RESEARCH ARTICLE

Prognostic Value of MGMT Promoter Methylation and TP53 Mutation in Glioblastomas Depends on IDH1 Mutation

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Abstract

Several molecular markers have been proposed as predictors of outcome in patients with glioblastomas. We investigated the prognostic significance of O[6]-methylguanine-DNA methyltransferase (MGMT) promoter methylation and TP53 mutation status dependent on isocitrate dehydrogenase 1 (IDH1) mutation in glioblastoma patients. A cohort of 78 patients with histologically confirmed glioblastomas treated with radiation therapy and chemotherapy were reviewed retrospectively. We evaluated the prognostic value of MGMT promoter methylation and TP53 mutation status with regard to progression-free survival (PFS) and overall survival (OS). It was revealed that mutations in IDH1, promoter methylation of MGMT, TP53 mutation, age, Karnofsky performance status (KFS), and extension of resection were independent prognostic factors. In patients with an IDH1 mutation, those with an MGMT methylation were associated with longer PFS (p=0.016) and OS (p=0.013). Nevertheless, the presence of TP53 mutation could stratify the PFS and OS of patients with IDH1 wild type (p=0.003 and 0.029 respectively, log-rank). The MGMT promoter methylation and TP53 mutation were associated with a favorable outcome of patients with and without mutant IDH1, respectively. The results indicate that glioblastomas with MGMT methylation or TP53 mutations have improved survival that may be influenced by IDH1 mutation status.

Keywords: Glioblastomas - IDH1 mutation - MGMT promoter methylation - TP53 mutation - prognosis

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Introduction

Glioblastoma multiform (GBM), classified as World Health Organization (WHO) grade IV, is the most common malignant brain tumors in adults with an incidence rate of 23 per 100,000 persons (Stancheva et al., 2014). Their clinical course varies substantially, such that some patients succumb to progressive disease within weeks while others survive for a decade or more. Current treatments include surgery, radiation therapy and chemotherapy (Sathornsumetee et al., 2007; Koca et al., 2014; Pashaki et al., 2014). However, the median survival is still not optimistic. In spite of the existing classification, GBM subgroups are not homogeneous in terms of survival (Molenaar et al., 2014). Several prognostic factors have been established, including age, preoperative Karnofsky Performance Status (KPS), extent of resection, and also some molecular markers.

Recently, molecular markers were shown to be helpful in predicting prognosis and treatment response. Three gene markers that have attracted most interest in this respect are mutations in the isocitrate dehydrogenase 1 (IDH1) or 2 (IDH2) gene (Stancheva et al., 2014), hypermethylation of the O[6]-methylguanine-DNA methyltransferase (MGMT) promoter, and complete deletion of both 1p and 19q. Besides, tumor-suppressor gene TP53 mutation status also acts in the pathogenesis of gliomas and influences patients' prognosis (Stancheva et al., 2014). Mutations in IDH1 were shown to be a positive prognostic marker for GBM patient survival (Nobusawa et al., 2009; Sanson et al., 2009 Bleeker et al., 2010). Recently, methylation of the MGMT gene promoter appeared to be a predictive factor for the response of GBM patients to temozolomide and radiotherapy and their survival (Hegi et al., 2005; Stupp et al., 2009; Weller et al., 2009). However, these gene alternations that would potentially influence the outcome of patients with GBM may not act alone. It is reported that the combination of IDH1 mutations and MGMT methylation status predicts survival in GBM better than either IDH1 or MGMT alone (Molenaar et al., 2014). Thus, there may be a synergistic effect or a mechanistic link between IDH1 mutations and MGMT methylation (Wick et al., 2013). Similarly, approximately 64% of IDH1 mutated tumors also carry TP53 mutations (Tabatabai et al., 2010). Nevertheless, the correlation of MGMT promoter methylation and TP53 gene mutations and their effect on prognosis has not been established in astrocytoma (Groenendijk et al., 2011) and GBM (Jesien-Lewandowicz et al., 2009) yet.

