Original Article
The prognostic value of O\textsuperscript{6}-methylguanine-DNA methyltransferase gene promoter methylation detected by gel-based methylation-specific polymerase chain reaction in patients with glioblastoma multiforme: a systematic review

Cheng-Ta Hsieh\textsuperscript{1,2,3,4}, I-Chang Su\textsuperscript{1,2}, Chi-Jung Huang\textsuperscript{5,6}, Chih-Ju Chang\textsuperscript{1,2}, Jinn-Shyan Wang\textsuperscript{2}

\textsuperscript{1}Division of Neurosurgery, Department of Surgery, Cathay General Hospital, Taipei 10630, Taiwan R. O. C.; \textsuperscript{2}School of Medicine, Fu Jen Catholic University, New Taipei City 24205, Taiwan R. O. C.; \textsuperscript{3}Department of Chemistry, Fu Jen Catholic University, New Taipei City 24205, Taiwan R. O. C.; \textsuperscript{4}Graduate Institute of Basic Medicine, Fu Jen Catholic University, New Taipei City 24205, Taiwan R. O. C.; \textsuperscript{5}Department of Medical Research, Cathay General Hospital, Taipei 10630, Taiwan R. O. C.; \textsuperscript{6}Department of Biochemistry, National Defense Medical Center, Taipei 11490, Taiwan R. O. C.

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Abstract: Background: Temozolomide (TMZ) plays an important role in the treatment of glioblastoma multiforme (GBM). O\textsuperscript{6}-methylguanine-DNA methyltransferase (MGMT) gene promoter methylation has been described as a prognostic factor in patients with GBM treated by TMZ. However, the prognostic value of MGMT gene promoter methylation in GBM, as investigated by gel-based methylation-specific polymerase chain reaction (MS-PCR), remains unclear. Thus, this systematic review provides an overview of the prognostic value of methylated MGMT promoter in the patients with GBMs treated by TMZ as detected by gel-based MS-PCR. Method: The relevant literatures were conducted on PubMed, Medline and Cochrane databases. The outcomes including the median survival, median progression-free survival (PFS), survival rate, and PFS rate were extracted and analyzed. Results: Five studies were reviewed. The methylated MGMT promoter was described in 35% to 67.9% of patients. Median survival ranged from 16 to 43.6 months (methylated MGMT promoter) vs. 12.7-16.8 months (unmethylated MGMT promoter). Survival ranged from 62% to 73.9% (methylated MGMT promoter) vs. 41.2%-65% (unmethylated MGMT promoter) at 1 year and from 15.8% to 46% (methylated MGMT promoter) vs. 13.8%-21% at 2 years. Median PFS ranged from 7.1 to 21.9 months (methylated MGMT promoter) vs. 5.3-10 months (unmethylated MGMT promoter). PFS ranged from 52% to 68.9% (methylated MGMT promoter) vs. 40%-43.8% (unmethylated MGMT promoter) at 0.5 year and from 30% to 53% (methylated MGMT promoter) vs. 11.8%-24% at 1 year. Conclusion: The methylation status of MGMT promoter, as detected by gel-based MS-PCR assay, could offer prognostic value in patients with GBM treated by TMZ.

Keywords: Glioblastoma multiforme, gel-based methylation-specific polymerase chain reaction, O\textsuperscript{6}-methylguanine-DNA methyltransferase, malignant glioma, prognostic value

Introduction

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults [1]. The gold standard for treatment of GBM is surgical resection with feasible extent, followed by adjuvant chemotherapy and radiotherapy [2]. Most conventional chemotherapeutic agents are ineffective in GBM patients due to the blood-brain barrier (BBB) and, thus, the median survival is usually less than one year [3, 4].

Temozolomide (TMZ), a newly developed, oral alkylating agent used in the management of GBM, can pass through the BBB [5] with a better median survival [6]. Therefore, radiotherapy plus continuous daily TMZ, followed by six cycles of adjuvant TMZ, has become the gold standard adjuvant therapy in GBM [2, 6].

