ORIGINAL ARTICLE

Association of molecular marker O⁶Methylguanine DNA methyltransferase and concomitant chemoradiotherapy with survival in Southern Chinese glioblastoma patients

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Key words
Chemotherapy, adjuvant; DNA methylation; Disease-free survival; Glioblastoma; O⁶-methylguanine-DNA methyltransferase

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Objectives (1) To compare the survival of concomitant chemotherapy and radiotherapy with radiotherapy alone in Chinese patients with primary glioblastoma. (2) To determine the methylation status of O⁶Methylguanine DNA methyltransferase in Chinese primary glioblastoma, and to assess the prognostic value of O⁶Methylguanine DNA methyltransferase methylation status in such patients.

Design Retrospective correlative analysis.

Setting University teaching hospital, Hong Kong.

Patients Patients diagnosed with histologically proven primary glioblastoma in the period of March 2005 to June 2007 were recruited. Genomic DNA was isolated from formalin-fixed and paraffin-embedded sections of glioblastoma tissues. Methylation-specific polymerase chain reaction for O⁶Methylguanine DNA methyltransferase was performed. Patients' information at presentation was collected (age, performance status, steroid use, extent of resection, complications, radiotherapy data, use of chemotherapy). Primary outcome was measured by overall survival while secondary outcome was measured by progression-free survival. Overall and progression-free survivals were estimated by the Kaplan-Meier technique. Outcomes were assessed for groups with and without concomitant chemoradiotherapy and for groups with and without O⁶Methylguanine DNA methyltransferase methylation.

Results A total of 35 glioblastoma patients were recruited; 27 were male and 8 female. Their mean age was 50 years. In all, 17 received concomitant chemoradiotherapy, and 18 received radiotherapy only. Their median overall survival was 12 (range, 7-17) months and the median progression-free survival was 5 (range, 3-6) months. In the radiotherapy alone group, the median progression-free survival and overall survival was 4 (range, 3-5) months and 6 (range, 2-10) months, respectively. In the concomitant radiochemotherapy group, the median progression-free survival and overall survival was 6 (range, 2-10) months and 13 (range, 8-18) months, respectively. Fifteen (43%) of the tumour samples showed methylation of O⁶Methylguanine DNA methyltransferase. There was a trend towards overall longer survival in the group with methylated tumours compared to those with unmethylated tumours; respective values for median survival (ranges) were 17 (13-21) versus 10 (6-14) months (P=0.105).

Conclusions Our single-centre results indicated that Chinese glioblastoma patients who had received concomitant chemoradiotherapy showed a trend towards longer overall survival compared to those receiving radiotherapy alone. Approximately 43% of our Chinese glioblastoma samples showed methylation of O⁶Methylguanine DNA methyltransferase. O⁶Methylguanine DNA methyltransferase methylation may be a significant prognostic factor in Chinese glioblastoma patients.

分子標誌物MGMT和化學放射治療，與華籍膠質母細胞瘤患者存活期的關係研究

目的 以化學放射治療對放射治療，比較華籍初發性膠質母細胞瘤患者的存活期、確定腫瘤樣本其MGMT基因甲基化狀態，以及評估MGMT基因甲基化狀態對患者的預後價值。

設計 回顧相關性分析。

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患者 2005年3月至2007年6月期間，經組織學分析確診初發性膠質母細胞瘤的患者。從腫瘤的福馬林固定石蠟塊組織中提取基因體DNA後，為MGMT基因進行甲基化特異性聚合酶鏈式反應檢測。研究並收集有關患者資料，包括年齡、表現狀況、類固醇使用、切除術範圍、併發症，以及有關放射和化學治療數據。主要療效和次要療效分別以整體存活期和無惡化存活期測量，並以Kaplan-Meier存活分析評估。此外，研究以上述兩種療法，以及甲基化與非甲基化腫瘤患者的療效作比較。

結果 共35名膠質母細胞瘤患者參與研究，包括27名男性和8名女性。他們平均年齡為50歲，當中17名接受化學放射治療，其餘18名只接受放射治療。患者的整體存活期中位數為12個月（介乎7-17個月），無惡化存活期中位數則為5個月（介乎3-6個月）。放射治療組方面，無惡化存活期和整體存活期的中位數分別為4個月（介乎3-5個月）和6個月（介乎2-10個月），而放射化學治療組則分別為6個月（介乎2-10個月）和13個月（介乎8-18個月）。15個（43%）腫瘤樣本被驗出MGMT基因甲基化。與非甲基化腫瘤患者比較，甲基化腫瘤患者的整體存活期較長（P=0.105）：前者為10個月（介乎6-14個月），後者則為17個月（介乎13-21個月）。

