Integrated Analysis of the Clinical and Molecular Characteristics of IDH Wild-Type Gliomas in the Chinese Glioma Genome Atlas

Peng Wang
  Beijing Tiantan Hospital

Yanwei Liu
  Beijing Tiantan Hospital

Lin Zhi
  Beijing Tiantan Hospital

Xiaoguang Qiu (✉ qiuxiaoguang@bjtth.org)
  Beijing Tiantan Hospital   https://orcid.org/0000-0002-6806-9827

Research Article

Abstract

Purpose:

Current studies and guidelines suggest that the biobehavior of IDH-wild type (IDH-wt) lower-grade glioma (LGG, WHO II-III) is similar to IDH-wt glioblastoma (GBM). However, differences in their clinical and molecular characteristics have not been reported. This study aimed to analyze the clinical and genetic information of gliomas with IDH-wt.

Methods:

Four hundred patients with IDH-wt were enrolled in the study (LGG = 176, GBM = 224), and their clinical and genetic information was collected from the Chinese Glioma Genome Atlas (CGGA). We conducted an analysis of this information between the two groups of patients and drew conclusions thereof.

Results:

The median age of the LGG patients was 42 (40–47) years, while that of the GBM patients was 51 (47–53) years ($P < 0.05$). GBM patients were more likely to undergo total resection ($P < 0.05$) and had fewer epileptic seizure symptoms ($P < 0.01$). The median overall survival (OS) was 51.87 months for the LGG patients and only 14.5 months for the GBM patients ($P < 0.01$). The median progression-free survival (PFS) was 37.6 months for the LGG patients and only 9.3 months for the GBM patients ($P < 0.01$). LGG patients were more prone to PETN mutations ($P < 0.05$). Transcriptome analysis showed that the differentially expressed genes in LGG patients were mainly enriched in metabolic pathways and pathways in cancer and in the function of signal transduction and positive regulation of GTPase activity, while in GBM patients, they were mainly enriched in the PI3K-Akt signaling pathway and in the functions of apoptotic process and oxidation-reduction process.

Conclusions:

Our data indicate that these two groups of patients should be re-evaluated and treated differently, despite both having IDH wild type.

Introduction:

Gliomas are the most common and lethal type of primary malignant central nervous system (CNS) tumor, with an extremely poor prognosis. They comprise approximately 30% of all brain tumors and 80% of all malignant brain tumors[1]. According to the World Health Organization (WHO) classification of tumors of the CNS, malignant adult diffuse gliomas are classified into grades II to IV based on their histologic features. In the 2016 edition of the classification, gliomas were subdivided into more subtypes based on

molecular features such as 1p/19q codeletion and IDH mutational status[2–4]. With the addition of molecular pathology to the diagnosis of glioma by the WHO in 2016, the classification of glioma has undergone new changes, providing a new basis for the prediction of patient treatment and prognosis, thus improving the accuracy of treatment[5, 6]. With the newly proposed molecular pathology-based diagnosis, IDH, as a very important classification standard, has been widely used in the classification and diagnosis of glioma[7]. According to Yan et al.'s research, more than 80% of LGGs harbor an IDH mutation, including diffuse astrocytoma (grade II, 90%), pleomorphic xanthoastrocytoma (grade II, 14%), and anaplastic astrocytoma (grade III, 73%), while only 5% of primary GBMs contain IDH mutations. [2, 3, 8].

Current studies have mainly focused on a comparison between IDH mutant-type LGG and GBM. Few studies have been conducted on glioma with IDH-wt. According to the latest studies by the RTOG 9802 and Kosuke et al., the prognosis of IDH-wt LGG is substantially poor, with a median OS and PFS of 22.8 months and 8.4 months, respectively, close to those of GBM[9, 10]. In addition, in terms of molecular genetic background, IDH-wt LGG and GBM are similar, and some researchers believe that IDH-wt GBM may develop from IDH-wt LGG[11]. In addition, in the third cIMPACT-NOW report, the committee recommended reclassifying IDH1/2 wild-type diffuse lower-grade gliomas of WHO grade II and III (LGG) as diffuse astrocytic glioma, IDH1/2-wt with molecular features of glioblastoma[12]. Moreover, NCCN guidelines recommend that IDH wild-type LGG be treated using therapy typical for GBM[13]. However, according to the above reports and the latest literature, as well as our data, we found that IDH-wt LGG and IDH-wt GBM may have differences in their prognoses and molecular features such as TERT promoter mutation and EGFR amplification, which may lead to differences in treatment [12, 14].

