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Prognostic Impact of MGMT Promoter Methylation in Glioblastoma - A Systematic Review

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Abstract

MGMT promoter methylation is currently considered the main prognostic biomarker in glioblastoma, yet some concerns remain about its actual impact on outcome. The aim of the present study was to analyze literature data on this topic. Therefore, a systematic review and analysis of recently published glioblastoma cohorts examining the relationship between MGMT methylation and prognosis was performed. We found that only 19/28 studies (68%) confirmed the prognostic value of MGMT methylation and/or its role in predicting response to temozolomide. In these studies, however, the population showed significantly lower rates of unfavorable prognosticators as compared with studies where MGMT methylation was not prognostic/predictive. Moreover, studies demonstrating a better prognosis for MGMT methylated cases had significantly lower rates of deaths at 3 and 6 months. Multivariate analysis showed that the 3-month and 6-month deaths are significantly associated with the prognostic/predictive value of MGMT methylation, and that the percent of MGMT methylated tumors and of patients treated with alkylating drugs trend towards statistical significance if modeled with the 6-month but not with the 3-month mortality rate. These results suggest that the paucity of short-term survivors may represent a bias in studies focusing on MGMT methylation and prognosis, and that a temporal threshold may be necessary in order to demonstrate the clinical benefit of MGMT promoter methylation.

Keywords: Glioblastoma; MGMT; Survival; Epigenomics; Alkylating antineoplastic agents

Abbreviations: Alkyl: Treated with Alkylating Drugs; CI: Confidence Interval; Coeff: Coefficient; Debulk Surg: Debulking Surgery; ECOG: Eastern Cooperative Oncology Group Performance Status; EORTC: European Organisation For Research and Treatment Of Cancer; GBM: Glioblastoma; KPS: Karnofsky Performance Status; Meth: Methylated ; MGMT: O^6-Methylguanine DNA-Methyltransferase ; NA: Not Available or Not Applicable; NCIC: National Cancer Institute of Canada; OR: Odds Ratio; Pros: Prospective; PS: Performance Status; Pts: Patients; Ret: Retrospective; SE: Standard Error; TMZ: Temozolomide

Introduction

O^6-methylguanine DNA-methyltransferase (MGMT) is a DNA-repair protein that removes alkyl groups from the O^6 position of guanine and therefore blunts the cytotoxic effect of alkylating drugs, including temozolomide (TMZ) [1,2]. Epigenetic silencing of the MGMT gene through promoter methylation is associated with diminished DNA-repair activity [2]; this event has been linked with a better response to alkylating drugs and with an improved outcome in malignant glioma [1]. The 2-year analysis of the milestone multicenter randomized EORTC-NCIC trial confirmed the prognostic and predictive role of MGMT promoter methylation for response to TMZ in glioblastoma (GBM) [3]. The 5-year analysis of survival data from the same trial confirmed the prognostic role of MGMT promoter methylation but resized its predictive value for response to TMZ [4]. Recently, the prognostic role of MGMT promoter methylation in GBM has been confirmed in a large phase III trial comparing standard-dose and dose-intensified TMZ in newly diagnosed GBM [5].

Several issues have been raised on the actual role of MGMT promoter methylation in GBM, including the choice of the method to assess the methylation status [6] and the relationship between MGMT promoter methylation and reduced MGMT protein expression [7-9]. Apart from technical problems, however, an accurate analysis of the pertinent literature shows that the prognostic and predictive role of MGMT promoter methylation in GBM was not confirmed in a substantial number of cohorts [9-18]. The aim of the present study was to review published series that addressed the issue whether MGMT promoter methylation status may be correlated with survival of GBM patients.