The cytotoxicity of TMZ depends primarily on the methylation of O\textsuperscript{6}-gaunine in the genomic DNA [7]. In the DNA repair system, the function of O\textsuperscript{6}-methylguanine-DNA methyltransferase...
(MGMT) protein is considered to play an important role in the resistance to TMZ treatment [8]. Hegi et al. first described MGMT promoter methylation [as determined by gel-based methylation specific polymerase chain reaction (MS-PCR) analysis] as an independent favorable prognostic factor in patients with GBMs treated by TMZ [9]. However, the benefit of MGMT promoter methylation has not always correlated with clinical outcome [10, 11]. This result may be related to the CpG sites of MGMT promoter and the epigenetic mechanism, resulting in a diverse expression of MGMT protein [12, 13].

Although various new techniques have been used to investigate the methylation status of MGMT promoter, gel-based MS-PCR is generally accepted as a valid technique to derive prognostic information regarding its methylation status [10, 11]. The relationship between the methylation status of MGMT promoter detected by the gel-based MS-PCR and the clinical outcome of GBM patients treated by TMZ, however, remains unclear.

This systematic review and meta-analysis provides insight into the prognostic value of the methylation status of MGMT promoter [as detected by gel-based MS-PCR [9] in patients with GBMs treated with TMZ guided by Stupp’s protocol [6].

Methods

Search strategy

PubMed, Medline and Cochrane databases were searched until April 30, 2015 using different combinations of the following keywords: malignant glioma, GBM, MGMT promoter, MS-PCR, gel-based MS-PCR, TMZ, radiotherapy, survival, progression-free survival. Potentially relevant studies were identified from the reference lists of the studies obtained from the database search.

Study selection

Inclusion criteria were: (1) patients with newly diagnosed GBM; (2) treatment with the current guidelines as proposed by Stupp et al. [6]; and (3) MGMT promoter methylation status investigated by the same primer and gel-based MS-PCR as defined by Hegi et al. [9].

An English-only restriction was also imposed during our search.

Data extraction

Data were extracted from eligible studies by two independent reviewers. A third reviewer was consulted for resolution of any disagreements which arose between the two reviewers.

The following information was extracted from the studies that met the study criteria: author details, year of publication, study country, number of patients, age, percentage of males, performance status, surgical method used, specimens obtained, annealing condition of MS-PCR, median survival, median progression-free survival (PFS), survival rate, and PFS rate.

Outcome measures

Outcome measures included median overall survival (OS), median PFS, survival rate, and PFS. A meta-analysis was performed on the 1-year survival rates, 2-year survival rates, and 1-year PFSs.

Statistical analysis

Heterogeneity among studies was assessed using the I² statistic. The I² statistic indicates the percentage of observed between-study variability caused by heterogeneity.

Heterogeneity, as determined by the I² statistic, was defined as follows: 0 to 24% = no heterogeneity; 25 to 49% = moderate heterogeneity; 50 to 74% = large heterogeneity; and 75 to 100% = extreme heterogeneity. When heterogeneity was observed between studies (P < 0.1 or an I² statistic > 50%), a random-effects model was performed. Otherwise, a fixed-effects model was used when the p-value was > 0.1 or the I² statistic was < 50%. Pooled estimates for differences in median OS time were calculated, and a 2-sided p-value < 0.05 was considered statistically significant. All statistical analyses were performed using the statistical software Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

Results

Study selection

A total of 1,326 articles were identified initially during the search of relevant databases (Figure
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1). Among the 31 potential articles assessed for eligibility, 26 were excluded. 16 articles were excluded because the Stupp’s protocol [6] was not used for all patients. Nine articles were excluded because gel-based MS-PCR [as defined by Hegi et al. [9] was not utilized. One article did not use the same primer described by Hegi et al. [9]. Finally, five studies were included in this systematic review [9, 14-17].