Our study explored the differences between the two types of glioma by comparing the clinical and genetic information of 400 patients, which will provide a theoretical basis for better distinguishing the two types of patients and future precision medicine.

Methods:

Patients:

All patients with newly diagnosed gliomas enrolled in this study were obtained from the Chinese Glioma Genome Atlas (CGGA) database (http://cgga.org.cn/), and their clinical characteristics, such as age, sex, resection range, survival, and chemotherapy regimen, and molecular pathological information, such as 1p/19q deletion, MGMT methylation status, and PTEN mutational status, were collected for subsequent analysis. We also collected mRNA sequencing (mRNA-seq) data from 140 patients. All research performed was approved by the Tiantan Hospital Institutional Review Board (IRB). All the subjects were diagnosed with glioma by consensus, according to central pathology reviews by independent board-certified neuropathologists and further graded based on the 2007/2016 WHO classification. Written informed consent was obtained from all patients.

Identification Of Molecular Alterations:

IDH1/2 (IDH) mutational status was detected by pyrosequencing. We predicted 1p/19q codeletion status by FISH. To determine the mutation status of P53 and PTEN, we used an immunohistochemical method[15–18].

Data analysis:

To identify the gene sets related to particular biological processes present in IDH-wt LGG and GBM, gene expression profiling and gene set enrichment analysis (GSEA) were performed as described previously[19]. The analysis we conducted was based on that found on the Database for Annotation, Visualization, and Integrated Discovery (DAVID) website[20, 21]. Survival distributions were estimated with Kaplan-Meier survival analysis, and log-rank analysis was used to assess the significance of differences between stratified survival groups using GraphPad Prism 8.0 statistical software. The χ^2 test was performed by SPSS software (IBM SPSS Statistics 24). We found the gene sequencing data of the enrolled patients through the CGGA database, calculated the mean value of each gene and then used unpaired t test grouping to select genes of interest in IDH-wt LGG and GBM. We used R Studio to perform supervised cluster analysis on the screened differential genes to identify whether they were different from each other.

Results:

Clinical information analysis:

In terms of clinical information, we found that the mean age of the IDH wild-type LGG and GBM patients was 42 years and 51 years, respectively ($P < 0.01$). A total of 60.3% of IDH-wt GBM patients and 82/176 (46%) LGG patients underwent total resection ($P = 0.016$). Epileptic symptoms were noted for 50/224 (22.3%) IDH-wt GBM patients and 86/176 (48.8%) IDH-wt LGG patients, indicating that LGG patients are more likely to be complicated with epileptic symptoms ($P < 0.01$). There was no significant difference in the extent of invasion (multilobe LGG, 59/176, 33.5%; multilobe GBM, 74/224, 33%; $P = 0.31 > 0.05$) or sex ($P = 0.269 > 0.05$) between the two groups of patients. Data for all of the above patients are shown in Table 1.

Table 1
Characteristics of clinical and molecular in this study.

characteristics	II-III grade (IDH wild-type)	primary GBM (IDH wild-type)	P value
Total	176 (44%)	224 (56%)	NA
Age	42(40−47)	51(47−53)	< 0.01
Gender			0.269
Male	109(61.9%)	146(65.2%)	
Female	67(38.1%)	78(34.8)	
Resection			0.016
Total resection	82 (46.6%)	135 (60.3%)	
Subtotal resection	79 (44.9%)	78 (34.8%)	
NA	15 (8.5%)	11 (4.9%)	
Tumor location			0.310
Multi lobe	59 (33.5%)	74 (33.0%)	
Single lobe	80 (45.5%)	79 (35.3%)	
NA	37 (21.0%)	71 (31.7%)	
Symptom			< 0.01
Seizure	86 (48.9%)	50 (22.3%)	
No seizure	72 (40.9%)	153 (68.3%)	
NA	18 (10.2%)	21 (9.4%)	
Radiotherapy			0.087
Yes	144 (81.8%)	190 (84.8%)	
No	28 (15.9%)	22 (9.8%)	
NA	4 (2.3%)	12 (5.4%)	
Chemotherapy			< 0.05
Yes	84 (47.7%)	159 (71.0%)	
No	85 (48.3)	56 (25.0%)	
NA	7 (4.0%)	9 (4%)	
Median PFS (Mo)	37.6	9.3	< 0.01