Materials and Methods

Articles search

A search was performed in Pubmed database (http://www.ncbi.nlm.nih.gov/pubmed) using as keywords, "glioblastoma", "MGMT promoter methylation", "survival analysis", and as search operator "AND". The search was limited to articles published after 2005, i.e. the year in which TMZ chemotherapy began the standard for treatment of GBM patients [19]. This search retrieved 107 articles; through alternative searches, 2 more articles were found [11,20]. Of these 109 articles, 82 were excluded from analysis because of the followings, 1) data were not original (*N*=17); 2) Kaplan-Meier survival curves were not available (*N*=21); 3) series included less than 30 patients (*N*=10); 4) GBM patients were categorized using non-canonical criteria (*N*=23); 5) lower grade gliomas (*N*=3) and/or other tumors (*N*=3) were included;

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6) only *in vitro* or *in vivo* data were reported *(N=3)*, and 7) patients did not receive adjuvant radio-chemotherapy (*N*=2). We also included the recently published results of the phase III trial comparing standard-dose and dose-dense adjuvant TMZ for newly diagnosed GBMs (NCT00304031) [5]. Therefore, our final analysis included 28 studies, in which survival data were analyzed in relation with the MGMT methylation status.

Articles analysis

From the 28 studies addressing the question of the correlation between MGMT promoter methylation and prognosis, the following data were extrapolated, number of GBM cases, type of study (retrospective, prospective, trial), median age of patients (or mean age, if median not available), percent of patients with good performance status, percent of patients who underwent debulking *vs* biopsy surgery, percent of patients treated with TMZ or other alkylating agents, percent of cases in whom MGMT promoter methylation status was assessed, percent of cases with methylated MGMT promoter, and percent of deaths at 3 and 6 months from diagnosis of GBM. Whenever possible, this analysis was restricted to the subset of patients treated with radiotherapy plus adjuvant alkylating chemotherapy and in whom MGMT methylation status was assessed. Good performance status was defined as ECOG 0 or 1 or Karnofsky performance status (KPS) > 70. Debulking surgery was defined as any surgical operation for tumor removal excluding biopsy. Extrapolation of 3-month and 6-month survival data was performed based on the Kaplan-Meier curves. In those studies where overall survival was calculated from the date of randomization, deaths at 3 and 6 months from diagnosis were calculated based on study protocol details.

Statistical analysis

Comparison of clinical parameters (3-month and 6-month death rate, percent of retrospective studies, percent of patients with good performance status, percent of patients treated with debulking surgery, percent of patients treated with alkylating drugs, and percent of tumors with methylated MGMT promoter) among the different study groups was performed using the Chi-square test. Comparison of median age was performed using the Student's *t*-test. A multivariate logistic regression model was used to estimate the odds ratio of the prognostic/predictive significance of MGMT promoter methylation while adjusting for baseline variables that included number of cases, age, percent of methylated MGMT promoter, type of study (whether retrospective or not), treatment with alkylating drugs and deaths at 3 and at 6 months. The results are reported as 2-sided *P* values with 95% confidence intervals. Differences were considered significant at $P<0.05$. Statistical analyses were conducted using Stat View version 5 software (SAS Institute Inc., Cary, NC).

Results

Out of 28 studies that addressed the question of the correlation between MGMT promoter methylation and prognosis of GBM patients, 19 articles (68%) showed a direct relationship between the two variables (MGMT methylation predictive/prognostic of better prognosis), whereas in 9 articles (32%) such a relationship was not demonstrated (MGMT methylation no predictive/prognostic) (Table 1). The size of the study did not differ significantly between studies with MGMT predictive/prognostic and studies with MGMT not predictive/prognostic (136.4 ± 168.1 patients in the first group *vs.* 82.9 ± 32.8 patients in the second group, mean+standard deviation; $P=0.6759$, Mann-Whitney *U* test; 63.2% of studies with ≥70 patients in the first group *vs.* 55.6% in the second group; $P>0.9999$, Fisher's Exact Test). All the studies were analyzed for clinical variables of known prognostic value, like the age of patients, performance status, type of surgery (debulking *vs* biopsy), and for additional variables including the type of study (whether retrospective or not), adjuvant treatment with alkylating drugs, percent of MGMT methylated tumors, and 3-month and 6-month death rates. We found that only age was homogeneously distributed between studies where MGMT methylation was predictive/prognostic and those where MGMT methylation was not predictive/prognostic, whereas all the remaining parameters were significantly different (Table 2). In particular, clinical variables with known unfavorable prognostic value, like poor performance status and biopsy surgery, were significantly higher in studies where MGMT methylation was not predictive/prognostic (Table 2).

