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Role of *MGMT* Methylation Status at Time of Diagnosis and Recurrence for Patients with Glioblastoma: Clinical Implications

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Glioblastoma • Surgery • *MGMT* methylation • Heterogeneity • Recurrent glioblastoma

Abstract

Background. *MGMT* methylation status represents a powerful prognostic factor in newly diagnosed glioblastoma (GBM). Recently, its role in recurrent tumors has also been suggested; however, few data investigating the stability of this biomarker during the clinical course of the disease are available. In this study, we evaluated the rate of change of *MGMT* methylation status between diagnosis and first recurrence in patients who received tumor resection for recurrent GBM.

Methods. We included patients who received temozolomide concurrent with and adjuvant to radiotherapy after diagnosis of GBM and had a second surgery performed at least 3 months after radiotherapy completion. Other eligibility criteria were age ≥18 years and Eastern Cooperative Oncology Group performance status 0–2. We evaluated the *MGMT* methylation status by methylation-specific polymerase chain reaction.

Results. From our institutional data warehouse, 295 patients with recurrent GBM who underwent second surgery were evaluated. *MGMT* methylation status at both first and second surgery was available for 108 patients. MGMT was methylated in both surgeries in 38 patients (35.2%), while it was unmethylated in 43 patients (39.8%). We found a significant concordance between the first and the second *MGMT* methylation assessments ($K = 0.500$, $p < .001$), *MGMT* methylation being stable in 75% of the cases.

Conclusion. *MGMT* methylation presents relative stability during the clinical course of GBM. ***The Oncologist*** 2017;22:432–437

Implications for Practice: *MGMT* methylation is a prognostic factor in newly diagnosed glioblastoma. In this study, we evaluated the rate of change of *MGMT* methylation during the clinical course of the disease, and we found a significant concordance between the first and the second *MGMT* methylation assessments, with *MGMT* methylation being stable in 75% of the cases. Thus, re-testing this biomarker at recurrence does not provide further information for clinicians. *MGMT* methylation at first surgery, extent of resection at second surgery, and time between first and second surgery are significantly correlated with overall survival. Age and extent of resection are correlated with post-progression survival.

Introduction

Current standard treatment for glioblastoma (GBM) includes surgery followed by radiation therapy (RT) and chemotherapy with temozolomide (TMZ) [1]. Despite the improvement in overall survival (OS) achieved with combined TMZ with and adjuvant to radiotherapy (RT/TMZ), most of the patients experienced disease progression and median survival did not exceed 12–14 months, with a 5-year survival rate of 10% [1, 2].

Methylation of the *O-6-methylguanine-DNA methyltransferase* (MGMT) gene promoter has emerged as a strong

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prognostic factor for newly diagnosed GBM [3, 4]. MGMT encodes for a DNA repair enzyme that provides resistance to alkylating chemotherapies such as TMZ. Because MGMT transcription can be silenced by promoter methylation in tumor cells [4], it is widely assumed that MGMT promoter methylation in patient tumors causes decreased MGMT protein expression, thereby abrogating the DNA repair activity necessary for TMZ resistance.

Few studies have evaluated if *MGMT* methylation status of GBM might change during the course of care, and some have found contrasting results and variable rates of change (5%–40%) [5–8], even if different techniques for assessment are used (methylation-specific polymerase chain reaction [MSP], immunohistochemistry). Moreover, it remains unclear if *MGMT* methylation retains its role after disease progression [9]. We performed an analysis on our institutional data warehouse evaluating all consecutive GBM patients who underwent second surgery for recurrence after standard treatment with RT/TMZ [1] in order to investigate the rate of change of *MGMT* methylation status at the time of disease progression and the impact of *MGMT* methylation status on the clinical outcome in the recurrent setting.

Subjects, Materials, and Methods

Patients

We analyzed all consecutive GBM patients from our institutional data warehouse, which was built in 2006 and captured information about clinical characteristics, histology, and molecular biology and survival. Inclusion criteria were age $\geq$18 years Eastern Cooperative Oncology Group performance status 0–2, and recurrence after at least 3 months from combined RT/TMZ; patients must also have undergone a second surgical procedure for recurrent disease.