characteristics	II-III grade (IDH wild-type)	primary GBM (IDH wild-type)	P value
Median OS (Mo)	51.87	14.5	< 0.01
1p/19q codeletion			< 0.05
Yes	11 (6.3%)	0 (0%)	
No	51 (29%)	106 (47.3%)	
NA	114 (64.7%)	118 (52.7%)	
MGMT methylation			0.232
Yes	29 (16.5%)	69 (30.8%)	
No	42 (23.9%)	140 (62.5%)	
NA	105 (59.6%)	15 (6.7%)	
PETN			0.002
Yes(wild)	90 (51.2%)	79 (35.3%)	
No(mutant)	8 (4.5%)	26 (11.6%)	
NA	78 (44.3%)	119 (53.1%)	
P53			0.481
Yes(wild)	89 (50.6%)	88 (39.3%)	
No(mutant)	13 (7.4%)	17 (7.6%)	
NA	74 (42.0%)	119 (53.1%)	

Survival analysis:

To understand patient prognosis, we collected survival data from the enrolled cohort. The overall survival (OS) was 51.87 months for the IDH-wt LGG patients and only 14.5 months for the IDH-wt GBM patients (hazard ratio=0.4073, 95% CI of hazard ratio=0.3319 to 0.4999, P<0.0001). The median progression-free survival (PFS) was 37.6 months for IDH-wt LGG and only 9.3 months for GBM (HR=0.4272, 95% CI = 0.3486 to 0.5236, P<0.0001) (Figure 1). By Kaplan-Meier survival analysis, we found significant differences in survival between the two groups, even though the treatment strategy for IDH-wt LGG was less aggressive than that for IDH-wt GBM. We performed univariable Cox regression analysis on the patient prognostic data and found that in LGG, people with 1p/19q codeletion often had a better prognosis (P=0.006), and patients with MGMT methylation had a better prognosis (P<0.001). The prognosis of patients who did not undergo radiotherapy or chemotherapy was better (P=0.038). In LGG, the prognosis of grade 2 glioma was significantly better than that of grade 3 glioma (p<0.001). In GBM,

patients without PTEN mutations had a better prognosis (p=0.023). Intraoperative total tumor resection (p=0.015<0.05), postoperative radiotherapy (p=0.034) and postoperative acceptance with chemotherapy (p<0.001) were associated with a better prognosis.

Molecular pathological characteristics:

Among the patient information we collected, 1p/19q codeletion occurred in none of the IDH-wt GBM patients (0%) and in 11 IDH-wt LGG patients (21.6%). Compared with IDH-wt GBM patients (79, 35.3%), IDH-wt LGG patients (90 patients, 51.1%) were less likely to have PTEN mutations (P=0.002).

However, in our data, there was no significant difference in MGMT methylation status or P53 mutational status between the two groups of patients, with P-values of 0.232 and 0.481, respectively (Table 1).

Gene expression analysis:

We used R Studio to perform supervised cluster analysis on the gene sequencing data and obtained gene heat maps describing the levels of gene expression in the different patient groups (Figure 2). Through the obtained gene heat map, it can be seen that there are obvious differences in the gene expression levels and gene expression types between IDH-wt LGG patients and IDH-wt GBM patients.

We screened the IDH-wt LGG and GBM genes from the database and performed Gene Ontology (GO) analysis. The result of the analysis were completely different for gliomas of different grades (Figure 3). The differentially expressed genes in patients with IDH-wt LGG were mainly enriched in the signal transduction and positive regulation of GTPase activity functions, while those in patients with IDH-wt GBM were mainly enriched in the functions of apoptotic process and oxidation-reduction process.

We compared the gene expression of the patients and used the Kyoto Encyclopedia of Genes and Genomes (KEGG) database provided by the DAVID website for correlation analysis and found that the cellular pathways of IDH wild-type LGG and GBM were completely different in terms of magnitude and function[20, 21]. The differentially expressed genes in LGG were mainly enriched in metabolic pathways and pathways in cancer, while those in GBM were mainly enriched in the PI3K-Akt signaling pathway and HTLV-1 infection (Figure 4).