We then established a multivariate logistic regression analysis that accounted for number of patients, age, percent of MGMT methylated tumors, type of study, treatment with alkylating drugs, and either 3-month or 6-month death rate. This analysis showed that both deaths at 3 and at 6 months were significantly associated with the predictive/prognostic significance of MGMT promoter methylation in a study cohort ($P=0.0479$ and 0.0151, respectively; Tables 3 and 4). In the model accounting for 6-month death rate, both the percent of MGMT methylated tumors and the percent of cases treated with alkylating drugs trended towards statistical significance ($P=0.0646$ and 0.0536, respectively; Table 4).

In order to determine the specific weight of early deaths in MGMT-related prognosis, we assessed in each study the percent of deaths at 3 months both in MGMT methylated cases and in MGMT unmethylated cases (Table 5). Interestingly, we found that the 3-month mortality rate was significantly different both between the methylated cases of predictive/prognostic and the methylated cases of no predictive/prognostic series, and between the unmethylated cases of predictive/prognostic and the unmethylated cases of no predictive/prognostic series ($P=0.0003$ and 0.001, respectively; Table 6).

Discussion

MGMT promoter methylation and GBM prognosis: state-of-art

Since data from the milestone EORTC-NCIC trial became available [3], MGMT promoter methylation status has been regarded as the main prognostic biomarker for GBM [4,6]. There is a large number of publications that support this statement [3,5,20-36]. Methylation of the promoter of the MGMT gene is believed to result in lower levels of MGMT protein and therefore in a reduced removal of promutagenic alkyl adducts from DNA, hence strengthening the efficacy of alkylating drugs including TMZ [1,2]. However, there are some concerns on this theory. First, the association between MGMT promoter methylation and reduced MGMT protein expression in GBM is still a matter of debate in literature [7,8,9]. Moreover, issues have recently been raised on the reliability of methods used for assessing the MGMT promoter methylation status, that include, *i)* low precision of current techniques, *ii)* heterogeneous methylation of cytosine-guanine dinucleotides in the gene's promoter region, and *iii)* contamination of macrodissected specimens by nontumor cells [6].