結論 這項單一中心研究顯示，以化學放射治療華籍膠質母細胞瘤患者，其整體存活期較只接受放射治療的患者為長。此外，43%的腫瘤樣本被驗出MGMT基因甲基化，這或是診斷膠質母細胞瘤的重要預後因子。

Introduction

Glioblastoma multiforme (GBM) is a World Health Organization grade IV tumour and the most malignant type of astrocytoma. Surgery and radiotherapy are the mainstay of treatment historically. The role of chemotherapy was uncertain until the approval of temozolomide in 2005. Temozolomide is the first form of chemotherapy to show survival benefit (median survival of 12.1 months extended to 14.6 months) in a randomised phase III trial.[1] A follow-up retrospective molecular analysis of O^6Methylguanine DNA methyltransferase (MGMT) in the same group of tumours was published at the same time.[2] It was found that methylation of *MGMT* gene conferred chemopredictive responsiveness (to temozolomide) and was thus a prognostic marker in GBM patients.

In Hong Kong, it is estimated that the annual incidence of GBM is about 2.5/100 000 inhabitants. There are about 150 to 200 patients with newly diagnosed GBM every year. The prognosis of GBM is very poor, despite intensive research and aggressive clinical approaches. We previously reported a satisfactory response after the use of temozolomide in recurrent malignant glioma among Chinese patients.[3] We were, however, not certain about the response to this drug in Chinese patients with primary GBM. Most western literature reported a 40 to 50% methylation of MGMT in GBM tissues,[4,5] whereas some of us reported a 60% rate in Chinese oligodendrogliomas.[6] We had tried to validate the MGMT status in a group of recurrent GBM tumours in a previously reported cohort.[3] Among 13 patients with recurrent GBM, only three (23%) showed methylation of MGMT and we did not note any correlation in the response to chemotherapy (unpublished data).

This study was commenced in Hong Kong in February 2008, and was in the form of a retrospective correlative analysis of survival in Chinese primary GBM patients with respect to the use of concomitant chemoradiotherapy and molecular status of the tumour tissue MGMT status. One objective of the study was to compare the survival of patients after concomitant chemotherapy and radiotherapy versus radiotherapy alone. A second objective was to determine tumour methylation status of MGMT in these patients and assess its prognostic value for the treatment of Chinese patients with primary GBM.

Methods

Patients with primary GBM diagnosed by tissue histology from March 2005 to June 2007 were recruited and their clinical data were reviewed. Inclusion criteria were: age of 18 years or more, Chinese ethnicity, and Karnofsky performance score (KPS) of 60 or higher. Patients with secondary GBM (de-differentiated from lower-grade glioma) were excluded. Those with known syndromic diseases associated with development of glioma (ie neurofibromatosis type 1 or 2, Von Hippel–Lindau disease, Turcot syndrome, Cowden disease, Li-Fraumeni syndrome) or existing secondary cancers were also excluded.

Regarding the radiotherapy technique, a consistent protocol with either conformal radiotherapy or intensity-modulated radiotherapy was used. The first-phase irradiation delivered 45 Gy in 25 fractions to the gross tumour and oedema area with an addition of 2 cm margin. This was then followed by a cone-down field to irradiate the contrast-enhanced tumour with 2.5 cm margin up to a total of 59.4 Gy in 33 fractions. A proportion of patients were given chemotherapy as an adjunct to radiotherapy at the discretion of attending oncologists after considering the performance status as well as the financial situation of patients. The

chemotherapy regimen followed that described by Stupp et al[1] using temozolomide at 75 mg/m^2/day during radiotherapy, and 150-200 mg/m^2 on days 1-5 every 4 weeks for 6 cycles thereafter. Patient data at presentation including age, performance status, steroid use, extent of resection, complications, radiotherapy and chemotherapy data were recorded.

Genomic DNA was isolated from paraffin sections of GBM tissues isolated from formalin-fixed and paraffin-embedded sections of GBM tissues. Methylation-specific polymerase chain reaction (PCR) for MGMT was performed as described by our group previously.[6]

The percentage of methylation of *MGMT* gene was derived. Outcomes were measured by overall survival (OS) as primary outcome and progression-free survival (PFS) as a secondary outcome. Overall survival and PFS were assessed by the Kaplan-Meier method and the survival curves were compared using the log-rank test. Outcomes could be compared between concomitant chemoradiotherapy and the radiotherapy alone groups, and between methylated-MGMT and unmethylated-MGMT groups.