Discussion:

Following the release of the WHO's new glioma classification in 2016, there has been a growing trend toward precision medicine. Our research compared the clinical information, molecular pathology, and gene expression between IDH-wt LGG and GBM patients. According to the results of our analysis, we found that there were significant differences between the two groups in the above aspects.

clinical information

In terms of clinical information, the IDH-wt LGG patients were primarily 40-47 years old, with a median age of 42 years, while the GBM patients were primarily 47-53 years old, with a median age of 41 years. According to Ostorm et al.'s research, GBM is mainly found in elderly patients (65-75), and LGG is mainly found in younger patients (age<65)[22]. By comparing the two sets of data, we found that the median age of the GBM patients was much younger than that of the LGG patients (P<0.05), indicating that IDH-wt might lead to a tendency toward a younger age of onset in GBM. By collecting patient surgical data, we found that 60.3% of GBM patients but only 46% of LGG patients underwent total tumor resection. Therefore, we suggest that GBM patients are more inclined to undergo total tumor resection, while the course of LGG is longer, and the scope of tumor invasion may be wider; furthermore, there is no obvious MRI enhancement boundary, so surgery for total resection may be difficult. Epilepsy is one of the most common symptoms of glioma patients, so we collected the history of epilepsy of the enrolled patients and found that 50/224 patients with GBM (22.3%) and 86/176 patients with LGG (48.8%) had epilepsy. There was a significant difference between the two groups of patients (p<0.01). In summary, we concluded that there are significant differences between LGG and GBM in terms of clinical manifestations.

Survival analysis:

By analyzing the survival data, we found that the OS and PFS between IDH-wt LGG and GBM were completely different (P<0.001). The OS of LGG in our database reached 51.87 months, while that of GBM was only 14.5 months. The PFS of LGG was 37.6 months, and that of GBM was only 9.3 months. Although the treatment strategy for IDH-wt LGG is less aggressive than GBM, its OS and PFS were still better. According to the research data from RTOG 9802, the OS and PFS of LGG are 7.5 years and 4.4 years, respectively, which are significantly longer than what we reported. The reason may be that our LGG patient data was not subjected to risk stratification[23].

Molecular pathological characteristics:

Among the patients we collected, a total of 168 contained 1p/19q data, including 62 (36.9%) LGG patients and 106 (63.1%) GBM patients. None of the GBM patients (0%) and 11 of the LGG patients (17.7%) had 1p/19q codeletion. This result is consistent with the current major view[24]. According to the results of the chi-square test, the probability of 1p/19 codeletion was significantly different between IDH-wt LGG and GBM patients (P<0.05). A total of 203 of 400 patients had PETN mutation data (50.8%), 98 (48.3%) with LGG and 105 (51.7%) with GBM. Ninety (91.8%) LGG patients and only 79 (75.2%) GBM patients had wild-type PETN. By studying the previous literature, we found that the above data are consistent with previously reported data[25-28]. The chi-square test also showed that the probability of PETN mutational status was significantly different between IDH-wt LGG and GBM patients (P<0.001). In regard to p53 mutational status and MGMT methylation status, we could not find any significant differences. The chi-square test results showed that the P value for the difference in P53 mutational status between IDH-wt LGG and GBM was 0.481, while that for the MGMT methylation status was 0.232, indicating no statistically significant difference between the groups for either condition. Through the

above analysis, we believe that although only some of the abovementioned molecular pathological characteristics are different between IDH-wt LGG and GBM, significant differences were demonstrated in at least 1p/19q codeletion and PETN mutational status.

Gene expression analysis:

Based on the above differences in clinical and molecular pathology, we found 8879 differentially expressed genes from the CGGA database between the two groups through unpaired t test analysis (p<0.01). Through these differentially expressed genes, we conducted supervised clustering analysis and produced a gene heat map. Then, we performed GO analysis and KEGG pathway analysis and found that there were also differences in the signaling pathways and protein expression levels between LGG and GBM patients. The differentially expressed genes in LGG patients primarily performed the function of signal transduction and positive regulation of GTPase activity, while those among GBM patients primarily performed the functions of apoptotic process and oxidation-reduction process. In regard to KEGG pathway analysis, the differentially expressed genes in LGG were more enriched in metabolic pathways and pathways in cancer, while those in GBM were more enriched in the PI3K-Akt signaling pathway and HTLV-1 infection pathway.

Although IDH-wt LGG and GBM have many similarities, the current data and research are insufficient to show that they are the same, nor can IDH-wt LGG be treated the same way as GBM. Further research is needed to reclassify these diseases in greater detail. However, according to the recently released NCCN 2020 treatment guidelines, IDH-wt LGG should be treated more similarly to GBM. Therefore, whether this regimen is the most suitable treatment regimen should be further analyzed[13].

