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Aggressive Treatment in Glioblastoma: What Determines the Survival of Patients?

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Abstract

Background and Study Aims The exact reason of long-term survival in glioblastoma (GBM) patients has remained uncertain. Molecular parameters in addition to histology to define malignant gliomas are hoped to facilitate clinical, experimental, and epidemiological studies.

Material and Methods A population of GBM patients with similar clinical characteristics (especially similar resectability) was reviewed to compare the molecular variables between poor (overall survival [OS] $<$ 18 months, control cohort) and long-term survivors (overall survival $>$ 36 months, OS-36 cohort).

Results Long-term GBM survivors were younger. In the OS-36 cohort, the positive rate of isocitrate dehydrogenase (IDH) mutation was very low (7.69%, 3/39) and there was no statistical difference in OS between IDH mutant and wild-type patients. The results of 1p/19q codeletions are similar. Besides, there were no significant difference in MGMT promoter methylation, telomerase reverse transcriptase (TERT) promoter mutation, and TP53 mutations between OS-36 cohort and control cohort.

Conclusions No distinct markers consistently have been identified in long-term survivors of GBM patients, and great importance should be attached to further understand the biological characteristics of the invasive glioma cells because of the nature of diffuse tumor permeation.

Keywords
- glioblastoma
- long-term survival
- IDH1
- 1p/19q codeletions
- MGMT
- TERT

Introduction

Glioblastoma (GBM) is one of the most common and fatal primary malignancies of the central nervous system (CNS), with a median life span of 6 months to 1.5 years and a 5-year survival rate of $<$ 10%, in spite of comprehensive treatment with extensive excision of the tumor and adjuvant therapies, including concurrent chemoradiotherapy and other molecular-targeted therapies.[1,2] However, very few patients can survive long enough and present a substantial progression-free survival (PFS). The exact reason of long-term survival in GBM patients has remained uncertain.[3]

The objective of this study based on a population of GBM patients with similar clinical characteristics (especially similar resectability) was to compare the molecular variables between poor and long-term survivors and to try to discuss the potential mechanism influencing survival in this patient population.

Methods

Patient Recruitment and Data Acquisition

This is a retrospective study of patients with prefrontal or pretemporal GBM (presumed noneloquent areas) located in a single lobe (►**Fig. 1**), operated on from January 2008 through December 2012. More case illustrations can be obtained from the Supplementary Material. All available clinical, surgical, and radiologic data were collected via patient chart review. To increase homogeneity and ensure similar resectability, all the operations were performed by the same surgical team. Furthermore, we excluded tumors that were

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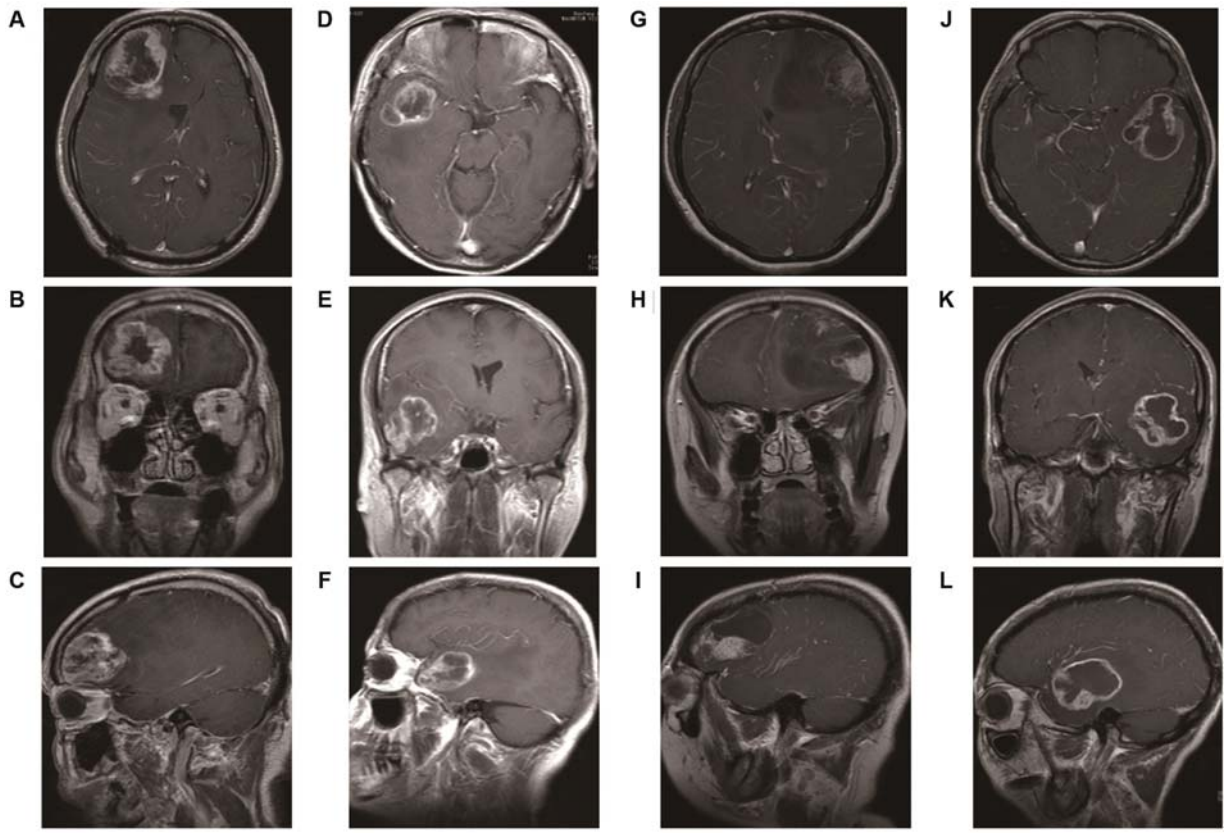