All patients underwent a postoperative computed tomography (CT) scan within 48 hours of surgery to determine the extent of tumor removal. Extent of resection for each patient was classified as complete (>95% resection by volume) or partial ($\leq$95% resection by volume). Patients who underwent biopsy (at first or second surgery) were also included. A review of patient charts was conducted to obtain demographic information, including age, sex, and description of surgical procedure. Histological evaluations were made on formalin-fixed, paraffin embedded tissues. Tumor tissue was classified and graded as GBM according to WHO 2007 guidelines. The *MGMT* methylation status was evaluated with the MSP [10].

We aimed to reduce the risk that treatment-related changes may hamper the results of the analysis of *MGMT* methylation on second surgical samples. After microscopic evaluation, the most representative block was selected between those used for diagnosis, and six 10-μm-thick sections were cut, followed by one Hematoxylin and Eosin control slide. The tumor area was marked on the control slide by a pathologist, and material was manually dissected under microscopic guidance from the corresponding 10-μm sections using a sterile blade. The total amount of neoplastic cells and the proportion of neoplastic cells versus "contaminant" non-neoplastic cells (i.e., endothelial, stromal, and inflammatory cells) was estimated by the pathologist in the area marked on the control slide. Only samples with at least 100 neoplastic cells and a proportion of neoplastic cells versus "contaminant" non-neoplastic cells greater than 5%, in the marked area, were then processed for the molecular analysis. The study was approved by the institutional review board of the Azienda USL of Bologna, Italy.

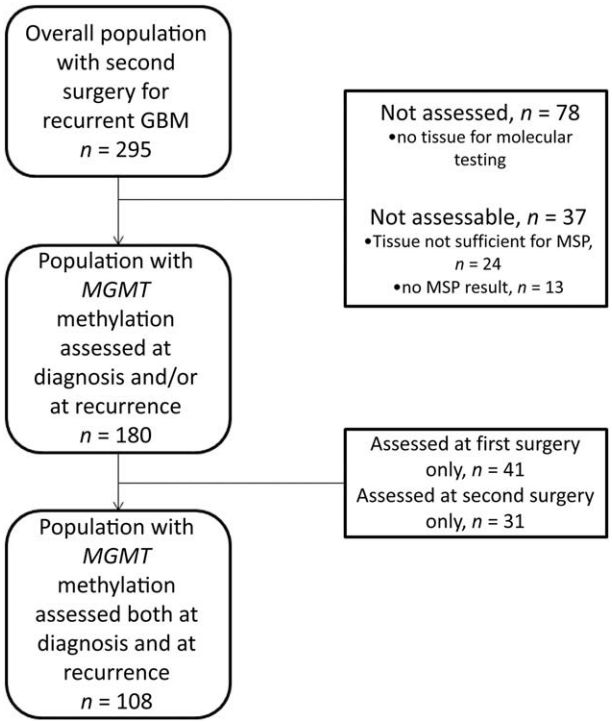

Figure 1. CONSORT flow chart.
Abbreviations: GBM, glioblastoma; MGMT, 0-6-Methylguanine DNA-methyltransferase; MSP, methylation-specific polymerase chain reaction.

Objectives

The aim of this study was to assess the rate of change of *MGMT* methylation status during the clinical course of GBM and the potential implications of these changes.

Statistical Analysis

Data are reported as means, ranges, and frequencies. Survival data (median survival times with 95% confidence interval [CI]) were computed by the Kaplan-Meier procedure and were analyzed by the means of the log-rank test and the Cox proportional hazards model. The significance level required to keep a variable in the multivariate forward stepwise model was 10%. Analyses were not corrected for multiple testing. The hazard ratios were computed together with their 95% CIs. Differences between *MGMT* methylation status obtained at first and second surgery were evaluated by the means of the McNemar test, and the concordance was evaluated with Cohen's Kappa Coefficient. Fisher's exact test was applied in order to evaluate if the rate of *MGMT* methylation status changes was similar among the two groups of methylated and unmethylated patients assessed at the first surgery. *MGMT* methylation status, extent of second surgery, chemotherapy after re-surgery, type of chemotherapy after re-surgery, time between first and second surgery, age, and performance status were considered in univariate and multivariate analysis. The time between first and second surgery was

