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Original Article

Genetic features and postoperative outcomes of patients with glioblastoma involving the corpus callosum

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Abstract: Aim: Glioblastoma mutliforme (GBM) with corpus callosum involvement is an important subtype of glioma; however, the prognosis and genetic factors, as well as the effectiveness of surgery and analysis, are unclear owing to the paucity of studies. Methods: This study attempted to identify genetic features and postoperative outcomes of patients with glioblastomas involving the corpus callosum. We retrospectively reviewed 238 glioma patients with histologically confirmed glioblastoma. Overall survival was analyzed, along with whether corpus callosum involvement and tumor genetic makers (TP53 mutations, MMP-9, PTEN, MGMT, EGFR, and IDH1). Results: A total of 43 (18.06%) GBM patients had corpus callosum involvement. Corpus callosum involvement was an independent factor in poor prognosis in patients with GBM (Hazards ratio (95% CI): (1.628 (1.119-2.383), P = 0.0000); mutant P53 was also associated with shorter survival for GBMs involving the corpus callosum (HR: 2.338 (1.123-4.869); P = 0.0184). Conclusion: Glioblastoma with corpus callosum involvement is a specific subtype for gliomas. Our results could be used in formulating surgical plans and clinical survival predictions.

Keywords: Corpus callosum, glioblastoma, genetic markers, brain tumor, glioma

Introduction

Gliomas are the most common type of malignant primary brain tumor, yet they currently have no effective treatment. Patients with glioblastoma multiforme who undergo maximal safe tumor resection and standard radiochemotherapy with temozolomide achieve a median survival of only 14.6 months [1, 2]. These tumors can develop de novo or via progression of a grade III glioma, which typically has a five-year survival rate of 24% [3]. Even the overall survival (OS) of high grade glioma patients (HGGs) was reported to be much shorter than that of other epithelial malignancies, such as colon or lung cancer [4]. Corpus callosum involvement of HGGs had the worst overall survival in recent studies [5-8].

The corpus callosum is made up of dense myelinated fibers and is the largest white matter structure in the brain. It is involved in many neurological diseases including refractory epilepsy and akinetic mutism [9, 10]. Involvement of corpus callosum is not an uncommon phenomenon, because the GBM can infiltrate the other hemisphere via the connecting fibers, finally developing into a butterfly glioma. A number of genetic and genomic alterations have already been clearly described in gliomas [11], including epidermal growth factor receptor (EGFR) amplification, methyl-guanine methyltransferase (MGMT) promoter methylation, and isocitrate dehydrogenase (IDH1) mutation. Patients may get a precise suggestion on chemotherapy strategy and prognosis based on these biomarkers [12].

A few studies have discussed the prognostic value of corpus callosum involvement in glioblastoma multiforme (GBM) [7-9], however, little objective data addresses the genetic factors of corpus callosum involvement in HGGs or GBM. In this study, we investigated the prognostic impact of IDH1 mutations, MGMT methylation, and EGFR overexpression in a prospective of cohort study of 238 GBMs, including 43 patients with corpus callosum involvement.

