Lower MGMT expression predicts better prognosis in proneural-like glioblastoma

Zhi-Cheng He1,2, Yi-Fang Ping1,2, Sen-Lin Xu1,2, Yong Lin1,2, Shi-Cang Yu1,2, Hsiang-Fu Kung1,2, Xiu-Wu Bian1,2

1Institute of Pathology and Southwest Cancer Center, Southwest Hospital, Third Military Medical University, Chongqing 400038, China; 2Key Laboratory of Tumor Immunopathology of Ministry of Education of China, Third Military Medical University, Chongqing 400038, China

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Abstract: Objective: To investigate the expression and significance of MGMT in different molecular subtypes of glioblastoma (GBM), and to evaluate the important role of MGMT and P53 in predicting the prognosis of GBM patients. Methods: MGMT expression was detected by immunohistochemical staining in 72 cases of GBM which had been classified as three molecular subtypes. The relationship between MGMT and P53, an important molecule for identification of proneural-like GBM, were further analyzed. The association between MGMT and patients' prognosis was analyzed with Kaplan-Meier method, which was further validated by the data from 513 cases of GBM in the TCGA database. Results: MGMT expression was lower in proneural-like subtype in 72 GBM cases (p < 0.001), and was negatively correlated with P53 (r=-0.6203, p < 0.001). This results was also verified by a validation group of 87 GBM cases (r=-0.2950, p < 0.001). Interestingly, low expression of MGMT predicted a better outcome in proneural-like subtype or P53 high-expression group (p < 0.05) but not in non-proneural-like subtype and P53 low-expression group. All of these results were verified by the data from TCGA database. Conclusion: MGMT can be used as an independent prognostic factor and plays an important role in molecular typing and diagnosis of GBM by combination with proneural-like subtype marker P53.

Keywords: Glioblastoma, molecular subtypes, MGMT, P53, prognosis

Introduction

Glioblastoma (GBM) is one of the most malignant tumors in the central neural system. Due to the high degree of malignancy and resistance to the treatment, the overall prognosis of GBM patients is very poor [1, 2]. However, there were obvious differences in survival time among patients [3]. Therefore, it is essential to seek the molecular markers for accurately judging the prognosis and guiding the individual treatment. Verhaak RG, et al [4] subclassified GBMs into proneural, classical, neural and mesenchymal subtypes according to the status of several genes including P53, isocitrate dehydrogenase 1 (IDH1), platelet derived growth factor receptor alpha (PDGFRα), epidermal growth factor receptor (EGFR), Neurofibromatosis type 1 (NF1) and cyclin-dependent kinase inhibitor 2A (CDKN2A) etc. This molecular typing method led to more accurate molecular diagnosis and guidance for individualized treatment. Le Mercier M, et al [5] further divided GBMs into three subtypes according to IHC staining of P53, PDGFRα and EGFR. This simplified approach made molecular classification of GBM more convenient and feasible in clinical pathological diagnosis [6, 8].

O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme which can effectively protect cells against alkylating agents that cause DNA damage through preventing of G:C→A:T gene mutations [9]. The status of MGMT determines the efficacy of temozolomide, one of the most commonly used chemotherapy drugs, and plays an important role in the treatment of gliomas [10]. However, the relationship between MGMT and GBM molecular typing as well as prognosis of each subtypes of GBM is not clear.

In this study, we investigated the relationship between MGMT expression and GBM subtypes,
and evaluated the prognostic significance of MGMT in each GBM subtype. The correlation between MGMT and proneural subtype marker P53, and the prognostic significance of MGMT in GBM with different P53 level were also determined. All the results were validated by the data from TCGA database.

Materials and methods

Tissue specimens

Seventy-two cases of GBM were obtained from Southwest Hospital, The Third Military Medical University, and divided into three molecular subtypes (Table 1). Among them, 57 cases with follow-up data were used to analyze the prognostic significance of MGMT. The disease-free survival time was calculated by the first recurrence or death of the patients [11]. 83 cases of GBM used for validating the results were also obtained from Southwest Hospital, The Third Military Medical University. All of the samples and clinical data were obtained with the written informed consent from patients or their guardians. This study was approved by the ethics committees of the Third Military Medical University (TMMU) and conducted according to the principles of Helsinki Declaration.