Table 1. Patient characteristics

Characteristics	Overall population ($n = 295$)		Only patients with both MGMT evaluations ($n = 108$)	
Age, mean (range)	50.7 (18–74)		50.8 (21–74)	
Gender				
Male	193	65.4%	69	63.9%
Female	102	34.6%	39	36.1%
KPS, median (range)	80 (70–100)		80 (70–100)	
MGMT at first surgery	$n = 178$		$n = 108$	
Methylated	79	44.4%	54	50.0%
Unmethylated	99	55.6%	54	50.0%
MGMT at second surgery	$n = 137$		$n = 108$	
Methylated	64	46.7%	49	45.4%
Unmethylated	73	53.3%	59	54.6%
Extent of second surgery				
Biopsy	28	9.5%	16	14.8%
Partial	141	47.8%	42	38.9%
Gross total resection	126	42.7%	50	46.3%
Type of chemotherapy after second surgery	$n = 290$		$n = 106$	
None	58	20.0%	24	22.6%
Temozolomide	97	33.4%	34	32.1%
Nitrosoureas	77	26.6%	29	27.4%
Other (bevacizumab or experiments agents)	58	20.0%	19	17.9%

evaluated as a dichotomous variable with a 6-month cut-off and a 12-month cut-off; we reported and considered only the 12-month cut-off because it was the variable selected by the forward stepwise procedure and it was the most correlated with the outcomes. The SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA) was used as a statistical package. Two-tailed p values less than .05 were considered significant.

Results

Patients' Characteristics

Two hundred ninety-five consecutive patients at first recurrence for GBM who underwent second surgery were evaluated (Fig. 1). Histology at the time of second surgery confirmed GBM in all the cases, with enough viable tumor tissue for accurate testing.

MGMT Methylation Changes

The *MGMT* methylation status was determined for 178 patients (60.4%) at the time of first surgery, with 79 methylated (44.4%) and 99 unmethylated (55.6%) tumors (Fig. 1). At the time of second surgery, *MGMT* methylation was evaluated for 137 patients (46.4%); it was methylated in 64 patients (46.7%) and unmethylated in 73 patients (53.3%). *MGMT* methylation status obtained both at first and second surgery was available for 108 patients (37%, Fig. 1). The patients' characteristics are summarized in Table 1. At the evaluation of second surgery samples, *MGMT* methylation status was changed in 16 of the 54 methylated patients (29.6%) and in 11 of the 54 unmethylated patients (20.4%); the changes were equally balanced in methylated and unmethylated patients at first surgery ($p = .374$, Fig. 2).

We found no differences between the first and the second assessment ($p = .441$), confirmed by the significant and positive concordance between the first and the second *MGMT* methylation assessments ($K = 0.500$, $p < .001$). *MGMT* methylation was stable in 75.0% of cases. To reduce the possibility that discrepancies are due to suboptimal tissue, we also analyzed concordance excluding 16 patients whose biopsy at first or second surgery may have yielded suboptimal samples. However, the rate of *MGMT* methylation stability was superimposable, 72.8%, and concordance between the first and the second *MGMT* methylation assessments was confirmed ($K = 0.459$, $p < .001$). Due to the small sample number of patients with discordance in *MGMT* methylation status, we were not able to find any peculiar characteristic of this population (age, original or relapse side).

OS

Among the 108 patients who have *MGMT* methylation status obtained both at first and second surgery, median survival from first surgery was 24.4 months (95% CI: 21.3–27.5), being 35.2 months (95% CI: 18.9–51.5) in patients with MGMT methylated at both surgeries, 27.3 months (95% CI: 21.4–33.2) in patients with MGMT methylated at first surgery and unmethylated at the second surgery, 23.3 months (95% CI 19.9–26.7) in patients with MGMT unmethylated at first surgery and methylated at the second surgery, and 20.1 months (95% CI 16.6–23.3) in patients with MGMT unmethylated at both surgeries.