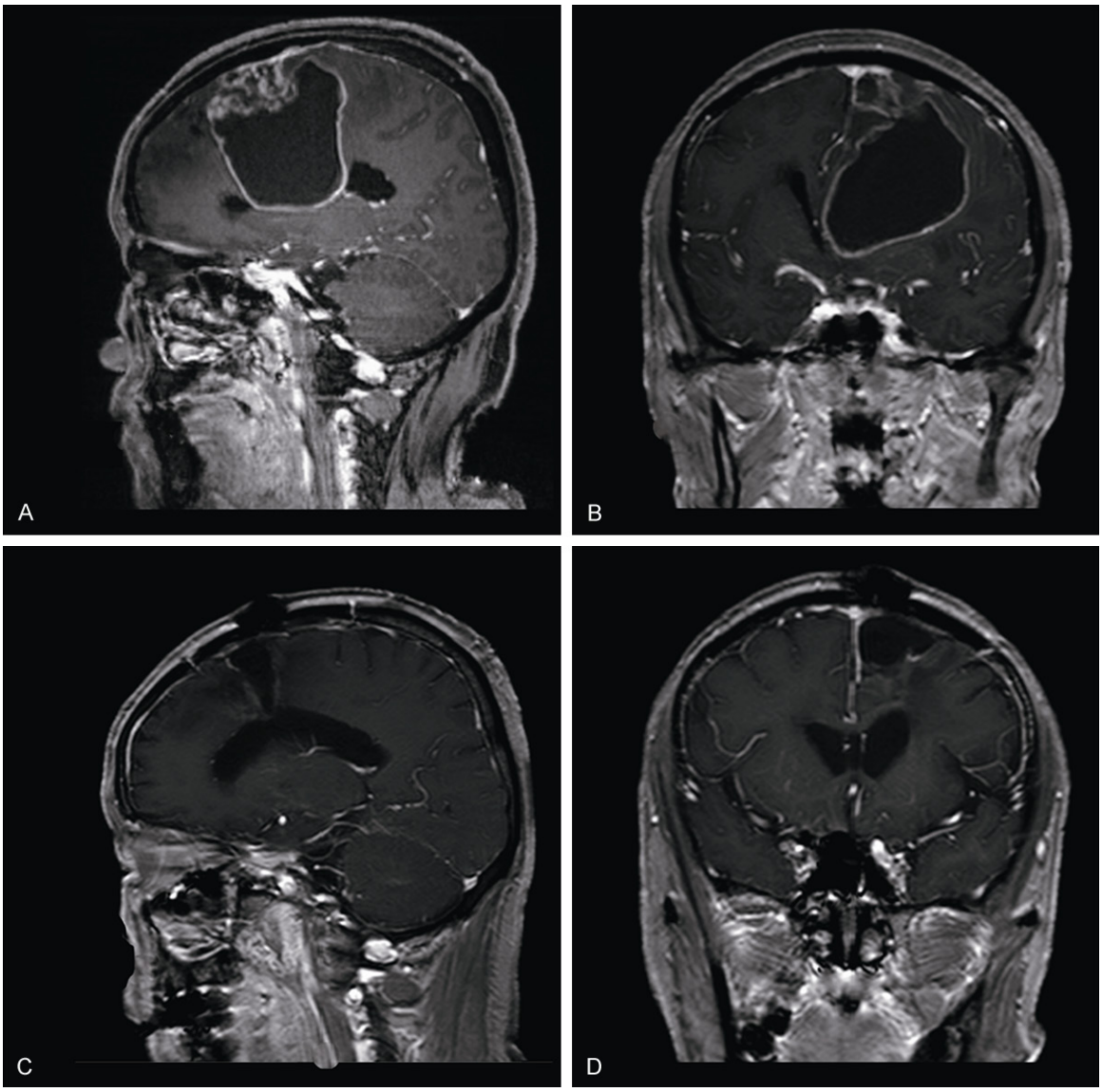

Figure 1. Typical MRI images of GBM with corpus callosum involvement. (A axial and B) sagittal preoperative views demonstrating a glioblastoma involving the corpus callosum. (C axial and D) sagittal postoperative MRI images.

Patients and methods

Participants

Medical records and magnetic resonance images (MRIs) form a total of 238 glioma patients were analyzed in this study. Requirement of informed patient consent was waived in this retrospective study, but approval was obtained from the ethics committee of West China Hospital, Si Chuan University. All patients included in this study were pathologically diagnosed with glioma between December 2011 and December 2013. The major criteria for inclusion were as followings: 1) histologically proven high grade glioblastoma, 2) tumor resection surgery, 3) pre-operative MRI images were available, 4) tumor genetic biomarkers were analyzed, 5) patient follow-up data could be acquired by telephone or out-patient department.

Evaluation of tumor boundary and lesion analysis

Imaging was performed using an Avanto 1.5T (Siemens, Erlangen, Germany) scanner. Images from each patient were manually re-examined, and the hyper-intense regions in the T1 contrast image for high grade gliomas were defined

Table 1. Eligible patients' characteristics of the study

Characteristics, number (%)	Corpus callosum involvement		*p* value
	Yes	No	
Number of patients	43	195	
Age at surgery (median)	52.7	55.3	0.584
Gender			0.725
Male	21 (48.9)	101 (51.8)	
Female	22 (49.1)	94 (48.2)	
Extent of resection			0.000*
Gross total	2 (4.6)	154 (78.9)	
Subtotal	41 (95.4)	41 (21.1)	
Preoperative KPS (group)			0.617
100-70	29 (67.4)	139 (71.3)	
< 70	14 (32.6)	56 (28.7)	
Pre-operative seizure			0.894
Yes	8 (18.1)	38 (19.9)	
No	35 (71.9)	157 (80.1)	
Adjuvant treatment			0.854
Chemotherapy	35 (81.3)	178 (91.2)	
Radiotherapy	21 (48.8)	101 (51.7)	

*Statistically significant.