The objective of the present study was to investigate

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the predictive value of MGMT promoter methylation and TP53 gene mutation for the survival in GBM treated with radiochemotherapy depends on IDH1 gene mutation. These findings may increase our understanding of the association between gene alternations and patient survival based on the combination of the IDH1 mutational status, MGMT methylation status, and TP53 gene mutation, which imply a role for target gene test in survival prediction.

Materials and Methods

Patients

Adult patients with newly diagnosed histologically confirmed GBM according to the WHO classification, who were treated with radiation treatment (RT) plus chemotherapy between September 2008 and March 2009, were enrolled in this retrospective study. All clinical relevant surgical, genetic, and pathological information for patients who were treated at our institute were obtained from a prospective database. Patients with a diagnosis of recurrent GBM were not included in the study. A total of 78 patients were included in the final analysis with sufficient clinical, pathology, and genetic information. The histopathological diagnosis was evaluated and confirmed by two independent neuropathologists blinded to the patients' clinical and radiological information. Gross total resection (GTR) was defined as the area that was >95% of the area with abnormal T2 hyperintensity before surgery. In this study, resections that were not GTR were considered residual tumor (<GTR). Progression-free survival (PFS) was defined as the time from surgery to tumor progression observed on post contrast magnetic resonance (MR) images. Overall survival (OS) was calculated as time from surgery to death. The overall follow-up duration ranged from October 2008 to the time of analysis in the present study. This study was approved by our institutional review board, and written consent was obtained from all enrolled patients.

Molecular biomarkers assessment

IDH1 mutation was determined using DNA pyro-sequencing, which we have described previously (Dunn et al., 2013). The methylation status of the MGMT promoter was determined by methylation-specific polymerase chain reaction (PCR) after sodium bisulfite DNA modification as described previously (Minniti et al., 2011). Immunoperoxidase staining for TP53 mutants was performed on formalin-fixed, paraffin-embedded tissue sections following the standard procedure introduced in our previous study (Li et al., 2008). The immunohistochemical expressions of TP53 protein were independently reviewed by 2 experienced pathologists and were then classified as the following: -, negative; +, isolated positive cells; ++, clusters of positive cells; and +++, mostly positive cells. The scales of positive cells were then used to score mutant TP53 expression levels that were documented from 0 (-) to 3 (+++).

Statistical analysis

Statistical processing of data was performed using SPSS 19.0 for Windows (IBM). Figures were constructed in Prism 6 (Graphpad Software). Patients with GBM were categorized into two subgroups in term of the MGMT methylation status and TP53 mutation status respectively depend on IDH1 mutation. Additionally, log-rank analysis of Kaplan-Meier data was performed to compare the PFS and OS of the cohort. Factors that were significant ($p<0.05$) in univariate analysis were tested with multivariate survival analysis based on the Cox proportional hazard ratio (HR) model.

Results

Patients

Seventy-eight consecutive patients (43 males and 35 females) with GBM who underwent RT plus chemotherapy between September 2008 and March 2009 were analyzed. Pretreatment characteristics for available patients are listed in Table 1. The median age was 45 years (range, 21-62 years), and median KPS was 80 (range, 50-90). Sixty-seven patients were considered to have gross total resection, 11 patients underwent incomplete resection on the basis of an intraoperative or immediate postoperative MR images. Adjuvant chemotherapy consisted of 6 cycles of TMZ in 33 patients and 12 cycles in 45 patients, respectively. Sixty-six patients had died at the time of analysis (March 2009).

Prognostic factors

Age (≥50 *vs* <50, $p=0.023$), KPS (KPS≥80 *vs*<80; $p=0.011$), extent of resection (GTR *vs* <GTR, $p=0.019$), IDH1 mutation ($p=0.032$), TP53 mutation ($p=0.028$), and MGMT methylation status ($p=0.012$) had an effect on PFS. Similarly, these factors also play a predictive role on OS except TP53 mutation (Table 2).