Fig. 1 (a–l) Prefrontal or pretemporal glioblastoma (presumed noneloquent areas) located in a single lobe.

in eloquent areas (motor, sensory, language cortices, or brain stem), tumors with extensive invasion (i.e., having a hemispheric diffusion or crossing the midline or invading multiple lobes), and deep-seated tumors (i.e., basal ganglia, thalamus). To be suitable for this study, it was required that the first histopathologic diagnosis was a GBM according to the 2007 WHO classification of CNS tumors confirmed by the central pathology review at the pathology department according to the 2016 WHO classification of CNS tumors.[4] Only patients older than 18 years without any history of adjuvant therapy (radiotherapy, chemotherapy, and molecular-targeted drugs) were analyzed. A total of 68 patients met the inclusion criteria.

The study design was approved by the ethical committee of our institution (Nanfang Hospital, Southern Medical University). All patients provided their written and informed consent for all surgical treatments and molecular studies of their tumor. All the tumor specimens were sent to Genetron Health Co. Ltd (Beijing, China) for testing.

General Treatment Strategy

Supratotal resection without causing an iatrogenic deficit was the surgical treatment goal. Monitoring of intraoperative motor and somatosensory evoked potentials, intraoperative mapping, and navigation were used according to surgeon preference. The surgical principle was to resect the parenchyma beyond the area of signal abnormalities on fluid-attenuated inversion recovery (FLAIR) weighted magnetic resonance imaging (MRI) (known to be invaded by tumor cells) until eloquent structures were detected by direct electrostimulation in awake patients. Furthermore, all patients who underwent supratotal resection returned to a normal life, and no patient suffered additional permanent neurologic deficit. All the patients were uniformly treated by radiotherapy and chemotherapy with concomitant and adjuvant temozolomide (Stupp protocol). Follow-up enhanced MRI and overall assessment of the patients were performed every 3 months after the operation. Once the tumor recurred, further treatment options (reoperation, Gamma Knife treatment, targeted therapy, or change of chemotherapy plan) was individualized according to the patient's desires and expectations. Considering the extent of resection in the previous operation, the second operation will definitely cause dysfunction to the patient.

DNA Extraction and Analysis for IDH1/IDH2 Mutation, MGMT Promoter Methylation, 1p/19q Codeletion, TERT Promoter Mutation, and TP53 Mutation

DNA Extraction

We extracted total deoxyribonucleic acid (DNA) from formalin-fixed, paraffin-embedded (FFPE) tissue by using QIAamp GeneRead DNA FFPE Kit (Qiagen 180134, Qiagen, Hilden, Germany) and quantified by UV absorption (NanoDrop, Thermo Fisher Scientific, Wilmington, Delaware, United States).