**Study characteristics**

The study characteristics of these five studies [9, 14-17] are summarized in Table 1. Only one study included international participants [9]. More than 80% of the patients with performance status (such as WHO performance status 0-2 or Karnofsky performance status ≥ 70%) were enrolled in three studies [9, 14, 15]. The complete resection rate of tumor varied from 20% to 65.4%. Formalin-fixed paraffin embedded GBM tissue was primarily used to isolate genomic DNA and to investigate the methylation status of MGMT promoter [9, 14, 15, 17]. Although five studies adopted the same primer as described by Hegi et al. [9], the annealing degree varied from 50 to 62°C.

**Outcomes**

The outcomes are summarized in Table 2. The median ages ranged from 53 years to 61 years and the percentage of males ranged from 55.6% to 66%. The methylated MGMT promoter was described in 35% to 67.9% of patients.

Patients with methylated MGMT promoter had a median survival ranging from 16 months to 43.6 months while patients who had unmethylated MGMT promoter had a median survival ranging from 12.7 months to 16.8 months. All five studies reported a longer median survival in patients with methylated MGMT promoter, and two statistically significant differences were observed regarding one-year and two-year survival rates and PFSs.

The one-year and two-year survival rates in patients with methylated MGMT promoter ranged from 62% to 73.9% and 15.8% to 46%, respectively. For patients with unmethylated MGMT promoter, the one-year survival rate ranged from 41.2% to 65%, and the 2-year survival rate ranged from 13.8% to 21%.

Patients with methylated MGMT promoter had a median PFS ranging from 7.1 months to 21.9 months, and patients who had unmethylated MGMT promoter had a median PFS ranging from 5.3 months to 10 months. Four studies reported a longer median PFS. Only two showed statistical significance.

In addition, the 0.5-year and one-year PFS rate in patients with methylated MGMT promoter ranged from 52% to 68.9% and 30% to 53%, respectively. For patients with unmethylated MGMT promoter, the 0.5-year PFS rate ranged from 40% to 43.8%, and the 1-year PFS rate ranged from 11.8% to 24%.

**Meta-analysis**

Survival rate: Three studies provided 1-year survival rates and a meta-analysis was conducted (Figure 2) [9, 16, 17]. A fixed-effects model was used for the meta-analysis because there was no heterogeneity among these studies (P = 0.81, I² = 0%). The 1-year survival rate was significantly higher in the patients with methylated MGMT promoter compared with
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### Table 1. Characteristics of five studies included in the systematic review

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Study design</th>
<th>Quality score#</th>
<th>Cases with methylations status</th>
<th>Performance</th>
<th>Surgery</th>
<th>Specimen</th>
<th>Annealing degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hegi et al. [9]</td>
<td>2005</td>
<td>International study</td>
<td>R</td>
<td>7</td>
<td>106</td>
<td>WHO performance status 0 (39%), 1 (47%), 2 (13%)*</td>
<td>Biopsy (17%), CR (39%), PR (44%)*</td>
<td>FFPE</td>
<td>59</td>
</tr>
<tr>
<td>Brandes et al. [14]</td>
<td>2008</td>
<td>Italy</td>
<td>NR</td>
<td>6</td>
<td>103</td>
<td>WHO performance status 0 (17%), 1 (75%), 2 (8%)</td>
<td>Biopsy (1%), CR (49.5%), PR (49.5%)</td>
<td>FFPE</td>
<td>60</td>
</tr>
<tr>
<td>Costa et al. [15]</td>
<td>2010</td>
<td>Portugal</td>
<td>NR</td>
<td>6</td>
<td>80</td>
<td>KPS ≥ 70: 82%</td>
<td>Biopsy (10%), Total or subtotal resection (90%)</td>
<td>FFPE</td>
<td>NA</td>
</tr>
<tr>
<td>Karayan-Tapon et al. [16]</td>
<td>2010</td>
<td>France</td>
<td>NR</td>
<td>6</td>
<td>80</td>
<td>WHO performance status 0-2 (64%), 3-4 (28%)</td>
<td>CR (65.4%)</td>
<td>Fresh frozen</td>
<td>62</td>
</tr>
<tr>
<td>Lam &amp; Chambers [17]</td>
<td>2012</td>
<td>Canada</td>
<td>NR</td>
<td>6</td>
<td>101</td>
<td>WHO performance status 0 (11%), 1 (36%), 2 (19%), 3 (4%)</td>
<td>Biopsy (32%), CR (20%), PR (48%)</td>
<td>FFPE</td>
<td>59</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete resection; PR, partial resection; NA, not available; PFS, progression-free survival; FFPE, formalin-fixed paraffin embedded; WHO, world health organization; KPS, Karnofsky performance status scale. *Adopted from Stupp et al. study. #Newcastle-Ottawa quality scale (NOS) (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) was used to assess the methodological quality of the included studies.

