MGMT Methylation: A Marker of Response to Temozolomide in Low-Grade Gliomas

Sibille Everhard, MSc,[1,2] Gentian Kaloshi, MD,[2,3]
Emmanuelle Crinière, MSc,[1,2]
Alexandra Benouaich-Amiel, MD,[2,3] Julie Lejeune, MSc,[2,3]
Yannick Marie, MSc,[1,2] Marc Sanson, MD, PhD,[1–3]
Michèle Kujas, MD, PhD,[2,3] Karima Mokhtari, MD,[2,3]
Khê Hoang-Xuan, MD, PhD,[1–3]
Jean-Yves Delattre, MD,[1–3] and Joëlle Thillet, PhD,[1,2]

The methylation status of the O6-methylguanine-methyltransferase promoter (MGMTP) was evaluated in 68 low-grade gliomas treated by neoadjuvant temozolomide. Methylated MGMTP was detected in 63 of 68 (92.6 %) patients and was a favorable predictor of progression-free survival as compared with unmethylated MGMTP tumors ($p < 0.0001$). Assessment of MGMTP status could help identifying low-grade gliomas patients more likely to respond to chemotherapy or to benefit from MGMT depletion strategies.

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The DNA repair protein O6-methylguanine-methyltransferase (MGMT) is a marker of resistance to chemotherapeutic alkylating agents including temozolomide (TMZ) in high-grade gliomas, particularly glioblastomas.[1–3] Some low-grade gliomas (LGGs) were also found hypermethylated for the MGMT promoter (MGMTP),[4–7] but the implication of MGMTP methylation with respect to drug sensitivity is unsettled. The objective of this study was to evaluate the frequency of MGMTP methylation in LGGs and to determine whether MGMTP status could help to predict LGG response to TMZ.

From the [1]Institut National de la Sante et de la Recherche Médicale, U711; [2]Université Pierre & Marie Curie, Faculté de médecine Pitié Salpétrière, Institut Fédératif de Recherche 70; and [3]Assistance Publique-Hôpitaux de Paris, Hôpital de la Pitié-Salpétrière, Service de Neurologie Mazarin, Paris, France.

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Address correspondence to Dr Thillet, INSERM, U711, Batiment de la nouvelle Pharmacie, 47 Bd de l'Hôpital, 75651 Paris cedex 13, France. E-mail: thillet@ext.jussieu.fr

Patients and Methods

Patients

Patients were selected from our database encompassing clinical information regarding patients with a primary brain tumor seen in our department since 1997. Inclusion criteria were required for inclusion in this study: histological diagnosis of LGGs (World Health Organization grade II) after central review, available cryopreserved tumor material, measurable disease on magnetic resonance imaging, available clinical and radiological follow-up to evaluate tumor response and progression-free survival (PFS), evidence of radiological progressive disease, initial treatment with TMZ without previous treatment for the tumor other than surgery, and informed consent obtained for molecular analysis. TMZ was administered orally from days 1 through 5 at a starting dose of 200mg/m^2, repeated every 28 days after the first daily dose of TMZ. In the absence of unacceptable toxicity (repeated grade IV blood toxicity despite a 25% dose reduction) or of disease progression, patients continued to receive TMZ for at least 12 cycles and up to 24 cycles, based on the clinical judgment of the referring physician. Follow-up was based on clinical examination and brain magnetic resonance imaging with gadolinium infusion repeated every 2 months.

Patients left the study at malignant transformation (histologically proved or suspected when rapidly growing foci of enhanced contrast appeared on imaging) or when tumor progression required a treatment other than TMZ, which was mainly radiotherapy.

Radiographic response to TMZ was evaluated on the measurable change of the product of the two largest perpendicular diameters of the tumor on the axial planes of the magnetic resonance imaging (T2/fluid-attenuated inversion recovery–weighted images), as reported previously.[8] In brief, partial response was defined as ≥50% reduction in the size of T2/fluid-attenuated inversion recovery nonenhancing tumor; minor response was defined as ≥25 to 50% reduction in the tumor size, and progressive disease was defined as greater than 25% increase in tumor size. Stable disease was defined as any other clinical status not meeting the criteria for partial response, minor response, and progressive disease that was observable at least 6 months.

Molecular Analysis

TUMORS SAMPLES AND DNA PREPARATION. DNA from frozen samples blocs was extracted using a standard protocol (Qiagen, QIAmp DNA Mini Kit).