Multiplex PCR-Based Next-Generation Sequencing

The Genetron Health Glioma 6 Gene detection panel was designed to detect gene mutations of IDH1 R132, IDH2

R172, TERT C228T, C250T, BRAF V600E, and codeletion of chromosomes 1p and 19q. Primers for these five variants of four gene and several segments of chromosome 1p and 19q as well as barcoding adapter DNA oligos were synthesized by Sangon Biotech and dissolved to 100 µmol/L with low (Tris-EDTA) TE buffer. Sequencing libraries were generated using multiplex polymerase chain reaction (PCR) methods: the first-round enrichment in a 20-µL reaction mixtures containing 10-µL KAPA2G Fast PCR (Roche KK5020), 0.6-µL primer mixtures, 20-ng sample gDNA template, and distilled water up to the desired volume. Initial denaturation was at 95°C for 2 minutes, followed by 10 cycles at 95°C for 30 seconds, 60°C for 90 seconds, and 72°C for 90 seconds, and a final elongation at 72°C for 10 minutes. Immediately after the reaction, 5 µl enriched DNA was then combined with 1 µl universal index primers, 10 µl KAPA2G Fast PCR, and distilled water up to a system of 20 µl, the second enrichment was performed at 95°C for 5 minutes, followed by 15 cycles at 95°C for 30s, 60°C for 90s, and 72°C for 90s, and a final elongation at 72°C for 15 minutes. Each reaction was cleaned once using Agencourt AMPure XP 60-mL kit (Beckman A63881) to remove unused primers, according to the manufacturer's specifications. The concentration of the barcoded PCR produced library was then measured by Qubit 3.0 (Thermo Fisher Scientific), and diluted to 100 pmol. About 20-µL pooled amplicons were sequenced on the Ion Proton System (Thermo Fisher Scientific).

DNA Pyrosequencing for MGMT Promoter Methylation

Bisulfite modification of the DNA was performed using the EpiTect Bisulfite Kit (Qiagen 59104). DNA was amplified by PCR Master Mix and purified from the total PCR products using CoralLoad Concentrate and subjected to pyrosequencing using PyroMark Q24 MDx System (Qiagen, 970032) in accordance to the manufacturer's instructions. The methylation values obtained were averaged across the four CpG loci tested within the MGMT promoter.

Data Analysis and Workflow

Local alignments of reads to the hg19 genome were performed using bowtie2 (version 2.2.4) in paired end mode. SAM alignment files were converted to BAM files, sorted, and indexed using samtools (version 0.1.19). BAM files were processed with bam-readcount and the outputs were processed with a custom written Perl script. We set >5% as our abnormal cutoff of five mutations of four genes and set > 65 and < 35% to distinguish the loss of chromosome 1p or 19q. The samples were considered MGMT promoter methylated with an average methylation of > 10%.

Statistical Analysis

The comparison between the frequencies of molecular alterations and clinical data in the long-term survivor group versus the control group was analyzed by Fisher's exact test or the χ^2 tests. Overall survival (OS) was calculated from the date of first surgery until death or last follow-up (for censored cases). PFS was calculated from the date of surgery to the date of first progression. Survival curves were compared using the log-rank test. Cox regression and multivariate logistic regression analyses were conducted. Hazard ratio (HR) and odds ratio (OR) with 95% confidence intervals (95% CIs) were determined. All statistical analyses were conducted using SPSS 13.0 for Windows.

Results

Patient Characteristics

We identified 39 patients with a survival of >36 months and a median follow-up of 69.8 months (OS-36 cohort). Of these patients, 18 patients were documented to have survived for 60 months (OS-60). The median PFS was 31.2 months (range: 9.0–117.0 months). PFS rates at 12, 36, 48, and 60 months were 69.4, 47.2, 36.8, and 14.7%, respectively. The OS-36 cohort was compared with a cohort of 20 GBM patients who survived for < 18 months (control cohort) and had a median follow-up of 21.4 months (9 patients with survival between 18 and 36 months were excluded). Of the control cohort, 11 patients had survived < 1 year. ►**Table 1** reports the demographic, clinical, and molecular characteristics of the two cohorts. Ten of 18 OS-60 patients are still alive. OS-36 patients were younger than the control cohort ($p = 0.015$), but there was no difference between the OS-60 group and the entire OS-36 group. Initial Karnofsky Performance Score (KPS) was similar between the two groups. The re-evaluation of the preoperative and postoperative MRI by three neurosurgeons confirmed the supratotal resection of tumor in all the patients (►**Fig. 2**). There were similar surgical interventions between OS-36 patients and the control patients. A total of 58 patients (58/68, 85.3%) had recurrence, of which 16 underwent reoperation, 12 received Gamma Knife treatment, 24 received conservative treatment (targeted treatment or replacement of chemotherapy regimen), and 6