Findings of the current study

Here, we performed a systematic review of the literature in order

Authors & Year	*N* cases	Type of study	Age (yrs)	Good PS (%)	Debulk surg (%)	Alkyl (%)	MGMT assayed (%)	MGMT meth (%)	Deaths (%)	
									3 mos	6 mos
MGMT methylation prognostic/predictive										
Balañà et al., 2011 [21]	70	ret[a,b]	64	88.6	85.7	98.6	100	38.6	5.7	17.1
Brandes et al., 2008 [22]	103	trial[a,b]	53	92	99	100	100	35	0	1.9
Cao et al., 2009 [23]	73	ret[a]	55	54.2	91.6	100	100	60.3	1.4	8.2
Crinière et al., 2007 [24]	77	ret[a]	58	63	NA	100	100	58.4	1.3	6.5
Dunn et al., 2009 [20]	109	ret[a,b]	55	84	76	100	100	53.2	3.7	15.6
Ellingson et al.., 2012 [25]	358	ret[a]	56.4	NA[c]	NA	NA	100	36	1.1	5
Etcheverry et al., 2010 [26]	50	pros[a]	57.5	NA[d]	NA	100	100	60	8	18
Felsberg et al., 2009 [27]	66	pros[a,b]	56	82	100	100	100	39.4	0	1.5
Gilbert et al.., 2013 [5]	760	trial[a,b]	NA[e]	100	97	100	100	32.1	0.8	6.7
Hegi et al., 2005 [3]	106	trial [a,b]	56	87.3	83.8	100	100	43.4	1.9	10.4
Karayan-Tapon et al., 2010 [28]	81	ret[a]	61	64.2	NA	100	100	67.9	0	2.5
Lai et al., 2011 [29]	141	trial + ret[a,b]	58	96.1	86.2	100	100	40.4	0.7	7.1
Lakomy et al., 2011 [30]	38	ret[a,b]	53	95	100	100	100	31.6	2.6	7.9
Metellus et al., 2011 [31]	61	pros[a,b]	59	34.4	100	100	100	37.7	0	1.6
Morandi et al., 2010 [32]	159	ret[a]	57	100	NA	100	100	44	1.9	6.3
Motomura et al., 2011 [33]	68	ret[a]	55	66.2	NA	100	100	33.8	1.5	2.9
Stupp et al., 2010 [34]	45	trial[a,b]	57	92	83	100	100	51.1	2.2	8.9
Weiler et al., 2010 [35]	41	trial[b]	56	100	90	100	95.1	41	0	2.4
Weller et al., 2009 [36]	185	pros[a,b]	61.5	84	90	100	100	43.8	2.7	7
MGMT methylation no prognostic/predictive										
Clarke et al., 2009 [10]	85	trial	56.3	73	78	100	56.5	18.8	0	4.7
Costa et al., 2010 [11]	80	ret	56	53.3	90	100	100	47.5	6.3	23.8
El Hindy et al., 2011 [12]	160	ret	58	NA	59.4	37.5	66.3	18.9	13.8	31.9
Iliadis et al., 2012 [13]	65	pros	59	90	90.8	100	35.4	43.5	1.5	7.7
Lam&Chambers, 2012 [14]	101	ret	56.5	47	68	100	100	49.5	3	15.8
Martinez et al., 2009 [15]	46	ret	60.6	NA	NA	100	100	32.6	2.2	8.7
Mellai et al., 2009 [9]	67	ret	60.3	NA	100	35	100	29.9	13.4	31.3
Schaich et al., 2009 [16]	63	ret	63	84	NA	100	100	34.9	0	4.8
Tang et al., 2012 [17]	79	ret	NA	57	100	100	100	32.9	5.1	15.2

[a], MGMT prognostic for longer overall survival; [b], MGMT predictive for longer progression-free survival when treated with alkylating drugs; [c], mean KPS 72.4; [d], median KPS 78.6. Alkyl, treated with alkylating drugs; [e], 26.8% of patients with age < 50 yrs. Debulk surg: debulking surgery; Meth: Methylated; MGMT: O^6-Methylguanine DNA-Methyltransferase; NA: Not Available; Pros: Prospective; PS: Performance Status; Ret: Retrospective

Table 1: Systematic review of studies analyzing the relationship between MGMT methylation and prognosis in glioblastoma patients.

Parameter	MGMT promoter methylation		*P*	OR (95% CI)
	Predictive/ prognostic	*No predictive/ prognostic*		
N cases	2591	746	NA	NA
Retrospective studies (%)	45.3	79.9	<0.0001[a]	4.7958 (3.946-5.8285)
Age (yrs)	57.3	58.3	0.1894[b]	NA
Good PS (%)	88.4	65.3	<0.0001[a]	0.2481 (0.1971-0.3123)
Debulking surgery (%)	92.6	79.6	<0.0001[a]	0.3115 (0.2399-0.4046)
Treated with alkylating drugs (%)	100	80.7	<0.0001[a]	0.0019 (0.0003-0.0134)
MGMT methylated	40.4	34.3	0.00617[a]	0.7699 (0.6403-0.9258)
Deaths at 3 months (%)	1.5	6	<0.0001[a]	4.3128 (2.7781-6.6954)
Deaths at 6 months (%)	6.9	18.1	<0.0001[a]	2.9952 (2.3546-3.8102)

[A]: Chi-Square Test; [B]: Student's *t* Test; CI: Confidence Interval; MGMT: O^6-Methylguanine DNA-Methyltransferase; NA:Not Applicable; OR: Odds Ratio; PS: Performance Status

Table 2: Univariate analysis of clinical data in studies assessing the relationship between MGMT methylation and prognosis.