### Table 2. Outcomes from studies included in the systematic review

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Cases</th>
<th>Median age (years)</th>
<th>Male Gender (%)</th>
<th>Median Survival (95% CI, months)</th>
<th>Survival Rate (%)</th>
<th>Median PFS (95% CI, months)</th>
<th>PFS Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hegi et al. [9]</td>
<td>2005</td>
<td>M: 46 U: 60</td>
<td>56* 64</td>
<td>M: 21.7 (17.4-30.4), U: 12.7 (11.6-14.4)</td>
<td>M: 73.9 (1 y)#, 46 (2 y)</td>
<td>M: 10.3 (6.5-14), U: 5.3 (5.7-6)</td>
<td>M: 68.9 (0.5 y), 39.1 (1 y)#</td>
<td></td>
</tr>
<tr>
<td>Lam &amp; Chambers [17]</td>
<td>2012</td>
<td>M: 50 U: 51</td>
<td>56.5 65</td>
<td>M: 20.9 (13.1-NA), U: 13.7 (11.5-17.7)</td>
<td>M: 62 (1 y)#, 46 (2 y)</td>
<td>M: 7.1 (5.2-9.9), U: 5.9 (5.1-7.7)</td>
<td>M: 52 (0.5 y)#, 30 (1 y)#</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NA, not available; PFS, progression-free survival; M, methylated group; U, unmethylated group; y, year; NS, not significant; NR, not reported.

*Adopted from Stupp et al. study. #Calculated from the Kaplan-Meier survival curve.
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Those without the methylation status (P = 0.005). The pooled-estimate of odds ratio was 2.06 (95% confidence interval (CI): 1.25 to 3.39).

Two-year survival rate data was provided in three of five studies (Figure 3) [9, 15, 17]. A fixed-effects model was used for the meta-analysis because there was no heterogeneity among these studies (P = 0.18, I^2 = 41%). The 2-year survival rates were significantly higher in patients with methylated MGMT promoter compared with those without the methylation status (P = 0.01). The pooled-estimate odds ratio was 2.19 (95% CI: 1.19 to 4.02).

Progression-free survival rate: Only two studies revealed 0.5-year PFS rate data and, thus, a meta-analysis could not be performed [9, 17]. One-year PFS rates were noted in three studies (Figure 4) [9, 16, 17] and a fixed-effects model was used for the meta-analysis (P = 0.94, I^2 = 0%). The 1-year PFS rate was significantly higher in patients with methylated MGMT promoter compared with those without the methylation status (P < 0.0001). The pooled-estimate odds ratio was 3.71 (95% CI: 2.06 to 6.66).

Discussion

GBM is the most common primary CNS tumor, consisting of 12-15% of all intracranial tumors and 50-60% of gliomas [18]. The prognosis of patients with GBM is extremely poor despite multimodal treatment such as surgery, chemotherapy and radiotherapy [1, 19].

Several prognostic factors have been identified in patients with GBM including age, performance status, extent of surgical resection, mental status, tumor features on magnetic resonance imaging, dose of radiation, adjuvant chemotherapy, and molecular status [20-22]. Although extensive resection of tumor is highly recommended, most GBMs show infiltration of functional or eloquent structures which affects
surgical consideration [21, 23]. Adjuvant therapies (including chemotherapy and radiotherapy) become important in patients with unresectable or residual GBM because conventional chemotherapy has no therapeutic benefit due to its inability to cross the BBB [5].