Table 2. Tumor genetic biomarkers associated with corpus callosum involvement

Genetic biomarkers	Corpus callosum involvement		*p* value
	Yes	No	
MGMT methylation (+/-)	25/18	101/94	0.010*
IDH-1 (+/-)	2/41	19/176	0.278
MMP-9 (+/-)	23/20	99/96	0.747
Mutant P53 (+/-)	37/6	182/13	0.111
EGFR (+/-)	17/26	43/153	0.012*
PTEN (+/-)	21/22	97/98	0.914

*Statistically significant.

as tumor lesions. Tumor boundaries were also based on the T1 contrast images. Two associated professors (W. X. and L. YH), who were blind to the patients' diagnoses, independently evaluated whether the corpus callosum was infiltrated by gliomas based on a voxel by voxel analysis. A typical MRI images of GBM with corpus callosum involvement was showed in **Figure 1**.

Genetic analysis

Immunohistochemical analyses for five makers (mutant P53, PTEN, MMP-9, IDH1, and EGFR) were performed on formalin-fixed, paraffin-embedded tissue sections according to the manufacturer's protocols. Each slide stained for these markers was reviewed by two independent neuropathologists; samples with discrepancies in scoring were discussed until consensus was arrived at. The evaluation criteria were as follows: -, negative; +, isolated positive cells (<5%); ++, clusters of positive cells (5%-10%); and (10%-30%), mostly positive cells (>30%). If no positive staining for IDH1 was observed, the tumors were further processed by direct sequencing (performed by the HUA DA Gene Company). The forward primers (From the HUA DA Gene Company, Beijing, China) were as follows:IDH1F:5-ACGGTCTTCAGAGAAGC-3 ; and IDH1R: 5-GGTGTAGATACCAAAAG-ATAAGAAT-3. MGMT methylation analysis was detected using methylation-specific polymerase chain reaction or real-time methylation-specific PCR (LabCorp, Santa Monica, California).

Patient follow-up and data analysis

Patients' follow-up data was acquired via telephone or out-patient department. The follow-up period was at least six months after surgery. There major indices were examined: seizure prognosis, PFS (progression-free survival), and OS (overall survival). The seizure prognosis after surgery was evaluated by Engel classification, dichotomized as class I (completely seizure free) versus classes II-IV (not seizure free). PFS was defined as the duration from diagnosis to disease progression by imaging evaluation; the latest follow-up date was used if no disease progression had been observed. OS was defined as the time between diagnosis to either death or the latest follow-up.

All statistical data were analyzed by SPSS version 20.0. A type I error of a = 5% was defined. Univariate analyses were performed using the chi-square test for dichotomous variables and the Mann-Whitney U-test for continuous non-parametric data.

Results

Patients characteristics

A total of 238 glioblastoma patients were eligible for this study, including 43 (18.06%) patients with corpus callosum involvement.

Table 3. Independent associations with survival

Variables	Hazards ratio (95% CI)	*p* value
Corpus callosum involvement	1.628 (1.119-2.383)	0.0000
MGMT methylation +	0.442 (0.2610-0.908)	0.1502
IDH-1 +	NA	NA
MMP-9 +	1.091 (0.5304-1.972)	0.6226
Mutant p53 +	2.338 (1.123-4.869)	0.0184
EGFR +	1.1579 (0.7807-3.194)	0.1444
PTEN +	0.8053 (0.4184-1.550)	0.2094
Extent of resection	NA	NA

Patients' characteristics are given in **Table 1**. The median age at diagnosis was 54.1 years old. The male to female ratio was approximately 1.05:1. The median follow-up period duration in our study was 10.7 months (range 6-17 months), the last follow-up was December 31, 2015.

Glioblastoma with corpus callosum involvement

There was no statistical difference associated with age, gender, KPS score, or pre-operative seizure frequency between glioblastoma patients with or without corpus callosum involvement. However, the extent of resection (EOR) was significantly associated with corpus callosum involvement, and most of corpus callosum-involved patients only received sub-total resection (95.4%), compared to patients with no corpus callosum involvement (21.1%, P<0.001). We next analyzed the genetic features with/without corpus callosum involvement, and corpus callosum involvement was not associated with MGMT methylation, IDH1, MMP-9, or PTEN. Interestingly, corpus callosum involvement was related to EGFR overexpression (**Table 2**).

