Abstract. The World Health Organization classification distinguishes four grades for gliomas. Grade III gliomas, which are brain malignant brain tumors with variable biological behavior and propensity, have been not widely investigated. The objective of the present study was to identify specific gene modules and valuable hubs associated with gliomagenesis and molecular signatures to assist in determining grade III glioma prognosis. mRNAseq and micro (mi)RNAseq data were used to construct a co-expression network of gliomas using weight gene co-expression network analysis, and revealed the prognostic molecular signature of grade III gliomas. The differently expressed miRNAs and mRNAs were identified. A total of 37 mRNAs and 10 miRNAs were identified, which were closely associated with the survival rates of patients with grade III glioma. To further understand the tumorigenesis, Cytoscape software was used to construct a network containing these differently expressed molecules. The result suggested that both the downregulated genes and upregulated genes are vital in the process of glioma deterioration, and certain genes are closely associated with clinical prognosis.

Introduction

Gliomas are common primary tumors of the central nervous system. According to the World Health Organization classification, grade I and II gliomas are benign tumors, whereas grade III gliomas are a class of malignant brain solid tumor with a median patient survival rate of 2-5 years (1). These represent 10% of primary brain tumors (2), which can infiltrate the surrounding brain parenchyma. Using standard therapeutic protocols, patients with malignant glioma have different pathological appearances and clinical outcomes. Treatments include surgery, radiation therapy, and chemotherapy; however, there is no specialized treatment available. There are insufficient molecular targets relevant in the choice of therapy, and their role in clinical trials requires validation. Novel therapeutic methods based on the specific mechanism of high grade glioma carcinogenesis are required to improve treatment efficiency and avoid the side effects of traditional treatment.

In order to better understand the mechanisms underlying complicated diseases, building and analyzing biological networks associated with the intractable diseases are becoming an efficient approach. Instead of individual genetic determinants, network approaches provide an insight into the pathogenesis of complex diseases by examining interacting gene sets and pathways (3,4). The network analysis of expression profile data has been able to identify genes modules associated with tumorigenesis. In addition, this method can be used to understand the mechanisms underlying gliomagenesis at the system and gene level.

Weighted gene co-expression network analysis (WGCNA) was been widely used to examine the changes of transcriptome expression patterns in various diseases, which identifies clusters (modules) of highly correlated genes, and summarizes clusters using the module eigengene or an intramodular hub gene (5-8). Correlation networks facilitate network-based gene screening methods, which can be used to identify candidate biomarkers or therapeutic targets. These methods have been successfully applied in complex biological contexts, including cancer, mouse genetics, yeast and genetics, and the analysis of brain imaging data (7). In addition, the algorithm of WGCNA can simplify the problems of multiple testing, which are unavoidable in standard gene-centric methods of microarray expression profiling data analysis; consequently it is a useful systematic analysis method, which focuses on the coherence function of network modules (9).

The Cancer Genome Atlas (TCGA) project has provided a comprehensive means to improve the ability to diagnose, treat and prevent cancer through an improved understanding
of the genetic basis of the disease. By the end of 2015, TCGA had analyzed the genomic, epigenomic and gene expression profiles of >10,000 specimens from >25 types of tumor (10). This substantial data provides opportunities to identify the mechanism and prognostic molecular signatures of cancer in a comprehensive manner. The lower grade glioma (LGG) group data in TCGA includes the grade II and grade III glioma gene expression profiles and relevant clinical data of those samples. To better understand the mechanism underlying clinical heterogeneity, the present study combined the LGG micro (mi)RNAseq and mRNAseq data of TCGA to identify the relevant network of tumorigenesis and prognostic genes in clinical traits. 

Materials and methods

Patient characteristics and integrated mRNA and miRNA profiles. The clinical, and mRNAseq and miRNAseq data for 83 patients with grade III glioma were downloaded from the LGG cohort. The corresponding normal cohort data were obtained from five TCGA glioblastoma (GBM) normal control samples. The TCGA-Assembler download level-3 RNASeqV2 gene expression data, miRNA-seq data samples of and the clinical information of the patients were used (DirectoryTraversalResult_Sep-18-2015.rda). The raw count mRNAseq data of 83 glioma grade III patients and raw read miRNA data of five GBM normal patients (TCGA-06-AABW-11A-31R-A36H-07, TCGA-06-0678-11A-32R-A36H-07, TCGA-06-0675-11A-32R-A36H-07, TCGA-06-0681-11A-41R-A36H-07, TCGA-06-0680-11A-32R-A36H-07 and TCGA-HW-7493-01A-11R-2027-07), were selected.