Immunohistochemical staining

MGMT and P53 protein expression were detected by the general two-step method. Briefly, the antigen repair was done with the EDTA Tris by high temperature autoclave for 2.5 minutes. Then, the sections were put into 3% hydrogen peroxide to inactivated endogenous peroxidase for 10 minutes. After washing three times with PBS, the mouse against human MGMT (ZSGB-BIO ORIGENE, Beijing, China) and mouse against human P53 (ZSGB-BIO ORIGENE, Beijing, China) antibody were added to the sections overnight at 4°C. The secondary antibody (Dako Cytomation, Glostrup, Denmark) were incubated at 37°C for 30 minutes. Finally, the sections were colorated by DAB (Dako Cytomation) and counterstained with hematoxylin. Mouse immunoglobulin (IgG) was used as isotype control. All immunohistochemical staining was performed under the same condition. Two pathologists observed and quantified the sections independently. The number of positive cells was defined as 0 (<5%), 1 (5-25%), 2 (26-50%), 3 (51-75%) or 4 (>75%), and the intensity of 3, 2, 1, 0 means strong, medium, weak and negative. A total score of 0-12 was gained by the multiplying of these two factors. Score 0-3 was expressed as a negative or low expression. Score 4-12 was expressed as a positive or a high expression [12].

TCGA database analyses

All cases of 513 GBM were obtained from TCGA (The Cancer Genome Atlas) database of NIH (Institutes of Health National). The expression of MGMT and P53 were divided into high and low expression group by using X-tile software [13, 14].

Statistical analyses

Statistical analysis was performed by using SPSS16.0 statistical software. Non paired t test was applied to analyze the expression of MGMT in different molecular subtypes. Spearman correlation analysis was used for evaluation of the relationship between MGMT and P53. Pearson χ² test was applied to analyze the relationship between MGMT ex-

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Table 1. Clinical characteristics and MGMT status of GBM specimens

University (TMMU) and conducted according to the principles of Helsinki Declaration.
expression and clinicopathological parameters. Kaplan-Meier survival plots and log-rank test was used to analyze the prognosis of GBM specimens from our hospital and TCGA database. $P$ value $<0.05$ was considered statistically significant in all tests.

Figure 1. The expression of MGMT was lower in proneural-like subtype than in classical and other subtype. A. IHC staining of MGMT in all subtypes of GBM. B. Quantitative analysis of correlation of molecular classification of GBM with MGMT expression.

Figure 2. MGMT was negatively correlated with P53 expression in GBM. A. IHC and HE staining of MGMT and P53. B. The relationship between MGMT and P53 in 72 cases of GBM. C. The relationship between MGMT and P53 in 83 cases of GBM.
MGMT and GBM molecular subtypes

Results

The expression of MGMT was lower in proneural-like subtype GBM

Immunohistochemical staining of P53, PDGFRA, and EGFR was used to identify the molecular subtypes of GBM as described previously [5]. Among the 72 cases of GBM, 36 of them were proneural-like subtype (P53 or PDGFRA positive), 13 cases were classical-like subtype (P53 and PDGFRA negative, EGFR positive), and 23 cases were other subtype (P53, PDGFRA and EGFR negative) (Figure 1A). Among 36 proneural-like GBMs, 9 cases (25%) were positive for MGMT. The other 27 cases (75%) were MGMT negative. Among 13 classical-like cases, 12 of them (92.3%) were MGMT positive, and 1 of them (7.7%) were MGMT negative. As for the 23 cases of other type GBMs, 18 cases (78.3%) were MGMT positive and the remaining 5 cases (21.7%) were negative. Statistically, MGMT expression in proneural-like subtype was lower than that of other two types. However, there was no significant difference of MGMT expression between classical subtype and other subtype (Figure 1B). There were also no significant differences between MGMT expression and other clinical parameters (Table 1).

MGMT was negatively correlated with P53 expression

P53 was expressed higher in proneural-like GBM than other subtypes and was proposed as a marker of this subtype [4, 5]. We found that P53 expression was negative when MGMT expression was positive, and P53 expression was positive when MGMT expression was negative in majority of the 72 GBMs. In 34 MGMT negative cases, 29 cases were P53 positive (85.3%). Consistently, in the 38 MGMT positive cases, 31 cases of them were negative for P53 (81.6%). The typical pictures are presented in Figure 2A. A negative correlation was also found between MGMT expression and P53 expression (Figure 2B r=-0.6203, p < 0.001).
We then used 83 cases of GBMs to validate these results. As expected, the results were consistent with the previous ones. 48 cases (76.2%) of 64 MGMT negative cases were P53 positive. 11 cases (57.9%) of 19 MGMT positive cases were negative for P53 ($r=-0.2950$, $p<0.01$).