Survival analysis with tumor location and genetic features

Corpus callosum involvement has been shown to be an independent poor predictor of survival in patients with GBM (HR: 1.628 (1.119-2.383); P = 0.0000). Results of the Hazard Ratio (Mantel-Haenszel) analysis of the genetic features in GBM with corpus callosum involvement are summarized in **Table 3**. Only mutant P53 was associated with shorter survival (P = 0.0184, HR: 2.338 (1.123-4.869)). There was no statistically significant correlation between the other biomarkers and poor OS (**Figure 2**).

Discussion

To the best of our knowledge, this is the first study focusing on genetic features and postoperative outcomes of patients with glioblastoma involving the corpus callosum. Moreover, combining analysis with certain tumor location and genetic features would be helpful to precisely predict overall survival in GBM patients and plan individual treatment strategies.

Similar to some previous studies [7-9], the incidence of corpus callosum involvement in our research was 18.0%. However, this percentage was much higher than the 2.9% reported by Dziurzynski [13], and we believe that this discrepancy derived from the different inclusion criteria. In our study, the inclusion criterion was defined as tumor infiltration of any part of the corpus callosum, but the "butterfly glioma" reported by Dziurzynski. was defined as a contiguous enhancement in the bilateral corona radiata.

One key finding of this study is a statistically significant shorter overall survival in glioblastoma patients with corpus callosum involvement compared to those without involvement. This was consistent with previous studies, and moreover, corpus callosum involvement was an independent risk factor for GBM patients, with an HR of 1.61. There may be two possible explanations why corpus callosum involvement in GBM patients was an independent factor in poor prognoses. The extent of resection in GBM patients with corpus callosum involvement was relatively low; only 2 patients (4.6%) had a gross total resection in our cohort. The EOR was reported as an independent prognostic factor in many studies [14-16], and we believe the GBM could recur from the residual tumor tissue due to the aggressive growth, which could explain the short survival of those patients. Another possible reason relates to the origin of the glioma cells; [17, 18] many studies reported that neural stem cells and glial progenitor cells could be found in the subventicular zone (SVZ) and corpus callosum, and Jafri suggested that glioblastoma patients with SVZ involvement have decreased overall survival [17]. In our study, 95% of patients with corpus callosum involvement also showed tumors that had invaded the SVZ.