Table 1 Comparison between long- and short-term glioblastoma survivors

	OS-36 cohort ($n = 39$)	Control cohort ($n = 20$)	*p* value
Median age at diagnosis (y)	42	58	0.015[a]
KPS at diagnosis	80	70	0.076[a]
Gender (% males)	58	62	NS[b]
prefrontal (% cases)	21 (53.85%)	9 (45.00%)	NS[a] NS[a]
Pretemporal (% cases)	18 (46.15%)	11 (55.00%)	
Median OS (mo)	58	16	<0.001[c]

Abbreviations: control cohort: short-term survivors (<18 months); KPS, Karnofsky Performance Score; NS, not significant; OS, overall survival; OS-36 cohort: long-term survivors (>36 months).
[a]Mann–Whitney *U* test.
[b]Fisher's exact test.
[c]Log-rank test.

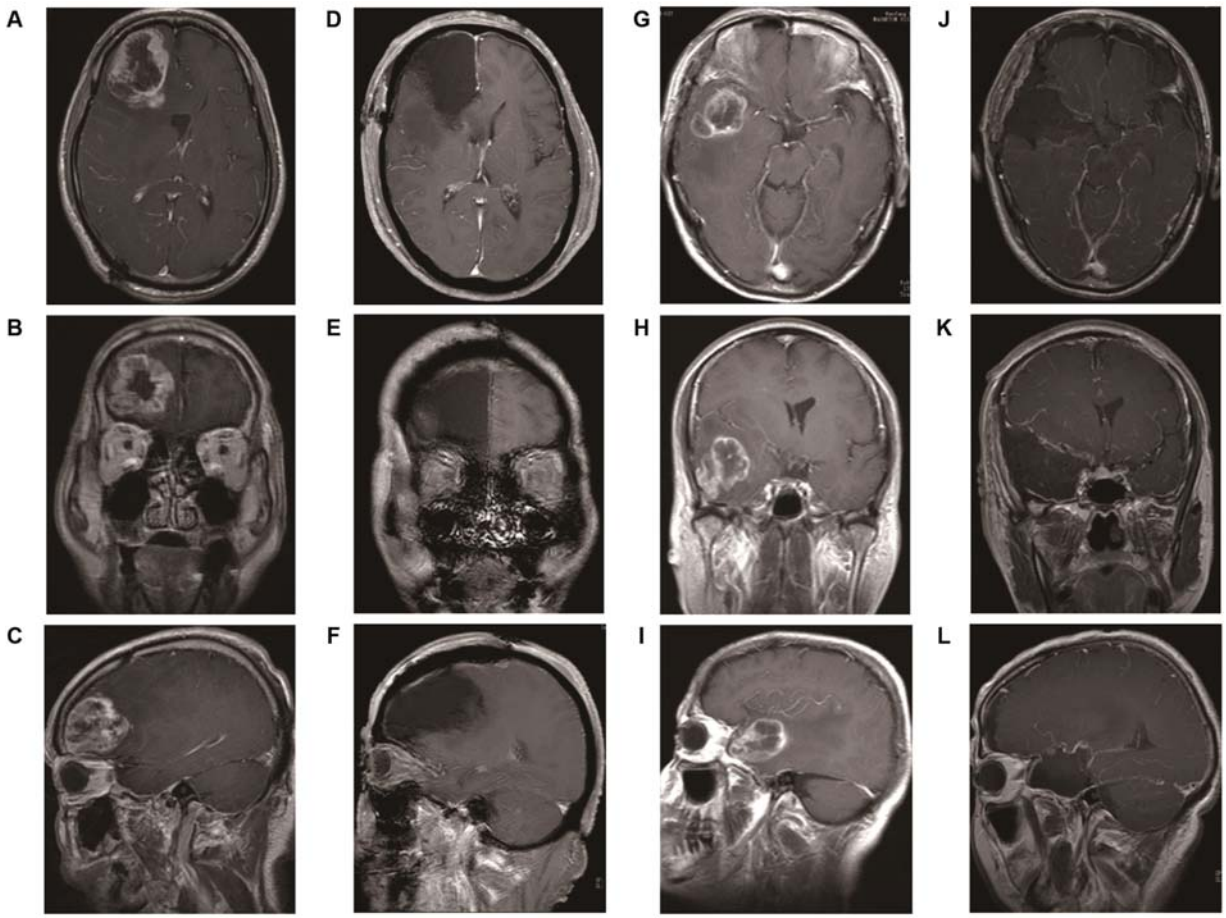

Fig. 2 (a–l) Re-examination of magnetic resonance imaging within 72 hours after operation.