Multivariate analysis showed that age<50 ($p=0.010$), KPS≥80 ($p=0.035$), IDH1 mutation ($p=0.006$), TP53 mutation ($p=0.012$), and MGMT promoter methylation ($p=0.031$) were significant prognostic factors associated with longer PFS (Table 3). As for OS, besides the above-mentioned factors, GTR were of favourable prognostic

Table 1. Characteristics of All Patients (n=78)

Characteristics		Number (%)
Number of patients		78
Age (years)	Median (Range)	45 (21-62)
Gender	Male	43 (55.1)
	Female	35 (44.9)
KPS	≥ 80	54 (69.2)
	< 80	24 (30.8)
Site of tumor	Frontal	30 (38.5)
	Temporal	24 (30.8)
	Occipital	6 (7.7)
	Parietal	18 (23.0)
IDH1 status	Mutated	37 (47.4)
	Wild type	41 (52.6)
MGMT promoter methylation	Methylated	43 (55.1)
	Unmethylated	35 (44.9)
TP53 status	Mutated	40 (51.3)
	Wild type	38 (48.7)
Extent of resection	GTR	67 (85.9)
	<GTR	11 (14.1)

Table 2. Univariate analysis of survival outcomes[a]

Characteristic	PFS			OS		
	p value[b]	HR	95%CI	*p* value[b]	HR	95%CI
Age≥50	0.023	1.738	1.083-2.553	0.027	1.792	1.062-2.693
KPS<80	0.011	2.892	1.265-3.671	0.005	2.961	1.519-3.235
GTR/<GTR	0.019	1.715	1.142-2.798	0.002	2.348	1.704-3.129
IDH1 mutation	0.032	0.321	0.107-0.960	0.021	0.346	0.136-0.881
TP53 mutation	0.028	0.409	0.179-0.932	0.103	0.545	0.261-1.142
MGMT promoter methylation	0.012	0.326	0.131-0.816	0.017	0.368	0.172-0.865

[a]PFS = Progression-free Survival, OS=Overall Survival, HR=Hazard Ratio, CI=Confidential interval; [b]A *p* value of 0.05 denoted significance

Table 3. Multivariate Analysis of Survival Outcomes[a]

Predictor	*p* value[b]	HR	95%CI
PFS			
Age≥50	0.01	1.743	1.120-3.275
KPS<80	0.035	3.36	1.054-4.138
GTR/<GTR	0.052	1.808	0.989-2.836
IDH1 mutation	0.006	0.124	0.128-0.553
TP53 mutation	0.012	0.149	0.144-0.664
MGMT promoter methylation	0.031	0.595	0.320-0.831
OS			
Age≥50	0.018	1.736	1.113-3.560
KPS<80	0.024	2.484	1.083-5.355
GTR/<GTR	0.039	1.922	1.015-3.146
IDH1 mutation	0.012	0.257	0.134-0.689
TP53 mutation	0.022	0.402	0.253-0.748
MGMT promoter methylation	0.018	0.59	0.218-0.706

[a]PFS=Progression-free Survival, OS=Overall Survival, HR=Hazard Ratio, CI=Confidential interval; [b]A *p* value of 0.05 denoted significance

significance for OS (*p*=0.039) as well (Table 3).

PFS and OS analysis

At a median follow-up time of 47 months (range, 6-65 months), Median PFS were 11 months and median OS was 17 months. Treatment response was achieved in 35 patients, including 13 complete responses and 22 partial responses. IDH1 mutation was detected in 37 (47.4 %) of 78 patients, MGMT promoter was methylated in 43 (55.1%) of 78 patients, and TP53 mutation was detected in 40 (51.3%) of 78 patients.