Lower MGMT expression predicts better prognosis in proneural-like GBM

We further analyzed the prognostic significance of MGMT expression and found that lower expression of MGMT predicted better prognosis in proneural-like subtype GBM (Figure 3A, $p<0.05$), but not in other and classical-like subtypes (Figure 3B). In addition, we divided the GBM specimens into two groups based on P53 expression and found that low expression of MGMT predicted better prognosis only in P53 high-expression group (Figure 3C and 3D). In TCGA database, we also found that MGMT expression predicted patient’s survival time more effective in proneural subtype and P53 high-expression group than in the other two.
MGMT and GBM molecular subtypes

The expression of MGMT in proneural subtype was lower and the prognosis was better in TCGA database.

In the TCGA database, proneural subtype GBM was found to express lower MGMT than non-proneural subtype GBM (Figure 5A, p < 0.001). Accordingly, patients survival times were longer in proneural subtype than other subtypes (Figure 5B, p < 0.05).

Discussion

The alkylation antineoplastic drugs temozolomide (TMZ) is a first-line treatment for GBMs, and the efficacy of TMZ is determined by MGMT status. Low expression of MGMT can increase the sensitivity of GBM patients to TMZ and so enhance the treatment efficacy [9]. In this study, we divided the GBM into three subtypes according to Le Mercier's method [5], and found that most of proneural-like GBMs expressed lower MGMT than classical-like and other subtype GBMs. Furthermore, lower MGMT expression predicts better prognosis in proneural-like but not in the other two subtype GBMs. These results partly explained the reason of patients with proneural-like GBM usually survive longer.

P53 mutant is proposed as a marker of proneural-like GBMs [4, 5]. This molecule is a tumor suppressor gene and physiologically acts as an important regulatory factor in cell cycle regulation, DNA repair, cell differentiation as well as cell apoptosis [16, 17]. P53 mutation has been proved to be an important event in development of variety tumors [18]. The mutant type P53 protein has been demonstrated to exhibit a carcinogenic effect and can be detected by the immunohistochemistry stain due to its prolonged half-life [19]. In this study, we detected mutant P53 and found that its expression was negatively correlated with MGMT expression. This result is consistent with the finding of proneural-like GBMs exhibiting low MGMT level. It has been reported that MGMT can prevent intracellular P53 mutation by effectively protecting G:C→A:T gene mutation [20]. On the other hand, wild type P53 has been found to inhibit intracellular MGMT expression through the pathway of MEK-ERK-MDM2-P53 [21]. Thus, there might be a feedback regulation loop between MGMT and P53. Indeed, P53 inhibitors could strengthen TMZ sensitivity by shortening the G2-M phase arrest [22]. However, whether MGMT and P53 present an antagonistic effect in the drug resistance of GBM needs to be further studied.

The characters of proneural subtype GBMs are similar to that of secondary GBMs, including TP53 mutation, PDGFRα abnormality, IDH1 mutation, and long survival time [23]. The tumor cells of proneural subtype GBMs were also enriched with the oligodendrocytic signature [24]. We found the MGMT expression is low in proneural subtype. The main reason for this may be P53 and MGMT have an antagonistic effect, or some other unknown mechanisms.

We found that patients with proneural subtype GBM usually survival longer than the other sub-
MGMT and GBM molecular subtypes

In summary, we found MGMT acts as an independent prognostic factor in GBM and plays an important role in molecular typing as well as diagnosis of GBM combined with proneural-like marker P53.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiu-Wu Bian and Hsiang-Fu Kung, Institute of Pathology and Southwest Cancer Center, Key Laboratory of Tumor Immunopathology of Ministry of Education of China, Southwest Hospital, Third Military Medical University, 30 Gaotanyan Avenue, Shapingba, Chongqing, China. Tel: +8613708373857; Fax: +862368754431; E-mail: bianxiuwu@263.net (XWB); hkung@cuhk.edu.hk (HFK)

References


MGMT and GBM molecular subtypes