Table 2 Comparison of markers in long- and short-term glioblastoma survivors

Marker	OS-36 cohort ($n = 39$)	Control cohort ($n = 20$)	p value
MGMT promoter methylation	69.23% (27/39)	55.00% (11/20)	NS
TERT promoter mutation	74.36% (29/39)	70.00% (14/20)	NS
TP53 mutations	15.38% (6/39)	10.00% (2/20)	NS

Note: For comparisons, we used the Mann–Whitney *U* test.

patients gave up further treatments. The postoperative median survival time of patients who underwent reoperation was 7.8 months (3–14 months), and we found that the postoperative survival time of most reoperation patients was < 1 year. Ten patients in the OS-60 cohort have not suffered progression: 8 are alive, 1 died of heart failure, and the other patient died from a traffic accident.

Molecular Markers and Prognostic Factors for OS-36

The results of molecular analyses are summarized in ►**Table 2**. In the OS-36 cohort, the positive rate of IDH mutation was very low (7.69%, 3/39) and there was no statistical difference in OS between the IDH mutant and wild-type patients. That is to say, most IDH and wild-type patients can still obtain long-term survival. The results of 1p/19q codeletions are similar. The relative frequencies of TP53 mutations, 1p/19q codeletions, and TERT promoter mutations were similar in OS-36 and control patients. Next, we asked which factors influenced the likelihood to become an OS-36 patient and conducted a multivariate logistic regression analysis. We included age (≤ 50 vs. > 50 years), KPS (≥ 80 vs. < 80), and five genetic alterations (MGMT promoter methylation, IDH1/IDH2 mutation, TP53 mutation, 1p/19q codeletions, and TERT promoter mutations) as the main factors. The most important prognostic factor for surviving for at least 36 months was only age ($p = 0.025$).

Discussion

There have been documented cases of GBM patients that showed a long-term survival (longer than 3 years), representing ~3 to 5% of GBM patients. However, GBM patients surviving longer than 5 years are more rare, representing $< 0.5\%$ of patients.[5,6] Thirty-nine of 68 (57.4%) of the participants in our pilot study have survived longer than 3 years, and 8 patients of the OS-60 group have survived until the time of this report, roughly 6 to 8 years. The eight patients undergoing regular radiologic and neurologic examinations

do not show any signs of disease progression. The factors that contribute to long-term survival are still unknown, including patient characteristics, histologic attributes, treatment regimen, and genetic alterations. These factors, especially the latter two, are the most discussed.

Aggressive radical tumor resection or even supracomplete resection is considered first-line treatment for GBM patients.[7–9] New technology for surgical operation of gliomas such as intraoperative MRI, intraoperative visualization, neuronavigation, cortical mapping, and functional imaging has renewed interest in a more radical resection of these tumors,[10–13] and it has been assumed that patients with supratotal resection of tumors may enjoy prolonged survival.[14,15] Even accumulating data have suggested that more aggressive surgical resection may potentially improve the efficacy of the subsequent chemotherapy and radiation.[16] However, Salford et al[17] analyzed the common characteristics of long-term survivors with malignant gliomas. The authors reported of cases of long-term survival in patients who underwent supratotal resection, but long-term survival also occurred in patients who underwent subtotal resection or even in patients who received only biopsy. Interestingly, in this series of patients, although they have similar clinical characteristics (as shown in the section of **Patient Recruitment and Data Acquisition**), survival time varies considerably between patients: some patients survive longer than 8 years, while others shorter than 1 year. In fact, GBMs at the time of diagnosis are widely diffused in respect to a highly invasive phenotype.[18] The infiltrated area around the highly cellular and contrast-enhancing mass may differ from 1 mm to several centimeters relying on neighboring anatomic structures.[18] Single invasive cells have been demonstrated to spread up to 6 cm from the hypercellular area of the tumor and even into the contralateral hemisphere through some fiber tracts, whereas compact myelinated fiber tracts tangential to the tumor mass may show little infiltration. A recurrent tumor may occur most likely from the invasive cells that preserve the ability to revert to resume a proliferative cellular program. To some extent, distinct morphologic phenotypes of the invasive cells may also have an impact on the pattern of dissemination besides distinct anatomic routs. Although the importance of surgical resection in reducing tumor burden, relieving symptoms, and confirming pathologic diagnosis is unequivocal, there is still controversy regarding the effect of surgical extent of resection on survival in GBM because randomized prospective clinical trials comparing different extent of resection will be impossible to be seen.