Although the results of our analysis are relatively significant, this study included data from the CGGA database, which contains data only on Asians, which may cause a certain bias in the gene expression. In addition, our study only included newly diagnosed patients and excluded recurrent GBM patients, which may also produce a certain bias for this later group. Currently, treatment regimens for IDH-wt LGG in the NCCN treatment guidelines tend to be consistent with GBM, and the 3rd version of cIMPACT-NOW indicates that when IDH-wt diffuse astrocytoma has certain molecular pathological characteristics, it can be considered a WHO grade IV glioma; however, through our analysis of clinical information, molecular pathology and gene expression, we found that there are still many differences between IDH-wt LGG and GBM[12, 13]

Conclusion:

Through the above analyses, we found that there are differences between LGG and GBM in terms of prognosis, epilepsy, resection range, PTEN mutational status and biological behavior. These differences imply that whether the current treatment regimen is ideal still needs to be explored further.

Declarations:

References:

1. Perry, A. and P. Wesseling, *Histologic classification of gliomas.* Handb Clin Neurol, 2016. **134**: p. 71-95.
2. Wang, P., et al., *Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas.* Oncogene, 2013. **32**(25): p. 3091-100.
3. De Carli, E., X. Wang, and S. Puget, *IDH1 and IDH2 mutations in gliomas.* N Engl J Med, 2009. **360**(21): p. 2248; author reply 2249.
4. Yan, H., et al., *IDH1 and IDH2 mutations in gliomas.* N Engl J Med, 2009. **360**(8): p. 765-73.
5. Louis, D.N., et al., *The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary.* Acta Neuropathol, 2016. **131**(6): p. 803-20.
6. Louis, D.N., et al., *The 2007 WHO classification of tumours of the central nervous system.* Acta Neuropathol, 2007. **114**(2): p. 97-109.

7. Camelo-Piragua, S. and S. Kesari, *Further understanding of the pathology of glioma: implications for the clinic.* Expert Rev Neurother, 2016. **16**(9): p. 1055-65.

8. Han, C.H. and T.T. Batchelor, *Isocitrate dehydrogenase mutation as a therapeutic target in gliomas.* Chin Clin Oncol, 2017. **6**(3): p. 33.

9. Kros, J.M., et al., *Evidence-Based Diagnostic Algorithm for Glioma: Analysis of the Results of Pathology Panel Review and Molecular Parameters of EORTC 26951 and 26882 Trials.* J Clin Oncol, 2015. **33**(17): p. 1943-50.

10. Bell, E.H., et al., *Comprehensive Genomic Analysis in NRG Oncology/RTOG 9802: A Phase III Trial of Radiation Versus Radiation Plus Procarbazine, Lomustine (CCNU), and Vincristine in High-Risk Low-Grade Glioma.* J Clin Oncol, 2020. **38**(29): p. 3407-3417.

11. Aoki, K., et al., *Prognostic relevance of genetic alterations in diffuse lower-grade gliomas.* Neuro Oncol, 2018. **20**(1): p. 66-77.

12. Brat, D.J., et al., *cIMPACT-NOW update 3: recommended diagnostic criteria for "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV".* Acta Neuropathol, 2018. **136**(5): p. 805-810.

13. Nabors, L.B., et al., *Central Nervous System Cancers, Version 3.2020, NCCN Clinical Practice Guidelines in Oncology.* J Natl Compr Canc Netw, 2020. **18**(11): p. 1537-1570.

14. Eckel-Passow, J.E., et al., *Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors.* N Engl J Med, 2015. **372**(26): p. 2499-508.

15. Bao, Z.S., et al., *RNA-seq of 272 gliomas revealed a novel, recurrent PTPRZ1-MET fusion transcript in secondary glioblastomas.* Genome Res, 2014. **24**(11): p. 1765-73.

16. Zhang, W., et al., *Genome-wide DNA methylation profiling identifies ALDH1A3 promoter methylation as a prognostic predictor in G-CIMP- primary glioblastoma.* Cancer Lett, 2013. **328**(1): p. 120-5.

17. Cai, J., et al., *Loss of ATRX, associated with DNA methylation pattern of chromosome end, impacted biological behaviors of astrocytic tumors.* Oncotarget, 2015. **6**(20): p. 18105-15.

18. Hu, X., et al., *Multigene signature for predicting prognosis of patients with 1p19q co-deletion diffuse glioma.* Neuro Oncol, 2017. **19**(6): p. 786-795.

19. Subramanian, A., et al., *Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles.* Proc Natl Acad Sci U S A, 2005. **102**(43): p. 15545-50.

