Association of molecular marker O6-Methylguanine DNA methyltransferase and concomitant chemoradiotherapy with survival in Southern Chinese glioblastoma patients

Danny TM Chan 陳達明
Michael KM Kam 甘冠明
Brigette BY Ma 馬碧如
Stephanie CP Ng 吳志萍
Jesse CS Pang 彭頡先
Claire KY Lau 劉嘉怡
Deyond YW Siu 蕭容媛
Benedict SL Ng 伍兆龍
XL Zhu 朱獻倫
George G Chen 陳 功
HK Ng 吳浩強
WS Poon 潘偉生

**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 O6-Methylguanine DNA methyltransferase in Chinese primary glioblastoma, and to assess the prognostic value of O6-Methylguanine 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 O6-Methylguanine 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 O6-Methylguanine 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 O6-Methylguanine 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 O6-Methylguanine DNA methyltransferase. O6-Methylguanine DNA methyltransferase methylation may be a significant prognostic factor in Chinese glioblastoma patients.
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. A follow-up (median survival of 12.1 months extended to 14.6 months) of recurrent malignant glioma among Chinese patients.3 We had tried to validate the MGMT status in a group of GBM tissues,4,5 whereas some of us reported a 60% rate in Chinese oligodendrogliomas.6 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 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.1 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.

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
Primary glioblastoma in Chinese patients

Hong Kong Med J Vol 17 No 3 June 2011 www.hkmj.org

A 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. 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. O6Methylguanine DNA methyltransferase was shown to be the first molecular marker carrying both prognostic and chemosensitive value in such patients. The western literature often reported a 40 to 50% methylation of MGMT in GBM, which was taken to indicate a better prognosis and chemosensitivity. 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. As MGMT protein expression by IHC was criticised as showing inconsistent results and not correlating with survival outcomes, a methylation-specific PCR for MGMT was recommended. In our series of Chinese GBM patients, 43% had methylated MGMT, which

| TABLE: Patient characteristics in concomitant chemoradiotherapy group and radiotherapy alone group |
| Characteristic* | Concomitant chemoradiotherapy | 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 O6Methylguanine DNA methyltransferase

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
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

The study was partially (<50%) supported in budget by Schering Plough. The support did not influence the manner in which the investigators/authors conducted their study or analysed the results.