BISULFITE TREATMENT OF DNA AND NESTED METHYLATION-SPECIFIC POLYMERASE CHAIN REACTION. DNA methylation status of the MGMT promoter was determined by bisulfite modification and subsequent Nested methylation-specific polymerase chain reaction, a two-stage polymerase chain reaction approach, as described previously,[9] except that polymerase chain reaction cycles were reduced to 30 cycles. The sodium bisulfite treatment was conducted using the EZ DNA Methylation Kit (Zymo Research, Orange, USA). DNA treated with SssI methyltransferase (New England Biolabs, Beverly, MA) and modified by bisulfite treatment was used as a positive control. DNA extracted from

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lymphocytes of healthy individuals was used as a negative control.

GENETIC ANALYSES. Patients were investigated for mutation of TP53 gene, expression of TP53, and loss of heterozygosity (LOH) of chromosomes 1p, 19q, 9p, and 10q by microsatellite polymorphism analysis.[8,10] Four polymorphic markers were located on 1p36 and four on 1p32-21 for chromosome 1p. Four polymorphic markers were located on 19q13.4-13.1 (chromosome 19q), four on 9p24-21 (chromosome 9p), and four on 10q23.3-22.1 (chromosome 10q).

Statistical Analysis

χ^2 and Fisher's exact tests for small sample were used to test the association between the radiological response and MGMTP methylation and the association between molecular alterations and MGMTP status. PFS was defined as the time from start of chemotherapy until the first unequivocal sign of radiological or clinical progressive disease. Patients who had no evidence of disease progression were treated as censored for the analysis of PFS. Probability estimates for PFS were calculated using Kaplan–Meier methodology. The log-rank test was used to test for equality of PFS distribution. Two-sided p values less than 0.05 were considered significant.

Results

Patient Characteristics and Response to Temozolomide

Sixty-eight patients fulfilled the inclusion criteria; their main characteristics are indicated in Table 1. Molecular and histological characteristics of patients according to their response to TMZ are shown in Table 2. Patients received a median number of 12 cycles of TMZ (range, 2–24 cycles) during a median follow-up of 19 months (range, 5–58 months). Response to TMZ was assessable in all patients. Five patients had a partial response (7%), 27 had a minor response (40%), 29 were stable (43%), and 7 had progressive disease (10%). Hence, the objective response rate was 47%. The median overall PFS was 28 months (95% confidence interval [CI], 20-Inf) for the whole group. The 1-year PFS rate was 77%. Patients who had an objective response to TMZ had a median PFS of 32.4 months (95% CI, 28-Inf), as compared with 21.5 months for patients with stable disease (95% CI, 17.5-Inf) and 4 months for patients with progressive disease (95% CI, 3-Inf) ($p < 0.0001$). Clinically, seizures improved in 30 of 55 patients who had seizures at the onset of TMZ, including 7 patients without objective radiological response.

MGMT Promoter Methylation Status

MGMTP methylation status could be determined for all the 68 LGG tumors obtained as frozen samples (Fig). Methylation of the MGMTP was found in 63 of the 68 tumors (92.6 %). As shown in Table 1, patients with methylated (M-MGMTP) and unmethylated MGMTP (U-MGMTP) were comparable for age, histology, and previous surgical treatment.

Correlations among Responses to Temozolomide, MGMT Promoter Status, and 1p-19q Loss

As shown in Table 2, M-MGMTP patients had a longer median PFS (29.5 months; 95% CI, 21.5-Inf) than U-MGMTP patients (6 months; 95% CI, 5-Inf; $p < 0.0001$). Furthermore, patients with M-MGMTP tumors had a higher rate of response to TMZ and a lower rate of progressive disease than patients with U-MGMTP tumors, although the difference did not reach statistical significance ($p = 0.12$).

Patients with 1p-19q loss also had a higher rate of objective response (68 vs 34%; $p = 0.02$) and median PFS (35 vs 23 months; $p = 0.04$) compared with patients without 1p-19q loss.

There was no significant association between MGMTP status and LOH on 1p (n = 61), 19q (n = 61), combined LOH on 1p-19q (n = 61), or for the others genetic alterations (LOH on 9p [n = 59], 10q [n =

Table 1. Main Characteristics of Patients according to Their MGMT Promoter Methylation Status

Characteristics	Total (N = 68)	U-MGMTP (N = 5)	M-MGMTP (N = 63)	*p*
Age, yr				0.60
Median	41	35	41	
Range	24–78	24–70	24–78	
Histology, n (%)				0.64
Oligodendrogliomas II	42 (62)	3 (60)	39 (62)	
Oligoastrocytomas II	18 (26)	1 (20)	17 (27)	
Astrocytomas II	8 (12)	1 (20)	7 (11)	
Type of resection, n (%)				1
Gross total resection	17 (25)	1 (20)	16 (25)	
Partial resection/biopsy	51 (75)	4 (80)	47 (75)	
1p-19q status (n = 61)				0.43
Intact, n (%)	39 (64)	4 (80)	35 (62.5)	
Lost, n (%)	22 (36)	1 (20)	21 (37.5)	

MGMTP = O6-methylguanine-methyltransferase promoter.