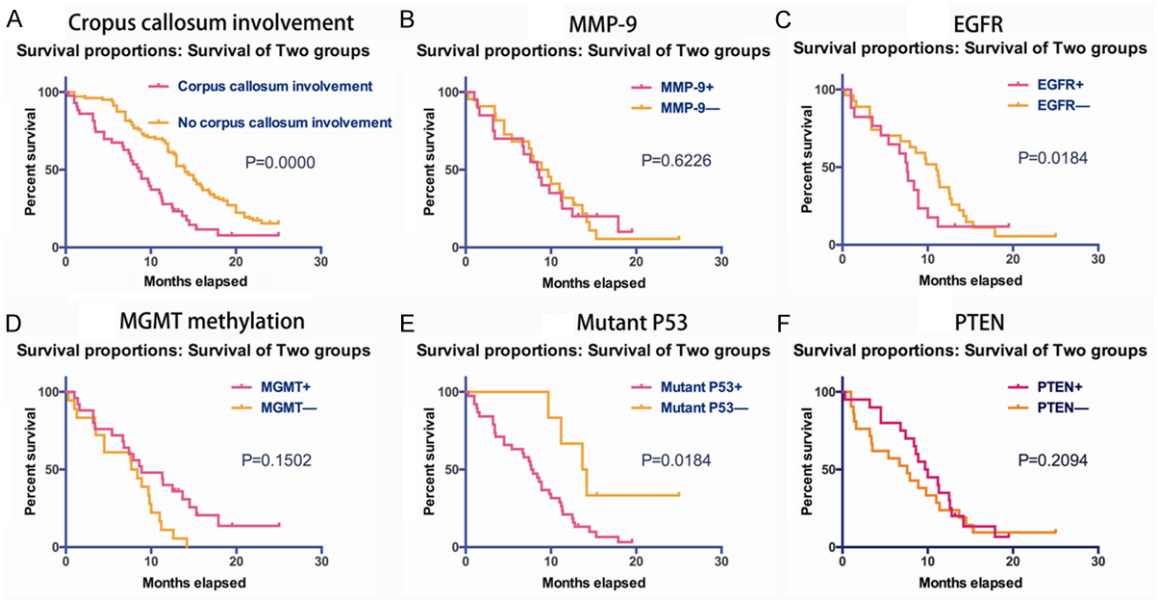

Figure 2. Kaplan-Meier plots represent overall survival, stratified by (A) corpus callosum involvement, (B) MMP-9 status, (C) EGFR status, (D) MGMT methylation, (E) mutant P53, (F) PTEN status for all GBMs involving the corpus callosum analyzed in this study involving the corpus callosum analyzed in this study.

We next analyzed the relationship between genetic features and postoperative outcomes of patients with glioblastoma involving the corpus callosum. A total of six biomarkers were identified, but due to the limited number of patients and the rarity of IDH1 mutation in primary GBMs, only 2 patients with corpus callous involvement also showed IDH1 mutation. Due to this paucity of data, we did not conduct a survival analysis of IDH1 mutation in GBMs with corpus callosum involvement. We did find that mutant P53+ protein was associated with poorer OS (HR: 2.338 (1.123-4.869); P = 0.0184). Similar results were reported by Wang YY and his colleagues, in voxel-based neuroimaging analysis of low grade gliomas; [19] however, the mutant p53 protein was not suggested as an independent prognostic biomarker in previous studies [20, 21]. This may be because previous studies did not divide the groups according to whether the glioblastoma infiltrated the corpus callosum.

One interesting result was that MGMT methylation was not related to the patients' OS. Two reasons may explain this. MGMT methylation status determined whether tumor cells would be responsive to temozolomide [22], but in our sample, some patients could not complete standard radiochemotherapy because of short survival period or poor KPS score. Another reason is that some patients could not complete the standard STUPP scheme due to financial problems. This decreased number of patients receiving standard chemotherapy may have caused the negative MGMT methylation status result [23].

Two potential limitations to this study should be considered. Firstly, PFS was not analyzed with respect to tumor location and genetic biomarkers; this was because GBMs with corpus callous involvement often did not get total resection. Moreover, most of these patients underwent craniotomy, which was generally accompanied with neurologic deficits or mental disorders, and the PFS acquired from MRI images or the latest follow-up was inaccurate compared to OS. Additionally, our sample size was relatively small, and we could not analyze the IDH1 mutation, which has a very significant impact on OS in GBM patients with corpus callosum involvement.

Conclusion

We identified anatomic correlations between genetic lesions on patients with glioblastomas with corpus callosum involvement. In addition, we demonstrated that corpus callosum involvement could be an independent prognostic factor for GBM patients. We also identified that

presences of mutant p53 protein is associated with overall survival in GBM patients with corpus callosum involvement. Our results, together with other recent evidence, could be used in formulating surgical planning and clinical survival predictions.

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Disclosure of conflict of interest

None.

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References

[1] Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. Neuro Oncol 2012; 14 Suppl 5: 1-49.

[2] Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007; 114: 97-109.

[3] Hart MG, Grant R, Garside R, Stein K, Grant R. Temozolomide for high grade glioma. Cochrane Database Syst Rev 2008; 4: CD007415.

[4] Mathis KL, Green EM, Sargent DJ, Delaney C, Simmang CL, Nelson H. Surgical quality surrogates do not predict colon cancer survival in the setting of technical credentialing: a report from the prospective COST trial. Ann Surg 2013; 257: 102-107.

[5] Wang T, Nelson RA, Bogardus A, Grannis FW Jr. Five-year lung cancer survival: which advanced stage nonsmall cell lung cancer patients attain long-term survival? Cancer 2010; 116: 1518-1525.

[6] Chaichana KL, Jusue-Torres I, Lemos AM, Gokaslan A, Cabrera-Aldana EE, Ashary A, Olivi A, Quinones-Hinojosa A. The butterfly effect on glioblastoma: is volumetric extent of resection more effective than biopsy for these tumors? J Neurooncol 2014; 120: 625-634.

[7] Chen KT, Wu TW, Chuang CC, Hsu YH, Hsu PW, Huang YC, Lin TK, Chang CN, Lee ST, Wu CT, Tseng CK, Wang CC, Pai PC, Wei KC, Chen PY. Corpus callosum involvement and postoperative outcomes of patients with gliomas. J Neurooncol 2015; 124: 1-8.