For patients with IDH1 mutation, presence of MGMT promoter methylation was associated with better outcomes. Median PFS was 17 months in patients with MGMT promoter methylated tumors and 8 months in those with MGMT promoter un-methylated tumors (*p*=0.016), with respective OS of 25 and 11 months (*p*=0.013). The median PFS and OS were 16 and 18 months in TP53 mutant tumors and 12 and 14 months in wild-type tumors respectively. However, the differences of PFS and OS between the TP53 mutant and wild-type tumors was not statistically significant (*p*=0.679 for PFS and *p*=0.554 for OS respectively) (Figure 1).

For patients without IDH1 mutation, presence of TP53 mutation was associated with better outcomes. Median PFS was 17 months in patients with TP53 mutant tumors and 7 months in those with TP53 wild-type tumors (*p*=0.003), with respective OS of 20 and 11 months (*p*=0.029). The median PFS and OS were 10 and 17 months in MGMT promoter methylated tumors and 9 and 13 months in un-methylated tumors respectively. However, the PFS and OS of MGMT promoter methylated and un-methylated

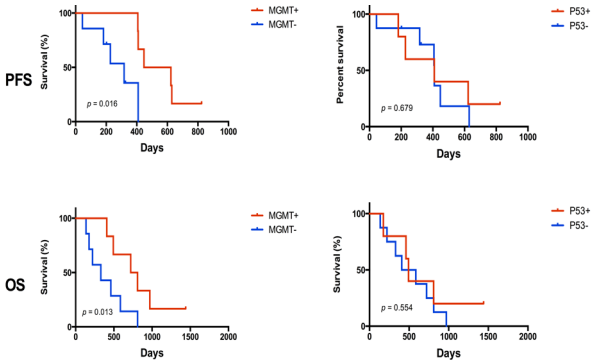

Figure 1. As Tumors with IDH1 Mutation, MGMT Promoter Methylation Showed Prognostic Value for PFS (*p*=0.016) and OS (*p*=0.013). While TP53 mutation failed to differentiate the survival of patients with GBM

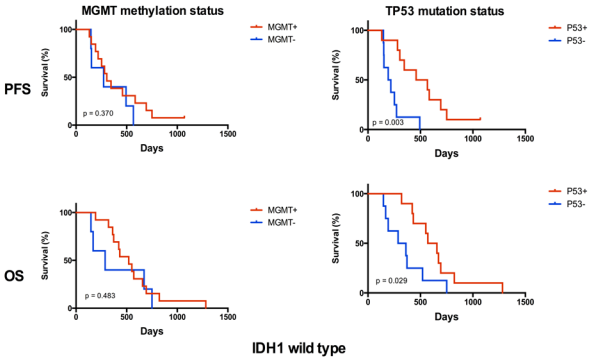

Figure 2. For Tumors without IDH1 Mutation, TP53 Mutation Status was able to Stratify the Survival (*p*=0.003 for PFS and *p*=0.029 respectively). However, MGMT promoter methylation was not predictive for survival outcome

tumors did not show statistical significance (*p*=0.370 for PFS and *p*=0.483 for OS respectively) (Figure 2).

Discussion

In this study we meant to evaluate the impact of clinical and some molecular prognostic factors in a series of patients with GBM treated with RT and adjuvant TMZ. Besides some clinical factors that have already been believed to be associated with prognosis, such as age, KPS, and extent of resection. IDH1 mutation status, TP53 mutation and MGMT promoter methylation status also showed predictive effect on survival in our population.