Written informed consent was obtained from surviving patients. For deceased patients, verbal consent was obtained by telephone from next of kin. The study was approved by the New Territories East Cluster–Chinese University of Hong Kong ethics committee in February 2008.

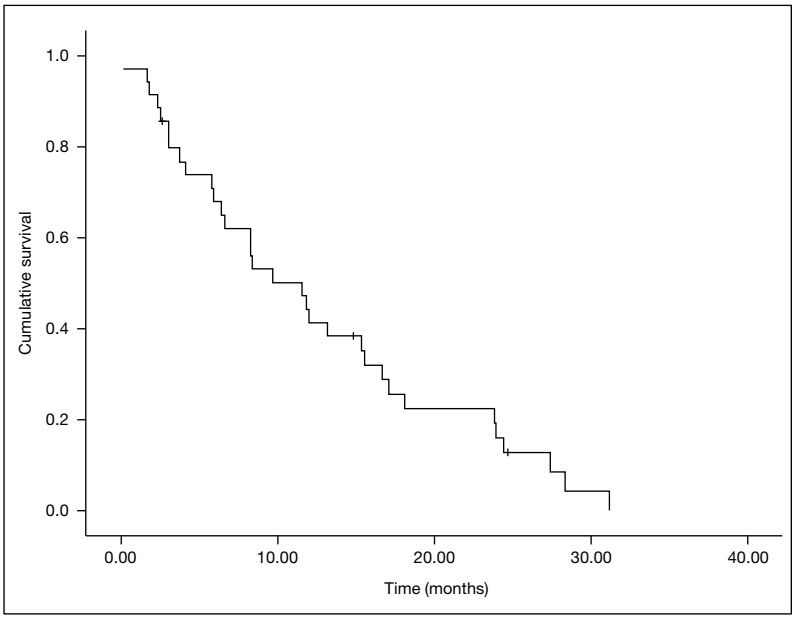

FIG 1. Overall survival of the whole group

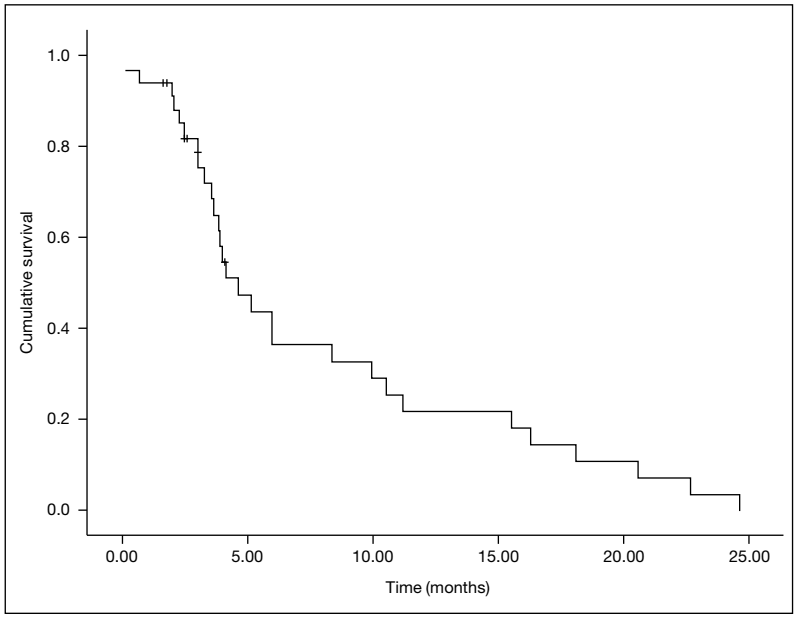

FIG 2. Progression-free survival of the whole group

Results

In all, 35 GBM patients were recruited, of whom 27 were male and 8 were female. Their mean age was 50 years. In the whole group of patients, the median OS was 12 (range, 7-17) months (Fig 1) and the median PFS was 5 (3-6) months (Fig 2).

Seventeen patients received concomitant chemoradiotherapy, and 18 received radiotherapy only. The two groups were comparable in terms of gender, age, performance status (KPS) at the time of presentation, and the type of surgery they had (Table). In the concomitant chemoradiotherapy group, they all completed their concurrent treatment, while eight continued with adjuvant chemotherapy. Six of the latter eight patients completed a full course of 6 cycles, while two completed 4 cycles. Fifteen (43%, 15/35) of the patients had had GBM tissue showing methylation of MGMT.

