. Immunohistochemical determination of MGMT protein expression has not proven reliable for diagnostic purposes. This brief review article aims to summarize the main aspects of *MGMT* promoter methylation testing in contemporary neuro-oncology, in particular its value as a clinically useful molecular marker, putting it into the context of other molecular markers of clinical use in gliomas of adult patients.

Keywords Glioma · O^6-methylguanine-DNA methyltransferase (MGMT) · Molecular diagnostics · Prognosis · Promoter methylation

The *MGMT* gene encodes a DNA repair protein that removes alkyl groups from the O^6 position of guanine, an important site of DNA alkylation [1]. Chemotherapy-induced alkylation at this site triggers cytotoxicity and apoptosis. Tumor cells that express high levels of the MGMT repair protein may thereby counteract the therapeutic effect of alkylating agents, including nitrosourea compounds and temozolomide that are most commonly used for the treatment of malignant gliomas. *MGMT* is epigenetically inactivated via hypermethylation of the 5′-CpG island in approximately 40% of primary glioblastomas and over 70% of secondary glioblastomas (Fig. 1) [2]. *MGMT* promoter methylation is also found in half of the diffuse and anaplastic astrocytomas as well as approximately two thirds of the oligodendroglial and mixed tumors [2]. CpG islands are defined as genomic regions that contain a higher than average frequency of CG dinucleotides (CpG sites) and are involved in regulation of gene transcription. CpG islands, including the one associated with the *MGMT* gene, often span the transcription start site of genes and contain crucial transcription factor binding sites. Aberrant methylation of CpG islands may impair gene transcription, which consequently leads to reduced or even complete loss of expression of the respective gene product

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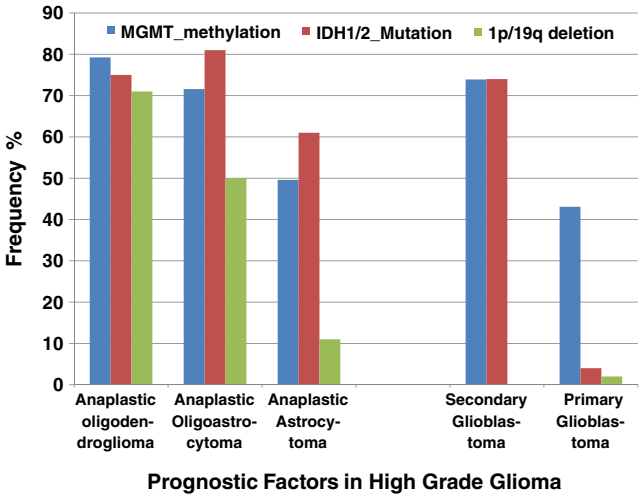

Fig. 1 Association of favorable prognostic factors with *MGMT* methylation in malignant glioma. The incidences of *MGMT* methylation, *IDH1* or *IDH2* mutations, and co-deletions of 1p/19 are displayed for different types of malignant gliomas in adults. *MGMT* methylation is associated with *IDH1* or *IDH/2* mutations in all types of malignant gliomas, with the exception of primary glioblastoma [2, 17, 27–29]

[3, 4]. In case of the *MGMT* 5′-CpG island, a region covering an *MGMT* enhancer element appears to be most critical for loss of transcription and MGMT protein expression, as suggested by luciferase reporter assays [5, 6]. In malignant gliomas, however, the *MGMT* promoter methylation patterns are highly heterogeneous from tumor to tumor and it is unknown which particular CpG sites or combinations thereof need to be methylated for silencing the gene and conveying benefit from alkyating agent therapy. The various assays that are in current use to evaluate *MGMT* status assess different numbers of CpGs at distinct locations within the *MGMT* promoter, typically covering between 3 and 20 of a total of 97 CpGs. Although in most instances results are expected to overlap, one has to be aware of the fact that different laboratories may obtain different results in individual patients. Such differences in test results do not need to be due to improper testing in one or the other laboratory, but may reflect a heterogeneous methylation pattern in the investigated tumor.

Methodological aspects of *MGMT* promoter methylation testing

The most commonly employed method, and also the technique originally described to convey the relevant clinical information, is methylation-specific PCR (MSP) analysis [7, 8]. This technique employs primers that specifically amplify fragments from either the methylated or the unmethylated sodium bisulfite-modified DNA sequence. In order to make the primers discriminative between both sequences they are designed to contain a maximum number of CpG sites that differ between methylated and unmethylated bisulfite-modified DNA. PCR products can be evaluated by gel-based approaches or quantitatively using real-time PCR assays that define a threshold for detecting methylation levels and thus permit a higher degree of standardization [9]. Such cut-offs, however, are just technically substantiated to date, and there is a need to validate cut-off points prospectively to establish clinically relevant methylation thresholds. A major advantage of *MGMT* methylation testing compared to testing at the gene or protein expression levels is that any methylation signal detected is representative of neoplastic glial cells only. RT-PCR analysis, western blotting or immunohistochemistry do not only detect MGMT expression from neoplastic glial cells but also from contaminating non-neoplastic cell populations, which can be very prominent in malignant gliomas and include microglia/macrophages, lymphocytes, reactive astrocytes, residual brain parenchymal elements and vascular cells. Without doubt, MGMT immunohistochemistry would clearly facilitate the diagnostic procedure, however, due to the described methodical limitations it does not uniformly reflect a tumor's *MGMT* methylation status and conveys a degree of uncertainty that is not acceptable for clinical decision-making [10, 11]. In fact, even when the extent of microglial contamination was considered in the evaluation of MGMT immunostaining, there was still no significant association of the immunohistochemical results with *MGMT* promoter methylation and patient survival [10]. Thus, either specificity and sensitivity of the antibodies used for immunohistochemical detection of MGMT is limited or other, yet to be identified molecular mechanisms contribute to the regulation of MGMT protein expression in glioblastomas in addition to promoter methylation [10]. Further research is needed to dissect possible reasons that may account for the lack of association of MGMT immunoexpression with *MGMT* promoter methylation and patient survival in glioblastomas.

