Commentary

“MGMT for pt mgmt”: Is Methylguanine-DNA Methyltransferase Testing Ready for Patient Management?

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O⁶-Methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme that removes alkyl adducts from the O⁶ position of guanine. In recent years, MGMT has attracted attention in multiple tumor types for two reasons: i) MGMT appears to modulate the responsiveness of cells to alkylating chemotherapies, and ii) it is a model gene for understanding epigenetic alterations in cancer, as its transcription can be down-regulated by promoter methylation.¹ In the setting of glioblastoma (GBM), the most common and most malignant form of brain tumor in adults, *MGMT* gene inactivation appears to be mediated through promoter hypermethylation.^{2–4} Moreover, *MGMT* promoter methylation has been shown to predict responsiveness of GBM to the oral alkylating agent temozolomide in combination with radiotherapy. In a key study by Hegi et al,⁴ *MGMT* methylation predicted longer median overall survival (18.2 months versus 12.2 months for tumors with unmethylated promoters), which was even more pronounced in the group treated with temozolomide plus radiotherapy (21.7 months versus 12.7 months).^{4,5} Additional studies have shown that the level of MGMT correlates with survival in GBM, and can potentially identify long-term survivors.^{6,7} Based on such findings, interest in *MGMT* promoter methylation assays for patient management has grown significantly. In this issue of *The Journal of Molecular Diagnostics*, Vlassenbroeck et al⁸ report a robust quantitative assay that should help in the interpretation of *MGMT* promoter methylation assays and should facilitate the comparison and implementation of such assays across laboratories.

A number of groups have published assays to detect *MGMT* promoter methylation, with most relying on methylation-specific PCR (MSP).^{4,9–11} MSP uses separate primer sets that discriminate methylated from unmethylated cytosines, since methylated cytosines are protected from, but unmethylated cytosines are susceptible to, bisulfite treatment-mediated conversion to uracil. The majority of such assays are reproducible and can be

adapted to the use of formalin-fixed, paraffin-embedded material.⁹

Alternative approaches include bisulfite sequencing, combined bisulfite restriction analysis (COBRA), mass spectrometry-based analysis, and pyrosequencing.^{12–14} Vlassenbroeck et al⁸ have compared the well evaluated MSP assay used by Hegi et al^{4,5} with a newly developed quantitative real-time MSP assay. Although prior studies have analyzed *MGMT* promoter methylation using quantitative PCR,^{15,16} the paper by Vlassenbroeck et al⁸ is one of the first studies to focus on brain tumors.¹⁷ The authors demonstrate a very good correlation between the gel-based MSP and the newly developed real-time MSP assay. The bimodal distribution using the quantitative assay strongly suggests biological relevance, and the statistically validated cut-offs make it a useful assay. Notably, a small number of cases scored positive using the qualitative gel-based assay but negative using the real-time assay, probably reflecting a slightly higher sensitivity of the gel-based assay. The major advantage of a real-time MSP assay for *MGMT* will be its reproducibility, as it is normalized to MSP for β -actin (*ACTB*). Reproducibility will help the assay's adoption across multiple laboratories and will be of importance to the MGMT molecular diagnostics field in general, as quality control and assurance issues have not been fully addressed. An interlaboratory comparison study certainly seems warranted, as well as the development of laboratory proficiency standards. Most importantly, however, it remains unclear which *MGMT* assay performs best in predicting temozolomide sensitivity in the setting of GBM. As such, a direct head-to-head comparison of standard MSP and the real-time MSP assay in the setting of a controlled clinical trial is essential, and such results are eagerly awaited.

Despite the promise of *MGMT* analysis, there remain numerous unresolved issues surrounding the biological relevance of *MGMT* promoter methylation. For example,

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we and several other groups have failed to detect coordinate decreases in MGMT protein expression by immunohistochemistry in cases with *MGMT* promoter methylation.¹⁸ In addition, immunohistochemistry commonly reveals intratumoral heterogeneity in the staining intensity of MGMT. Although it is possible that MGMT protein expression may not reflect newly transcribed protein available to remove alkyl groups, these findings raise the possibility that *MGMT* promoter methylation is not directly responsible for the biological properties of such tumors but is an epiphenomenon of a distinct more important genetic (or epigenetic) alteration. Of note, the *MGMT* gene is located on chromosome 10q, a region lost in the vast majority of GBM, implying that even those GBM without *MGMT* promoter methylation are most likely haploinsufficient for MGMT. The fact that *MGMT* promoter methylation predicts good prognosis independent of temozolomide also indicates a role of MGMT apart from the damage response.⁴

Another caveat in interpreting the biological importance of *MGMT* promoter methylation is the fact that other gene promoters are often methylated in the same tumors as *MGMT*. This phenomenon has been best described in colorectal tumors and is termed CIMP (CpG island methylator phenotype).^{19,20} Other co-regulated genes include several with clearly important roles in tumorigenesis such as *CDKN2A* (p16), *IGF2*, and *MLH1*. CIMP has also been associated with microsatellite instability and *BRAF* mutation,^{21,22} but the exact mechanism of tumorigenesis for different types of colon cancer still remains uncertain. Our group and others have shown that there are a number of other genes whose promoters are commonly methylated in brain tumors (*RUNX3*, *SOCS1*, *TES*, and *CDKN2A*).¹⁴ Thus, there may be a CIMP-like phenotype in brain tumors, although microsatellite instability and *BRAF* mutation are rare.^{23,24}

Unlike in embryonic development, the mechanisms of *de novo* promoter methylation and its maintenance in tumors are not well understood. It has been shown that DNA methyltransferases such as DNMT1 and DNMT3B are important for promoter methylation in tumors, but we do not know what drives their activation.²⁵ The field is only beginning to explore the connection between known genetic alterations in brain tumors, such as *EGFR* amplification or 10q loss, and DNMT activation.

Despite these caveats, *MGMT* promoter methylation appears to have taken hold as a biomarker to predict temozolomide sensitivity, and clinician expectations for routine implementation of MGMT assays have been raised. Practical concerns still exist in the routine adaptation of MSP *MGMT* analysis, including requirements for relatively large amounts of quality DNA for bisulfite treatment, often preventing analysis of small tumor biopsies used for diagnosis in unresectable GBMs. In addition, the problems of intratumoral heterogeneity¹⁸ of MGMT staining and methylation are well described. The data presented by Vlassenbroeck et al⁸ also indicate that there will remain a small number of problematic intermediate tumors, even with the well defined cutoffs enabled by the quantitative assay. Finally, it is important to highlight that the absolute clinical necessity of MGMT testing remains

unclear. In the Hegi et al study,⁴ temozolomide marginally improved survival even in *MGMT* unmethylated patients ($P = 0.06$). Thus, at the present time, with temozolomide being the most effective and best tolerated chemotherapeutic agent available to GBM patients, essentially all GBM patients will receive temozolomide as initial therapy along with radiation, regardless of their MGMT status. Nonetheless, we have had some demand from neuro-oncologists for *MGMT* MSP analysis, which is likely driven by the independent prognostic information and by the assistance MGMT status may have in fine-tuning administration of temozolomide and, at least potentially, other alkylating agents. At the very least, there is a clear need for stratifying patients by MGMT status for clinical trials of temozolomide or other alkylating agents in gliomas, as we progress in the era of genetically (and perhaps epigenetically) directed rational cancer therapy. As such, the article by Vlassenbroeck et al⁸ is a practical step toward determining whether MGMT analysis will eventually find a major role in patient management.

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