20. Huang da, W., B.T. Sherman, and R.A. Lempicki, *Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists.* Nucleic Acids Res, 2009. **37**(1): p. 1-13.

21. Huang da, W., B.T. Sherman, and R.A. Lempicki, *Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.* Nat Protoc, 2009. **4**(1): p. 44-57.

22. Ostrom, Q.T., et al., *The epidemiology of glioma in adults: a "state of the science" review.* Neuro Oncol, 2014. **16**(7): p. 896-913.

23. Shaw, E.G., et al., *Randomized trial of radiation therapy plus procarbazine, lomustine, and vincristine chemotherapy for supratentorial adult low-grade glioma: initial results of RTOG 9802.* J Clin Oncol,

2012. **30**(25): p. 3065-70.

24. Jenkins, R.B., et al., *A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma.* Cancer Res, 2006. **66**(20): p. 9852-61.

25. Limam, S., et al., *Prognostic significance of MGMT methylation and expression of MGMT, P53, EGFR, MDM2 and PTEN in glioblastoma multiforme.* Ann Biol Clin (Paris), 2019. **77**(3): p. 307-317.

26. Trabelsi, S., et al., *MGMT methylation assessment in glioblastoma: MS-MLPA versus human methylation 450K beadchip array and immunohistochemistry.* Clin Transl Oncol, 2016. **18**(4): p. 391-7.

27. Soomro, S.H., et al., *Molecular biology of glioblastoma: Classification and mutational locations.* J Pak Med Assoc, 2017. **67**(9): p. 1410-1414.

28. Gittleman, H., A.E. Sloan, and J.S. Barnholtz-Sloan, *An independently validated survival nomogram for lower-grade glioma.* Neuro Oncol, 2020. **22**(5): p. 665-674.
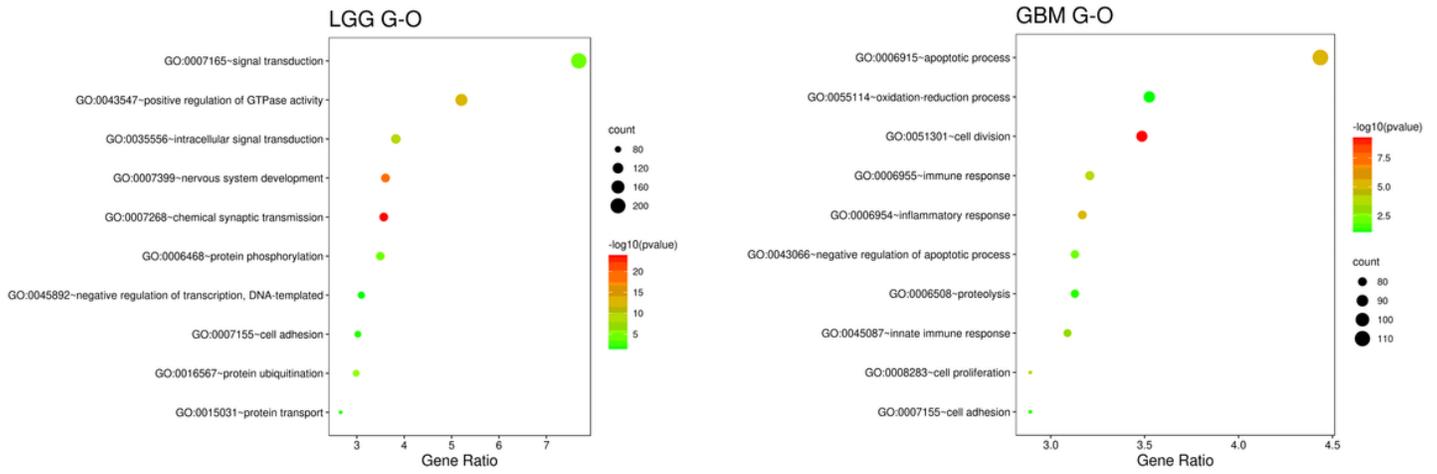
Figures
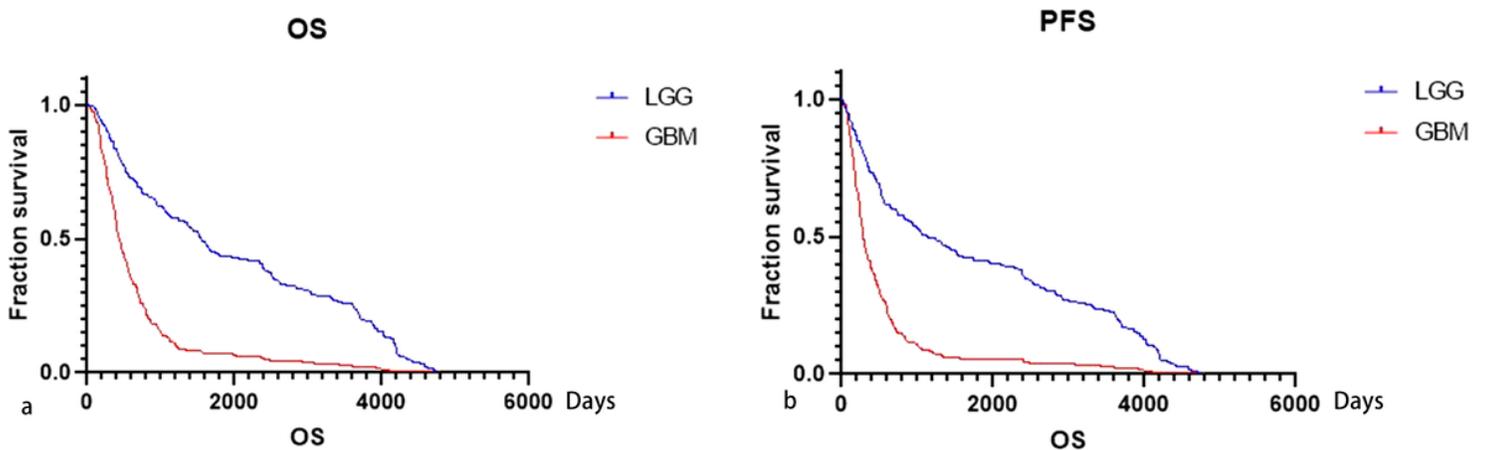
Figure 1