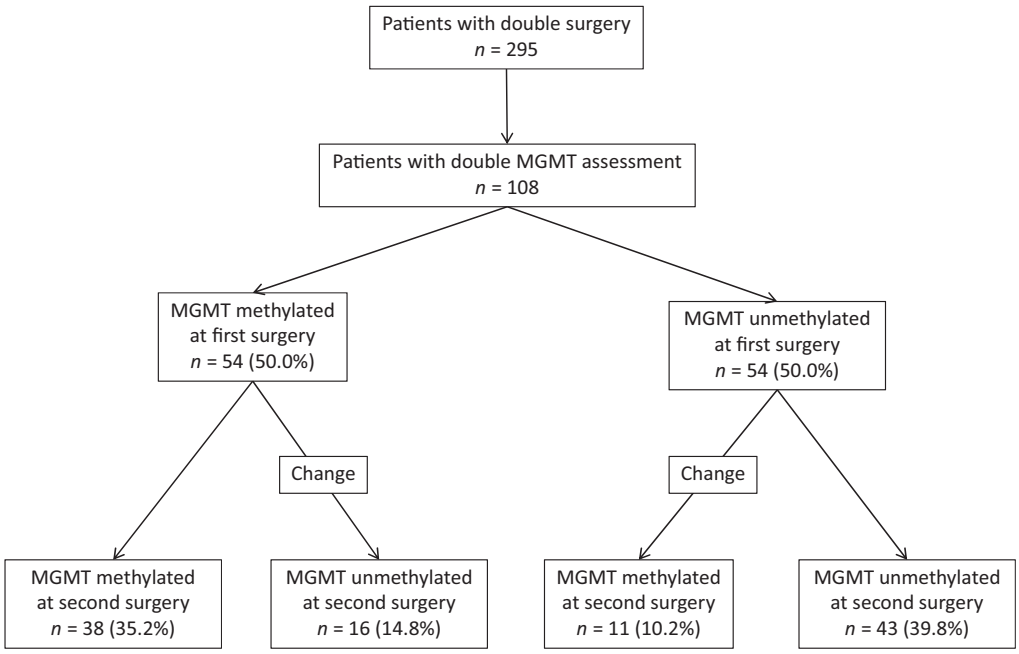

Figure 2. Changes in MGMT methylation status between first and second surgery.

Table 2. Multivariate analysis (only significant variables were included in this table)

	Survival from first surgery				Survival from second surgery			
Characteristic	***HR***	***95% CI***		***p***	***HR***	***95% CI***		***p***
MGMT at first surgery	0.567	0.412	0.779	< .001	—	—	—	—
Extent of second surgery				< .001				< .001
Partial vs. Biopsy	0.421	0.276	0.642	< .001	0.105	0.065	0.170	< .001
Complete vs. Biopsy	0.243	0.156	0.377	< .001	0.048	0.029	0.080	< .001
Complete vs. Partial	0.577	0.442	0.752	< .001	0.456	0.353	0.588	< .001
Age	1.012	1.001	1.023	.030	1.017	1.007	1.028	.001
Time between first and second surgery (12 months cut-off)	0.313	0.242	0.405	< .001	—	—	—	—

Abbreviations: —, not assessable; CI, confidence interval; HR, hazard ratio.

For all 295 patients, in univariate analysis, *MGMT* methylation at first ($p < .001$) and second surgery ($p = .008$), extent of resection at second surgery ($p < .001$), and time between first and second surgery ($p < .001$) were correlated with OS. Median OS was 32.1 months (95% CI: 27.1–37.1) and 21.1 months (95% CI: 19.1–23.1) in patients with MGMT methylated and MGMT unmethylated, respectively. Performance status was not significant ($p = .761$). In multivariate analysis, *MGMT* methylation at first surgery ($p < .001$), extent of resection at second surgery ($p < .001$), and time between first and second surgery ($p < .001$) were significantly correlated with survival (Table 2).

Survival from Time of Second Surgery

For all 295 patients, median survival after second surgery was 10.3 months (95% CI: 9.1–11.4). In univariate analysis, median survival after second surgery was not correlated with *MGMT* methylation obtained at second surgery (10.3 and 9.5 months in patients with *MGMT* methylated and MGMT unmethylated, respectively, $p = .205$), although it was correlated with *MGMT* methylation obtained at first surgery (11.9 and 9.3 months in patients with MGMT methylated and MGMT unmethylated, respectively, $p = .016$). In multivariate analysis, extent of resection at second surgery ($p < .001$) and age ($p = .001$) were significantly correlated with survival (Table 2).

Discussion

Change in biomarker expression in tumors is a phenomenon widely described in oncology. In breast cancer, a lack of concordance in receptor status between primary and recurrent tumors has been described in up to 40% of the cases [11]. However, similar solid data are not available for brain tumors, but some evidences come from a trial with anti-EGFRvIII immunotherapy showing the role of treatment in changing the pattern of biomarker expression [12].