The development of the new oral alkylating agent, TMZ, has shown significant survival benefit and minimal additional toxicity. Thus, concomitant radiotherapy with TMZ followed by up to six cycles of adjuvant TMZ has become the standard treatment for newly diagnosed GBM [6].

The MGMT protein, an excision repair enzyme, has been proven to be associated with tumor resistance as it may reverse TMZ cytotoxicity by removing the alkylation from the O6 position of guanine [12]. Immunofluorescence detection of the MGMT protein had been used in early studies [24, 25]. However, its clinical value remains controversial because of high interobserver variability or lack of distinction between normal brain and neoplasms [26].

The MGMT gene is located on chromosome 10q26. The CpG island at the 5' region of the promoter (from bp-552 to +289) includes 97 CpG sites which are usually unmethylated in normal tissue [27]. It is still unclear as to how many CpG sites in the CpG island have to be methylated to cause silencing of the transcription of the MGMT gene [11]. Therefore, many assays (including quantitative MS-PCR, combined bisulfate restriction analysis, pyrosequencing, MS-multiplex ligation-dependent probe amplification, or MS-high-solution-melting) are used to investigate a different fraction of CpG sites on the CpG island for methylation of promoter that is more closely associated with silencing of the MGMT gene [10]. Methylation of the MGMT promoter, as measured by MS-PCR analysis, was first confirmed to have benefit in patients with GBM treated with TMZ [9].

The methylation status of the MGMT promoter has been proposed as a powerful and independent prognostic factor during TMZ therapy [10]. However, the clinical prognosis does not always correlate with the methylation status of MGMT promoter, as investigated by gel-based MS-PCR, because of variations in the treated population and variable study conditions [10, 11]. Using criteria including the use of Stupp’s protocol [6] for TMZ and gel-based MS-PCR as defined by Hegi et al. [9], five studies which met these two criteria were investigated to determine the prognostic value of gel-based MS-PCR analysis in detecting methylation status of MGMT promoter in patients with GBM [9, 14-17]. In this systematic review, 35% to 67.9% of the patients had methylated MGMT promoter. Their median survival ranged from 16 months to 43.6 months. On the other hand, patients who had unmethylated MGMT promoter had a median survival ranging from 12.7 months to 16.8 months. A longer median survival in patients with methylated MGMT promoter was found in all five studies. Additionally, a longer median PFS was found in four studies [9, 14, 16, 17]. Patients with methylated MGMT promoter had a median PFS ranging from 7.1 months to 21.9 months, as compared with patients who had unmethylated MGMT promoter who had a median PFS ranging from 5.3 months to 10 months.

In the meta-analysis, the 1-year and 2-year survival rates were significantly higher in patients with methylated MGMT promoter compared with patients with unmethylation status. The pooled estimate of odds ratio in 1-year and 2-year survival were 2.06 and 2.19, respectively. The pooled estimate of odds ratio in 1-year PFS was 3.71. These results revealed that the methylation status of MGMT promoter [as detected using gel-based MS-PCR defined by Hegi et al. [9] could offer prognostic value in patients with GBM treated by TMZ guided by Stupp’s protocol [6].

This study had several limitations. The clinical outcomes may have been affected by patient age, performance status, and radiation dose. The small number of included studies and the comparatively low heterogeneity among the study populations made it difficult to draw significant conclusions. Despite these differences, the patients with methylated MGMT promoter, as detected by gel-based MS-PCR, had a significantly longer median survival and longer median PFS after treatment with TMZ.

**Conclusion**

Patients with methylated MGMT promoter had a significantly longer median survival and longer PFS after treatment with TMZ. The methyla-
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The prognostic value of gel-based MS-PCR, as detected by gel-based MS-PCR assay, could offer prognostic value in patients with GBM treated by TMZ.

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Disclosure of conflict of interest

None.

Address correspondence to: Jinn-Shyan Wang, School of Medicine, Fu-Jen Catholic University, 510, Zhongzheng Rd., Xinzhuang Dist., New Taipei 24205, Taiwan R. O. C. Tel: 886-2-29053438; Fax: 886-2-29052095; E-mail: 034407@mail.fju.edu.tw

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