Factor	Coeff	SE	Coeff/ SE	χ^2 value	*P*-value	OR	95% CI
constant	-21.889	13.486	-1.623	2.634	0.1046	3.118×10^{-10}	1.031×10^{-21}-94.322
N cases	0.001	0.015	0.079	0.006	0.9373	1.001	0.973-1.030
median age	0.322	0.223	1.445	2.089	0.1484	1.380	0.892-2.136
MGMT methylated cases	1.953	1.440	1.356	1.839	0.1751	7.053	0.419-118.755
Retrospective study	0.907	1.171	0.775	0.601	0.4383	2.478	0.250-24.586
Treated with alkylating drugs	-5.166	3.412	-1.514	2.292	0.1300	0.006	7.101×10^{-6}-4.585
Deaths at 3 mos	0.489	0.247	1.978	3.914	0.0479	1.631	1.005-2.648

CI: Confidence Interval; Coeff: Coefficient; MGMT: O^6-Methylguanine DNA-Methyltransferase; OR: Odds Ratio; SE: Standard Error

Table 3: Multivariate analysis of factors potentially affecting the predictive/prognostic role of MGMT methylation in a study cohort modeled with 3-month death rate.

Factor	Coeff	SE	Coeff/ SE	χ^2 value	*P*-value	OR	95% CI
constant	-36.921	20.620	-1.791	3.206	0.0734	9.234×10^{-17}	2.582×10^{-34}-33.018
N cases	0.006	0.018	0.351	0.123	0.7256	1.006	0.971-1.043
median age	0.520	0.331	1.572	2.473	0.1158	1.683	0.880-3.220
MGMT methylated cases	4.418	2.390	1.848	3.416	0.0646	82.894	0.765-8979.118
Retrospective study	0.113	1.515	0.075	0.006	0.9406	1.120	0.057-21.835
Treated with alkylating drugs	-9.991	5.176	-1.930	3.726	0.0536	4.583×10^{-5}	1.799×10^{-9}-1.167
Deaths at 6 mos	0.417	0.172	2.429	5.902	0.0151	1.517	1.084-2.124

CI: Confidence Interval; Coeff: Coefficient; Mgmt: O^6-Methylguanine Dna-Methyltransferase; OR: Odds Ratio; SE: Standard Error

Table 4: Multivariate analysis of factors potentially affecting the predictive/prognostic role of MGMT methylation in a study cohort modeled with 6-month death rate.

Authors & Year	*N* cases	Deaths at 3 months (%)		
		whole cohort	methylated pts	unmethylated pts
MGMT methylation prognostic/predictive				
Balañà et al., 2011 [21]	70	5.7	0	9.3
Brandes et al., 2008 [22]	103	0	0	0
Cao et al., 2009 [23]	73	1.4	0	3.4
Crinière et al., 2007 [24]	77	1.3	0	3.1
Dunn et al., 2009 [20]	109	3.7	3.4	3.9
Ellingson et al.., 2012 [25]	358	1.1	0	1.8
Etcheverry et al., 2010 [26]	50	8	6.7	10
Felsberg et al., 2009 [27]	66	0	0	0
Gilbert et al.., 2013 [5]	760	0.8	0.8	0.8
Hegi et al., 2005 [3]	106	1.9	2.2	1.7
Karayan-Tapon et al., 2010 [28]	81	0	0	0
Lai et al., 2011 [29]	141	0.7	0	1.2
Lakomy et al., 2011 [30]	38	2.6	0	3.8
Metellus et al., 2011 [31]	61	0	0	0
Morandi et al., 2010 [32]	159	1.9	0	3.4
Motomura et al., 2011 [33]	68	1.5	0	2.2
Stupp et al., 2010 [34]	45	2.2	0	4.5
Weiler et al., 2010 [35]	41	0	0	0
Weller et al., 2009 [36]	185	2.7	1.2	3.8
MGMT methylation no prognostic/predictive				
Clarke et al., 2009 [10]	85	0	0	0
Costa et al., 2010 [11]	80	6.3	5.3	7.1
El Hindy et al., 2011 [12]	160	13.8	NA	NA
Iliadis et al., 2012 [13]	65	1.5	NA	NA
Lam&Chambers, 2011 [14]	101	3	4	2
Martinez et al., 2009 [15]	46	2.2	6.7	0
Mellai et al., 2009 [9]	67	13.4	10	14.9
Schaich et al., 2009 [16]	63	0	0	0
Tang et al., 2011 [17]	79	5.1	3.8	5.7