[8] Bourekas EC, Varakis K, Bruns D, Christoforidis GA, Baujan M, Slone HW, Kehagias D. Lesions of the corpus callosum: MR imaging and differential considerations in adults and children. AJR Am J Roentgenol 2002; 179: 251-257.

[9] Agrawal A. Butterfly glioma of the corpus callosum. J Cancer Res Ther 2009; 5: 43-45.

[10] Rees JH, Smirniotopoulos JG, Jones RV, Wong K. Glioblastoma multiforme: radiologic-pathologic correlation. Radiographics 1996; 16: 1413-1438.

[11] Chan AK, Yao Y, Zhang Z, Shi Z, Chen L, Chung NY, Liu JS, Li KK, Chan DT, Poon WS, Wang Y, Zhou L, Ng HK. Combination genetic signature stratifies lower-grade gliomas better than histological grade. Oncotarget 2015; 6: 20885-901.

[12] Wang J, Su HK, Zhao HF, Chen ZP, To SS. Progress in the application of molecular biomarkers in gliomas. Biochem Biophys Res Commun 2015; 465: 1-4.

[13] Dziurzynski K, Blas-Boria D, Suki D, Cahill DP, Prabhu SS, Puduvalli V, Levine N. Butterfly glioblastomas: a retrospective review and qualitative assessment of outcomes. J Neurooncol 2012; 109: 555-563.

[14] Wang Y, Wang K, Wang J, Li S, Ma J, Dai J, Jiang T. Identifying the association between contrast enhancement pattern, surgical resection, and prognosis in anaplastic glioma patients. Neuroradiology 2016; 58: 367-74.

[15] Ibe Y, Tosaka M, Horiguchi K, Sugawara K, Miyagishima T, Hirato M, Yoshimoto Y. Resection extent of the supplementary motor area and post-operative neurological deficits in glioma surgery. Br J Neurosurg 2016; 13: 1-7.

[16] Sanai N, Berger MS. Glioma extent of resection and its impact on patient outcome. Neurosurgery 2008; 62: 753-766.

[17] Jafri NF, Clarke JL, Weinberg V, Barani IJ, Cha S. Relationship of glioblastoma multiforme to the subventricular zone is associated with survival. Neuro Oncol 2013; 15: 91-96.

[18] Sanai N, Alvarez-Buylla A, Berger MS. Neural stem cells and the origin of gliomas. N Engl J Med 2005; 353: 811-822.

[19] Wang YY, Zhang T, Li SW, Qian TY, Fan X, Peng XX, Ma J, Wang L, Jiang T. Mapping p53 mutations in low-grade glioma: a voxel-based neuroimaging analysis. AJNR Am J Neuroradiol 2015; 36: 70-76.

[20] You G, Huang L, Yang P, Zhang W, Yan W, Wang Y, Bao Z, Li S, Li S, Li G, Jiang T. Clinical and

molecular genetic factors affecting postoperative seizure control of 183 Chinese adult patients with low-grade gliomas. Eur J Neurol 2012; 19: 298-306.

[21] You G, Sha ZY, Yan W, Zhang W, Wang YZ, Li SW, Sang L, Wang Z, Li GL, Li SW, Song YJ, Kang CS, Jiang T. Seizure characteristics and outcomes in 508 Chinese adult patients undergoing primary resection of low-grade gliomas: a clinicopathological study. Neuro Oncol 2012; 14: 230-241.

[22] Cheng W, Li M, Jiang Y, Zhang C, Cai J, Wang K, Wu A. Association between small heat shock protein B11 and the prognostic value of MGMT promoter methylation in patients with high-grade glioma. J Neurosurg 2015; 6: 1-10.

[23] Stupp R, Taillibert S, Kanner AA, Kesari S, Steinberg DM, Toms SA, Taylor LP, Lieberman F, Silvani A, Fink KL, Barnett GH, Zhu JJ, Henson JW, Engelhard HH, Chen TC, Tran DD, Sroubek J, Tran ND, Hottinger AF, Landolfi J, Desai R, Caroli M, Kew Y, Honnorat J, Idbaih A, Kirson ED, Weinberg U, Palti Y, Hegi ME, Ram Z. Maintenance therapy with tumor-treating fields plus temozolomide vs temozolomide alone for glioblastoma: a randomized clinical trial. JAMA 2015; 314: 2535-2543.