A 5-year follow-up from the European Organization for Research and Treatment of Cancer (EORTC) trial demonstrated that for those receiving concurrent chemoradiotherapy the median OS was 14.6 months. That is to say although adjuvant temozolomide was a major advance in the treatment of GBM, this increased the median survival in multicenter trials by only 2.5 months.[19,20] Almost certainly not all the tumor cells are sensitive to radiochemotherapy, and a small fraction of cells resistant to radiochemotherapy can eventually result in tumor recurrence. Therefore, it is impossible for GBM patients to achieve long-term survival just based on adjuvant radiochemotherapy postoperatively.

The development of transcriptome provides an opportunity to further understand the molecular mechanisms underlying GBM migration and invasion. The use of molecular markers containing information on the diagnosis, therapy, and prognosis of tumors can well make up for the defects of histopathology and immunohistochemical markers. Therefore, molecular markers play an important role in the practice of modern neuro-oncology.[21] Owing to the value in diagnosis, prognosis, or prediction, IDH1mutations, 1p and 19q codeletions, and MGMT promoter methylation are the three molecular markers for routine assessment. Gliomas with IDH mutations have a better prognosis than IDH wild-type gliomas with the same histologic grade. The codeletion of 1p/19q is closely related to oligodendrocyte histology. Tumors with codeletion of 1p/19q have better prognosis and are more sensitive to radiotherapy or alkylating drug chemotherapy than the tumors of the same grade of malignancy without codeletion. Clinical studies have shown that GBM patients with MGMT promoter methylation have longer PFS and OS when treated with alkylated drugs. The mutation of TERT promoters, which are found in GBM and grade II oligodendroglioma, gives survival advantage to IDH1 wild-type GBM patients treated with standard chemoradiotherapy.[22] However, in this series of patients, there were no differences in IDH1 mutation, 1p/19q codeletion, and TERT promoter mutation between OS-36 cohort and control patients; although MGMT promoter–methylated tumors were a little more common in the OS-36 cohort, three patients without MGMT promoter methylation survived longer than 8 years, while five patients with MGMT promoter methylation had a median survival shorter than 1 year.

That is to say, although we confirmed certain clinical factors (age) to be associated with better OS, our study still cannot explain why the 36 GBM patients reported here had a long-term survival. Nonetheless, subsets of GBM patients can indeed be shown to have a better natural history and an improved response to treatment. After all, what determines the fate of the GBM patients? GBM is documented to be a result of an accumulation of numerous genetic mutations and epigenetic changes contributing to deregulation of multiple signaling pathways. Therefore, even tumors of the same histopathologic type may substantially differ in the clinical behavior or natural history. The complex and chaotic signal pathway system has made it almost impossible to design a treatment plan that is effective for every patient.[23] It remains impossible to determine which morphological or genetic markers are helpful in determining treatment options and long-term survival.[23,24] In fact, several decades ago, many neurosurgeons had been aware that the diffuse nature of malignant gliomas made it impossible to eradicate these tumors by super-radical resections using hemispherectomies. In a subset of GBM patients, whatever the aggressive treatments were taken, local invasiveness eventually results in tumor recurrence usually around the resected cavity, which is not significantly changed by adjuvant radio- and chemotherapy.[18] This raises the question of whether certain invasive cells activate the multiple cellular programs that

make it difficult to eliminate these cells by conventional treatments. Where these invasive glioma cells preferentially localized? What is the biological difference between the invasive glioma cells and the cells that can be eliminated by surgery or radiochemotherapy? What is the biological mechanism by which these invasive cells can escape from surgery and radiochemotherapy? Future research should be designed to place greater emphasis on these questions to explain why the survival time varies considerably between GBM patients.

Conclusion

No distinct markers consistently present in long-term survivors of GBM patients have been established and the mechanism behind this spectrum of survival outcomes ranging from months to years remains largely unknown. Perhaps great importance should be attached to further understanding the biological characteristics of the invasive glioma cells that determine the ultimate survival of GBM patients.

Conflict of Interest
None declared.

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