As a significant predictive indicator for prognosis in GBM, IDH1 mutation has been investigated in some

studies (Yan et al., 2012; Sarmiento et al., 2014; Stancheva et al., 2014). It has been demonstrated that patients with mutant IDH1 had significantly longer PFS and OS than those with wild-type IDH. The better prognosis of patients with mutant IDH1 may be partly attributed to the effect of IDH1 interaction with other clinical characteristics, including a younger age preference of IDH1 mutation, higher mutation incidence rate in low-grade glioma, higher GTR rate for IDH1 mutated tumor, and less aggressive biological behavior of tumors with mutant IDH1. Thus, it is reasonable to surmise that the prognosis of patients with mutant IDH1 might be favorable. MGMT promoter methylation has been reported as a favorable prognostic factor for survival and a predictive marker for benefit from alkylating agent chemotherapy in patients with GBM (Hegi et al., 2005; Stupp et al., 2009; Olson et al., 2011). It is also found that tumors with methylated MGMT promoter has longer PFS when treated with RT plus chemotherapy compared with patients treated with RT alone (Wick et al., 2013). In our study, the MGMT promoter methylation was significantly associated with longer PFS and OS for those who received RT and chemotherapy, conferring a reduction in risk for death. The tumor-suppressor gene TP53 encodes a protein that acts in the pathogenesis of many cancers. TP53 mutations have been reported not only mainly in low-grade gliomas (Butowski et al., 2006; Soussi et al., 2007), but also in primary and secondary glioblastomas (Ohgaki et al., 2009). Most TP53 aberrations resulted in decreased apoptosis in response to DNA damage, thus helping tumor growth and influencing patient's overall survival negatively (Ohgaki et al., 2007; Parsons et al., 2008; Tabatabai et al., 2010). Although it is accepted that the loss of p53 function plays a crucial role in glioma tumorigenesis (Munoz et al., 2011; Munoz et al., 2013), the prognostic value of p53 mutations has remained controversial and no consistent relationship with response to therapy or overall outcome has been reported (Stander et al., 2004; Levidou et al., 2010). In multivariate analysis, we found the mutational status of IDH1 and TP53, the methylation status of the MGMT promoter, age, preoperative KPS to be independent prognostic factors. The extent of tumor resection only showed prognostic value for OS, but not for PFS.

Besides the independent prognostic value of IDH1 mutation status, MGMT methylation, and TP53 mutation for patients with GBM. Some researches have evaluated the significance of the combination of gene predictor for survival prediction in patients with GBM. MGMT promoter methylation and IDH1 mutant status in the survival prediction in patients with GBM has earned much attention. It is showed that the combination of IDH1 mutations and MGMT methylation outperforms either IDH1 mutations or MGMT methylation alone in predicting survival of glioblastoma patients (Molenaar et al., 2014). Nevertheless, other research found that MGMT promoter methylation is a predictive biomarker in patients with IDH1wild-type, but not IDH1-mutant, malignant gliomas for those who received alkylating agent chemotherapy (Wick et al., 2013). The combination of IDH1 and TP53 mutation has also been evaluated for the survival of patients with GBM, but no expected conclusion regarding the prognostic value of the combined alternation of IDH1 and TP53 for outcome had been drawn (Stancheva et al., 2014). Because the study was carried out in the Bulgarian population that may be a limitation of the research. So the combined effect of IDH1 and TP53 mutation needs further investigation. The relationship of MGMT promoter hypermethylation and TP53 gene mutation also has been studied in tumor progression include endometrial cancer (Nagy et al., 2014). esophageal squamous cell carcinoma (Su et al., 2014), and nervous system tumors(Bello et al., 2004), such as astrocytoma (Groenendijk et al., 2011) and glioblastoma (Jesien-Lewandowicz et al., 2009). However, the effect of their combined alternation on prognosis of patients with GBM was not clear.