In the radiotherapy alone group, the median PFS and OS was 4 (range, 3-5) months and 6 (2-10) months, respectively. In the concomitant chemoradiotherapy group, the corresponding values were 6 (range, 2-10) months and 13 (8-18) months, respectively. Compared to those having radiotherapy alone, there was a trend towards increased PFS and OS in the concomitant chemoradiotherapy group (PFS, log-rank P=0.16; OS, log-rank P=0.15) [Figs 3 and 4]. There was a trend towards longer OS in those with methylated-MGMT (median, 17; range, 13-21 months) compared with unmethylated MGMT (10; 6-14 months) [P=0.105; Fig 5].

Discussion

One of the breakthroughs in neuro-oncology in last decade was the concomitant use of chemoradiotherapy in GBM. Such concomitant use of chemoradiotherapy has demonstrated a survival benefit in GBM for the first time in history. The

TABLE. Patient characteristics in concomitant chemoradiotherapy group and radiotherapy alone group

Characteristic*	Concomitant chemo-radiotherapy	Radiotherapy alone
No.	17	18
Gender		
Male	14	13
Female	3	5
Age (years)		
≥50	9	11
<50	8	7
Performance status		
KPS (pre-op)	70	73.3
KPS (post-op)	70	70
Type of surgery		
Excision	14	14
Biopsy	3	4
MGMT-methylated	9	6

* KPS denotes Karnofsky performance score, and MGMT O^{6}Methylguanine DNA methyltransferase

regimen consists of a daily low-dose prescription of the oral alkylating agent temozolomide during the course of radiotherapy. This is then followed by 6 cycles of high-dose chemotherapy using the same agent. This has become the standard of care since its approval in March 2005. Since 2001, we have been using temozolomide to treat recurrent malignant gliomas (anaplastic glioma and GBM) in Hong Kong. Since March 2005, concomitant chemoradiotherapy was advised for our GBM patients. Our previous experience showed that temozolomide had a very acceptable toxicity profile in our (Chinese) patients.[3] However, this drug is currently a standard drug (without safety net) under the Drug Formulary of the public health care system. Thus, other than medical factors, its use depends very much on the patient's financial situation. This has made comparison between our radiotherapy-alone and concomitant chemoradiotherapy groups difficult. The major prognostic factors were balanced in the two groups, although the intrinsic bias of different socio-economic status was unavoidable.

There is an on-going search for prognostic biomarkers and treatment-predictive indicators in GBM patients. O^{6}Methylguanine DNA methyltransferase was shown to be the first molecular marker carrying both prognostic and chemo-predictive value in such patients.[2,7] The western literature often reported a 40 to 50% methylation of MGMT in GBM, which was taken to indicate a better prognosis and chemosensitivity.[2,4,5] However, there were no such data in Chinese patients in the literature. There were two studies in Chinese literature that reported 52% and 61% MGMT protein expression based on immunohistochemistry (IHC) techniques.[8,9] As MGMT protein expression by IHC was criticised as showing inconsistent results and not correlating with survival outcomes,[10,11] a methylation-specific PCR for MGMT was recommended. In our series of Chinese GBM patients, 43% had methylated MGMT, which

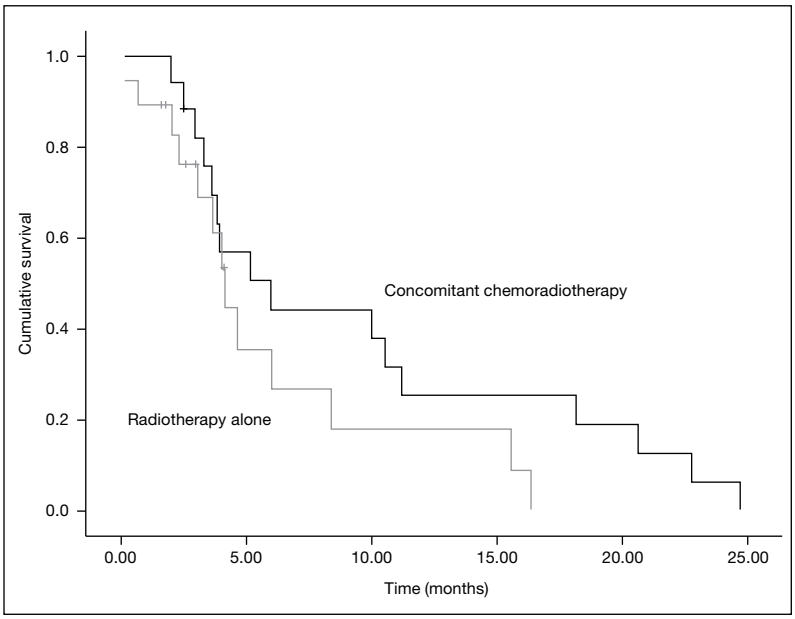

FIG 3. Progression-free survival of concomitant chemoradiotherapy versus radiotherapy alone
Concomitant chemoradiotherapy: 6 (2-10) months
Radiotherapy alone: 4 (3-5) months
P=0.16