MGMT: O^6-methylguanine DNA-methyltransferase; pts: patients

Table 5: Deaths at 3 months according to MGMT status in reviewed studies.

MGMT promoter methylation status	Deaths at 3 months (%)		*P*	OR (95% CI)
	Studies with MGMT methylation prognostic/predictive	*Studies with MGMT methylation no prognostic/predictive*		
Methylated	0.8	4.4	0.0003	6.0291 (2.2331 – 16.2776)
Unmethylated	1.9	4.6	0.001	2.4363 (1.2761 – 4.6516)

CI: Confidence Interval; MGMT: O^6-Methylguanine DNA-Methyltransferase; OR: Odds Ratio

Table 6: Mortality at 3 months according with MGMT status in reviewed studies.

to identify possible bias of GBM studies focusing on the prognostic/predictive role of MGMT promoter methylation. We included only studies on at least 30 patients, in order to rule out minor case series. Moreover, to further take into account the power of the study, we included the numerosity of the patients sample among the independent variables of our multivariate analysis. One main result is that in those studies where MGMT promoter methylation was not prognostic/predictive of better outcome, the patient population showed significantly higher percentages of unfavorable prognosticators, including poor performance status and biopsy surgery, as compared with studies where MGMT promoter methylation was prognostic/predictive of better outcome. Another important result of our analysis is that studies demonstrating a better prognosis of MGMT methylated cases show significantly lower rates of deaths at 3 and 6 months, and that the 3-month and 6-month death rates are significant independent variables associated with the prognostic value of MGMT promoter methylation.

Interpretation of the findings

There are several reasons that may explain these results. First of all, the inclusion criteria might differ between studies that demonstrated a prognostic role of MGMT methylation and those studies where MGMT methylation had no prognostic value. Although none of the reviewed studies identified the early deaths as exclusion criteria, some sort of case selection is suggested by the observation that the 3-month mortality rate was significantly lower in the unmethylated cases of MGMT prognostic/predictive studies than in the unmethylated cases of the MGMT no prognostic/predictive studies. Another possible explanation of our results is that the short-term survivors could not complete standard adjuvant treatments, particularly alkylating drugs, which are expected to be more effective in MGMT methylated patients. Results of multivariate analysis showing that MGMT methylation trends to statistical significance if modeled with the 6-month mortality rate but not with the 3-month mortality rate support the concept that a temporal threshold may be necessary in order to demonstrate any clinical benefit of MGMT promoter methylation. Our analysis thereby suggests that the predictive role of MGMT promoter methylation may apply to GBM patients with survival longer than 6 months and should not be extended to the short-term survivors.

Conclusion

We have identified a possible bias in GBM studies that show a positive predictive/prognostic significance of MGMT promoter methylation, namely the low rate of early deaths. Considering that the present study has been conducted on the limited literature currently available, these results do not warrant a delay in the start of TMZ treatment. However, in the first 6 months from diagnosis of GBM clinicians should be cautious in awarding clinical significance to MGMT promoter methylation.

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