MGMT methylation has a prognostic role in GBM patients [3]. What remains unclear is the stability of this epigenetic

alteration during the clinical course of the disease and thus if retesting *MGMT* methylation could provide useful information.

In our study, we included a large cohort of consecutive GBM patients who underwent a second surgical procedure for recurrent disease in order to analyze the role of *MGMT* methylation assessment of at the time of recurrence. Because postsurgical treatment may have an impact on genetic and epigenetic alterations, we included only GBM patients who received the same treatment, TMZ concurrent with and adjuvant to RT, and we avoided pseudoprogressions because second surgery was performed at least 3 months after RT completion. Moreover, we evaluated the stability of *MGMT* methylation status according to the extent of second surgery (complete or partial versus biopsy). Our study presents some limitations, particularly the retrospective nature of the data, as well as the use of postoperative CT with and without contrast enhancement instead of postoperative magnetic resonance imaging to assess the extent of surgery.

We showed that *MGMT* methylation status remains stable during the clinical course of GBM in the majority of patients, like in other findings from smaller cohorts [6, 7], and thus retesting this biomarker at recurrence does not provide further information. Moreover, in our study, OS from diagnosis is correlated with *MGMT* methylation status obtained at first surgery, but not at recurrence, confirming the role of *MGMT* methylation at diagnosis. Univariate analysis suggested that *MGMT* methylation at diagnosis could also predict survival after disease progression, as suggested by other groups [9]. However, this finding was not confirmed by multivariate analysis. Potential explanations could be that *MGMT* methylation affects OS by increasing progression-free survival but not post-progression survival, or that our sample size was not sufficient to show a role in multivariate analysis. However, 25% of patients showed discordant *MGMT* methylation status at diagnosis and at recurrence.

Different mechanisms can explain change in biomarker expression during the clinical course of neoplasms, such as pre-analytical and analytical errors, intratumoral heterogeneity, and selective pressure of cytotoxic treatments. We excluded that the availability of tissue could be the reason of these discrepancies, because when only patients with greater tissue available were considered (partial or complete resections at both surgeries), the rate of discordant cases was similar (27.2%).

Other potential explanations of this phenomenon could be tumor heterogeneity or technical issues in MSP (i.e., the time from resection to fixation and the process of fixation itself). Tumor heterogeneity for *MGMT* methylation has been investigated by different groups [13–17], with contrasting results. However, larger studies suggested that *MGMT* methylation was homogeneous in tumors [15], at least in frozen samples [13]. Therefore, a technical issue could contribute to discordant results in some cases.

Moreover, it should be considered that MSP could suffer from two technical issues: (a) the number of investigated CpG islands is limited: even if the primers and the beacon probes used for MSP are designed for evaluating the methylation status of the main clinically relevant CpG islands [3, 18], not all the CpG islands in the MGMT promoter region are investigated; for this reason, we are not aware of the methylation status of the entire MGMT promoter, and (b) the amount/quality of input DNA: the MSP is a real-time technique, and the results are evaluated during a "log-linear phase"; if on one hand this approach allows a semiquantitative evaluation of the methylation level, then on the other hand, the results could be influenced by low quantity or low quality of input DNA (e.g., due to over-fixation). Because promoter methylation-mediated gene silencing depends strongly on the location of the methylated CpGs, further improvement in *MGMT* methylation techniques (i.e., HumanMethylation450 - HM-450K BeadChip) [19] could improve results and concordance between samples.

Conclusion

Our study suggests that *MGMT* methylation is stable over time in the majority of the patients. Moreover, the assessment of this biomarker at recurrence barely seems informative for GBM patients.

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Disclosures

Maria Pia Foschini: Roche, Devicor Mammotome (RF). The other authors indicated no financial relationships.

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For Further Reading:

Alba A. Brandes, Marco Bartolotti, Alicia Tosoni et al. Practical Management of Bevacizumab-Related Toxicities in Glioblastoma. ***The Oncologist*** 2015;20:166–175.

Implications for Practice:

Given the widespread use of bevacizumab in clinical practice, it is important to raise clinicians' awareness of the potential risks of this treatment. Our aim was to provide an overview of the most common side effects of bevacizumab and to suggest a practical approach for their management.