Table 2. Molecular and Histological Characteristics of Patients according to their Response and Progression-Free Survival after Treatment with Temozolomide

Characteristics	Response			*p*	PFS (mo)	*p*
	PR = MR	S	PD			
MGMTP						$<10^{-5}$
Unmethylated	1	2	2	0.12	6	
Methylated	31	27	5		29.5	
1p19q (n = 61)						0.04
Intact	13	21	5	0.02	23	
Lost	15	7	0		35	
Histology						0.52
Oligodendrogliomas II	20	18	4	0.77	28	
Oligoastrocytomas II	7	9	2		20	
Astrocytomas II	5	2	1		34	

PR = partial response; MR = minor response; S = stable; PD = progressive disease; PFS = progression-free survival; MGMTP = O6-methylguanine-methyltransferase promoter.

60], mutation of *TP53* gene [n = 29], and expression of *TP53* [n = 35]).

Discussion

This study indicates that the MGMTP gene is frequently methylated in LGGs, and that a methylated status is a favorable predictor of PFS in LGGs treated with neoadjuvant TMZ.

The 92.6% rate of MGMTP methylation found in this large series is higher than previously reported figures in LGGs[4,6,7] (47.5–78.5%). However, the study that reported the lower MGMTP methylation rate was performed on DNA extracted from paraffin-embedded blocs.[4] In our experience, the frequency of M-MGMTP is lower in paraffin-embedded compared with frozen tumors (unpublished results), and caution is therefore needed to interpret the literature on MGMTP status when paraffin conservation was used.

These results suggest that MGMTP methylation is an "early" epigenetic event in the progression of glial tumors, as previously found for some genetic alterations such as *TP53* mutation or chromosomes 1p-19q loss.[11,12]

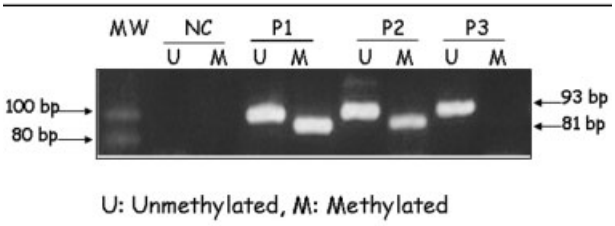

Fig. Unmethylated (U-MGMTP) and methylated O6-methylguanine-methyltransferase promotor (M-MGMTP) gel products of three patients (P1 and P2 have a M-MGMTP status and P3 has a U-MGMTP status) and the negative control (NC, containing polymerase chain reaction mix, primers and water). In all samples considered as methylated for MGMTP, both methylated and unmethylated templates were present.

In the absence of treatment, the prognostic impact of the methylation status of MGMTP on the natural history of gliomas is unclear, but a recent study in LGGs showed that untreated patients with M-MGMTP tumors had a shorter PFS than patients with U-MGMTP tumors.[13]

Treatment with TMZ could reverse this natural trend, because we found that M-MGMTP patients had a significantly longer PFS after TMZ than U-MGMTP patients. These results are in agreement with a recent study showing that low MGMT protein expression is associated with response to TMZ in a series of 9 low-grade oligodendrogliomas.[14]

Thus, LGG data appear to recapitulate the findings in malignant gliomas, where a M-MGMTP status is correlated with increased response[3,15] and PFS[1,15] after treatment with nitrosoureas[15] or TMZ.[1,3] Whether MGMTP methylation status also favorably influences overall survival in TMZ-treated LGG, as it does in glioblastomas, remains unknown because the low number of deaths in our series prevents a reliable analysis of the survival end point.

Others molecular predictors of response to treatment have been identified previously in LGGs and in anaplastic oligodendrogliomas. The most important of them is chromosome 1p ± 19q loss, which is associated with an increased rate of response and PFS in patients treated with nitrosoureas[16,17] or TMZ.[8,14] Our patients did not escape to this rule, but further analysis of the relations among chromosome 1p-19q loss, MGMTP status, and chemosensitivity is hampered by the low number of U-MGMTP patients in LGGs.

Although confirmation by prospective trials is needed, this study suggests that evaluation of the MGMTP methylation status could be useful during the

initial workup of LGGs to help in selecting patients more likely to respond to chemotherapy or those who could benefit from MGMT depletion strategies.

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