The Kaplan–Meier estimates for overall survival (OS) (a) and progression-free survival (PFS) (b) indicate that IDH-wt LGG was associated with longer overall survival (p = 0.011) and longer progression-free survival (p<0.01) than IDH-wt GBM.
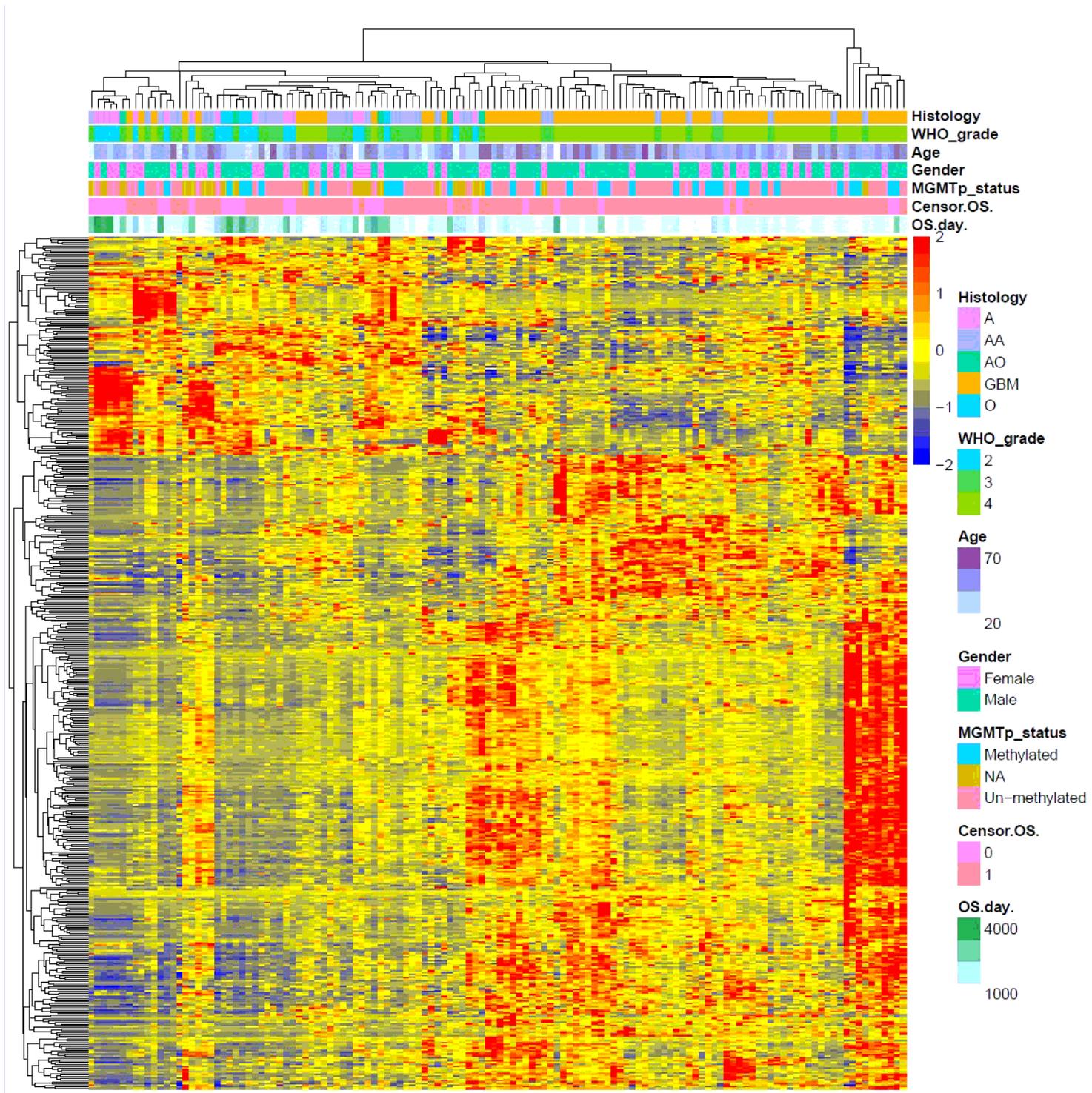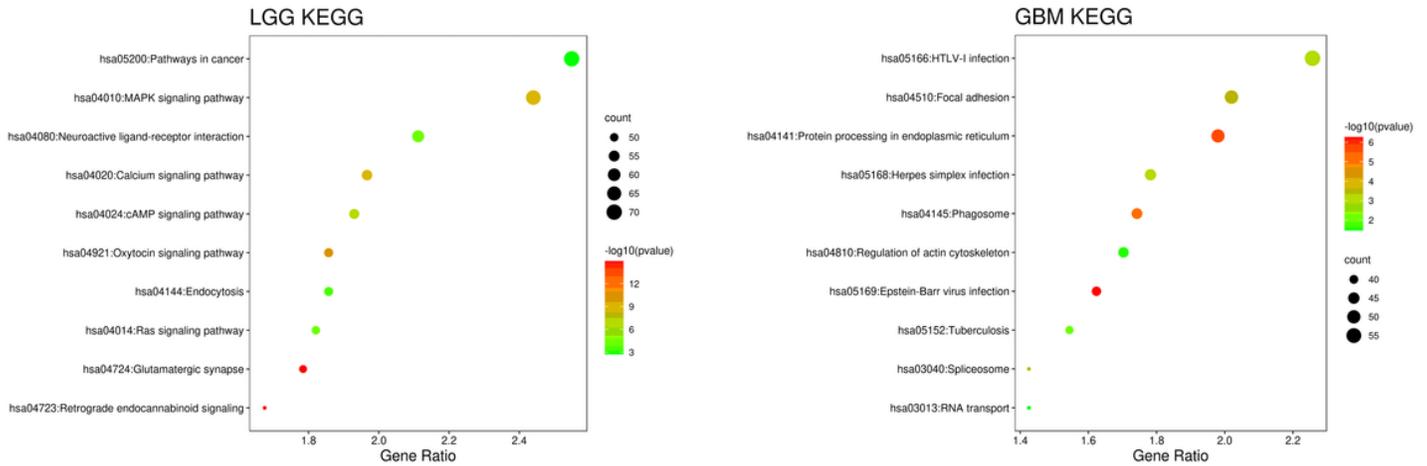
Figure 2

Supervised cluster analysis of the gene sequencing data. We obtained gene heat maps describing gene expression in the different patient groups and found that there were obvious differences in gene expression levels and gene expression types between IDH-wt LGG patients and IDH-wt GBM patients.

Figure 3

Functional enrichment analysis of associated genes, indicating the functional roles of the gene sets in the different subgroups. Enrichment results for biological processes were obtained from the GO database. The orders of the biological processes listed in the bubble chart are based on the number of targets annotated in biological process (BP).

Figure 4

Patient gene expression analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database provided by the DAVID website for correlation analysis. The orders of the biological processes listed in the bubble chart are based on the number of targets annotated in biological process (BP).