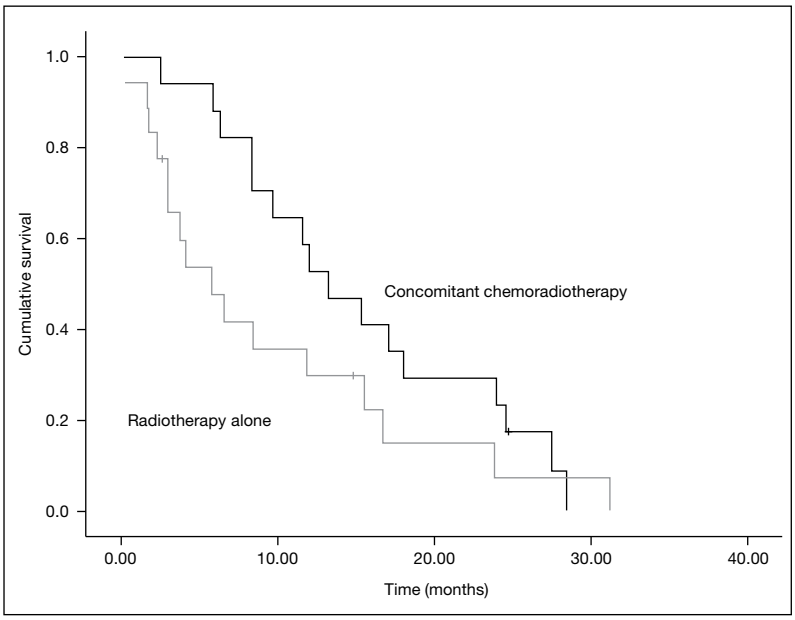

FIG 4. Overall survival of patients who received chemoradiotherapy versus radiotherapy alone
Concomitant chemoradiotherapy: 13 (8-18) months
Radiotherapy alone: 6 (2-10) months
P=0.15

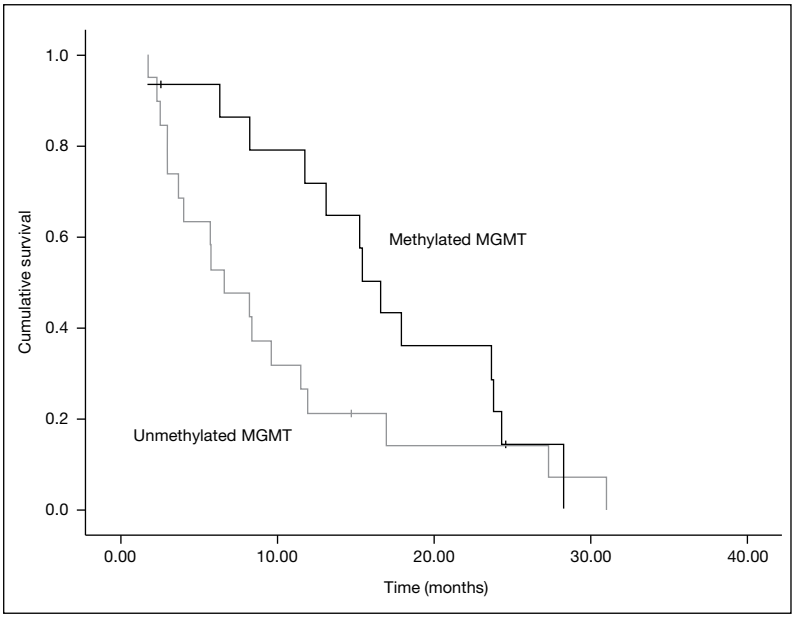

FIG 5. Overall survival of methylated O⁶Methylguanine DNA methyltransferase (MGMT) versus unmethylated MGMT
Methylated MGMT: 17 (13-21) months
Unmethylated MGMT: 10 (6-14) months
P=0.105

was in the range reported in western literature. We were able to show a trend towards increased OS in the methylated group, though the difference was not statistically significant. Due to the small sample size of our cohort, we were unable to carry out any analysis for chemosensitivity. An extended multicentre analysis involving larger patient numbers is underway to answer these questions.

Conclusions

Our single-centre results suggest that Chinese GBM patients who received concomitant chemoradiotherapy showed a trend towards longer OS than those receiving radiotherapy alone. Approximately 43% of our patients showed methylation of MGMT, which may be a significant prognostic factor in Chinese patients with GBM.

Disclaimer

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