According to our findings, it was suggested that the combination of IDH1 and MGMT, or IDH1 and TP53 analysis was able to predict survival in patients with GBM treated with radiochemotherapy. Specifically, for patients with mutated IDH1, tumors with IDH1 mutation and MGMT methylation had the more favorable prognosis compared to that with mutated IDH1 and unmethylated MGMT promoter (p=0.016 for PFS and p=0.013 for OS respectively), so MGMT methylation was predictive for the response to TMZ in IDH1-mutated glioblastomas, which in concordance with previous studies (Tabatabai et al., 2010; Wick et al., 2013). While TP53 mutation status failed to stratify the survival of both PFS and OS. Our result concerning the predictive value of IDH1 and MGMT methylation was in concordance with previous study (Carrillo et al., 2012; Molenaar et al., 2014). Nevertheless, based on the IDH1 wild type status, MGMT promoter methylation could not differentiate the outcome of patients with GBM treated with radiochemotherapy but TP53 mutation status showed predictive effect on the prognostic survival, patients with TP53 mutation experienced a longer PFS and OS than patients without mutated TP53 (p=0.003 for PFS and p=0.029 for OS respectively).

The two gene predictors, mutations in IDH1 or methylation of the MGMT promoter, perform well in different populations with various prevalences of alterations in IDH1 and MGMT and different median ages and overall survival. Glioblastoma has been classified into some molecular subtypes, including proneural, neural, classical, and mesenchymal, based on expression-profiling studies (Phillips et al., 2006; Lee et al., 2008; Verhaak et al., 2010). IDH1 mutation and TP53 alternation occurs frequently in proneural glioblastomas with a better outcome and younger age. Moreover, the favorable prognosis of proneural subtype is confined to tumors with the glioma CpG island methylator phenotype (G-CIMP) that is associated with IDH1 mutations (Noushmehr et al., 2010). In addition, the G-CIMP status is found to correlate with MGMT promoter methylation in glioblastoma (van den Bent et al., 2011) and low-grade glioma (Turcan et al., 2012). This suggests that IDH1 mutations may interact with MGMT methylation. As IDH1 mutation is considered an early genetic event in tumorigenesis, and both IDH1 mutation and MGMT methylation status generally do not change during the treatment. The robustness of IDH1 mutation and MGMT methylation may drive other genetic changes in tumor cells, tumors accompanied by IDH1

mutation and methylated MGMT may consequently have different genetic characteristics compared to tumors unaccompanied by the alternations, which may lead to their varied biological features and patients' survival. As previous reported that the survival time of glioblastoma patients with only IDH1 mutation is shorter than that for patients with both IDH1 mutation and MGMT methylation (Hartmann et al., 2010; Juratli et al., 2012; SongTao et al., 2012). It is suggested that the IDH1-mutated patients is not homogeneous and that the prognosis is not only depend on IDH1 mutation but also on MGMT methylation, so there may be a underlying mechanistic link between IDH1 mutations and MGMT methylation and needs to be further explored (Wick et al., 2013). Patients in our cohort all received chemoradiation treatment. In addition, IDH1 mutation was found predominantly in younger patients and 64% IDH1 mutated tumors also carried TP53 mutations, an investigation over survival of patients with and without TP53 mutation did not show any significant difference (Tabatabai et al., 2010). However, our study found that the TP53 mutation was able to stratify the outcome of patients without IDH1 mutation treated with chemoradiation therapy. The underlying mechanism or linkage still needs to be investigated.

Our study has some limitations. First, we retrospectively enrolled patients from a single institute; therefore, the prognostic role of IDH1 mutation, MGMT promoter methylation and TP53 mutation status require confirmation by a prospective multi-center investigation. Although the study was carefully conducted, molecular subtypes of glioblastoma, primary or secondary glioblastoma were not classified. Several studies confirmed that the target gene alternations and prognosis varies between these subgroups. Future studies should investigate the differences of prognosis and treatment responses between these subgroups of glioblastoma.

In conclusion, we retrospectively reviewed 78 patients with glioblastoma and identified IDH1 mutation, MGMT promoter methylation and TP53 mutation as significant prognostic factor. Furthermore, the MGMT promoter methylation and TP53 mutation were associated with favorable outcome of patients with and without mutant IDH1 respectively. Our results imply that glioblastomas with MGMT methylation or TP53 mutation are associated with improved survival that may be subject to IDH1 mutation status, and this effect should be considered in future investigations.

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