Clinical relevance of the *MGMT* promoter methylation status in gliomas

As mentioned above, the main use of *MGMT* as a molecular marker is its predictive value regarding the response of malignant gliomas to alkylating chemotherapy using either nitrosourea compounds [12], temozolomide [8], or a combination of both [13]. In the European Organisation for Research and Treatment of Cancer (EORTC)/National Cancer Institute of Canada (NCIC) 22981/26981 trial [8, 14], patients treated with radiotherapy and temozolomide survived significantly longer when they had a methylated *MGMT* promoter [8]. While data from this EORTC/NCIC 22981/26981 trial and another large prospective patient

cohort [15] found that *MGMT* promoter methylation was predictive for longer survival only in those patients who received temozolomide, a recent retrospective and single institution analysis reported that *MGMT* promoter methylation may also be predictive of response to radiotherapy and linked to longer survival in the absence of adjuvant chemotherapy in glioblastoma patients [16]. While the prognostic role of *MGMT* in glioblastoma patients not treated with chemotherapy is a matter of debate, recent data from anaplastic glioma trials, namely the NOA-04 and the EORTC 26951 trials, both found that *MGMT* promoter methylation predicted prolonged survival irrespective of the initial treatment, i.e., radiotherapy, chemotherapy or a combination of both [17, 18]. The prognostic role of *MGMT* promoter methylation in patients with diffusely infiltrating low-grade gliomas who do not receive adjuvant therapy is unclear. In a study of 49 variably treated patients with low grade gliomas, *MGMT* promoter methylation was reported to be a negative prognostic factor for progression-free survival [19]. In contrast, treatment with temozolomide in a phase II study of low-grade glioma patients reported a better outcome in patients with *MGMT* promoter-methylated tumors [20].

Coincidence of *MGMT* promoter methylation with other molecular alterations

The reason for the predictive role of *MGMT* promoter methylation for response to temozolomide and other alkylating agents can be easily derived from the functional aspects of the MGMT repair protein (see above). In contrast, the prognostic role of *MGMT* promoter methylation in patients with anaplastic gliomas receiving only radiotherapy is rather unexpected from a functional point of view. However, in contrast to primary glioblastomas, *MGMT* promoter methylation in diffuse gliomas and secondary glioblastomas is frequently associated with other prognostically favorable genetic alterations (Fig. 1). In oligodendroglial neoplasms, a strong association of *MGMT* hypermethylation is observed with 1p/19q codeletions [21–23] and *IDH1* mutations [24]. In diffusely infiltrating astrocytic gliomas—with the exception of primary glioblastoma—*MGMT* promoter hypermethylation and *IDH1* mutations frequently coincide [24]. Further studies are needed to more closely dissect which of these changes contribute most to the positive prognostic effect in anaplastic gliomas or if even the particular constellation of *MGMT* methylation with concomitant 1p/19q codeletions and *IDH1* mutations is associated with a higher sensitivity to cytotoxic therapy and a more favorable outcome. The situation may be more complex, though, than suggested by merely focusing on a single molecular marker such as *MGMT*. Noushmehr et al. recently described a glioma CpG island methylator phenotype (G-CIMP) that identified a set of prognostically favorable gliomas showing promoter hypermethylation of a specific set of genes [25]. This G-CIMP correlated to a similarly favorable proneural gene signature described in a preceding study by Phillips et al. [26] and was tightly associated with *IDH1* mutation. Interestingly, this G-CIMP was more prevalent among diffuse low-grade and anaplastic gliomas, in particular oligodendroglial tumors. While *MGMT* promoter methylation was not identified as being part of this hypermethylator phenotype, the exact association of *MGMT* methylation with this prognostically favorable signature has not yet been tested. However, the study suggests that particularly in anaplastic and low-grade gliomas prognostically favorable effects may be brought about not only by *MGMT* but by the aberrant methylation of other genes, possibly including yet to be identified candidate genes whose silencing may contribute to increased radiosensitivity of glioma cells. These observations further emphasize a different pathogenesis of diffuse gliomas and secondary glioblastoma as opposed to the majority of primary glioblastoma.

Summary and outlook

MGMT promoter methylation has been established as a clinically important molecular marker in neuro-oncology. While treatment decisions in the routine setting are not yet based on this marker, the *MGMT* promoter methylation status is now used as an important stratification or selection parameter in ongoing clinical trials. In glioblastoma patients, *MGMT* promoter methylation is predictive for the response to alkylating chemotherapy and associated with longer survival in patients treated with radiotherapy as well as concurrent and adjuvant temozolomide. Recent data from trials on anaplastic glioma patients indicate that *MGMT* promoter methylation in this tumor group is a favorable prognostic marker that is independent from the type of therapy, i.e., radiotherapy or alkylating chemotherapy. The high coincidence of *MGMT* methylation with other genetic aberrations, such as 1p/19q deletions and particularly *IDH1* or *IDH2* mutations, and potentially epigenetic silencing of yet unknown radioresistance genes may indicate a more complex molecular phenotype of clinical significance and has to be further dissected in subsequent studies.

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