Statistical analyses. Expressed data close to zero were eliminated, and round numbers of all arrays were selected. The normal group were compared with the grade III glioma group, and the ‘DESeq’ package in R software (3.3.0; www.r-project.org) was used to identify the differentially expressed genes (DEGs), miRNAs with a fold change >2.0, and adjusted P-value of P<0.05.

The WGCNA was used to identify the co-expression modules (5,7,11). WGCNA was implemented in the Bioconductor package (bioconductor.org/biocLite.R). The DEGs were applied to identify the gene modules of highly correlated genes using WGCNA. A total of 2,036 of the DEGs were selected and Pearson's correlation was calculated for all pairs of selected genes. The correlation matrix was converted into an adjacency matrix with a power function, so that the connection strength between two genes, \( x_i \) and \( x_j \), was defined as: \( a_{ij} = \beta \times (1 + \text{cor}(x_i, x_j))^{|\beta|} \). Where \( x_i \) and \( x_j \) represent the expression values of probes, and parameter \( \beta \) was determined by the criterion that the resulting adjacency matrix approximately fit a scale-free topological feature according to a previously proposed model-fitting index (11). The row index \( u \) (\( u=1, \ldots, m \)) represents sample measurements. The adjacency matrix was further transformed into a topological overlap matrix, which captures not only the direct interaction between two genes, but also their indirect interactions through all the other genes in the network. In the present study, two functions of adjacency matrices were defined. First, the Topological Overlap Matrix (TOM) is defined as follows:

\[
TOM_{ij} = \frac{\sum_{u} a_{iu} a_{uj} + a_{ij}}{\min(k_i, k_j) + 1 - a_{ij}}
\]

where is the node connectivity. A second function was used as a distance matrix in the hierarchical clustering of the transcript units for module detection, and was defined as follows:

\[
\text{Dissim}_{ij} = 1 - TOM_{ij}
\]

Using the clusterProfiler package of Bioconductor, the functions of different module genes were annotated by gene ontology (GO; www.geneontology.org) and Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg) analysis. Finally, the co-expression network of DEGs was established and visualized using Cytoscape software. For clarifying the role of miRNAs in grade III glioma, the differentially expressed miRNA data and mRNA data were merged to construct the co-expression network.

In order to identify prognostic mRNA and miRNA signatures, by combining the clinical data of the patient hub genes in TCGA, life curves were constructed for those samples with DEGs by 'survival' in R package. All analyses were performed using R software (version 3.3.0) and Bioconductor (version 3.2).

Results

DEGs. A total of 2,036 differently expressed mRNAs and 50 miRNAs were confirmed using the ‘DESeq’ package in R. The heatmap constructed using the differently expressed mRNAs is shown in Fig. 1. The mRNAs with lfoldchange>2 and miRNAs with lfoldchange>2 are shown in Tables I and II.

Hub genes. The 2,036 genes were clustered into five modules (Fig. 2) using WGCNA. In addition, the co-expression network of DEGs was established using WGCNA and visualized using Cytoscape software. In the network, BUB1B, KIFIC1, TOP2A, BUB1, SLC12A5, ESCO2, ESP1, EPR1, KIF15, CASC5, SGOL1, NUSAP1, CCNB2, NUF2, TTK and KIF2C were central in the network (Fig. 3). It was found that the network included two centers, with downregulated genes and upregulated genes constituting the regulatory network, respectively. BUB1B, KIFIC1, TOP2A, BUB1, ESP1 and EPR1 were at the center of the upregulated gene network; SLC12A5, VSNL1, SULT4A1, TMEM130, SNAP25 were central of the downregulated expression gene network. However, when the data of the differently regulated mRNAseq and miRNAseq were merged to construct the co-expression network, SLC12A5, MAL2, VSNL1, A2BP1, EPB49, SULT4A1, TMEM130, ADAM11, SNAP25, Clorf115, DNM1 and SYT1 were central in the network, and miR-128 and miR-129 were involved. (Fig. 4). It was hypothesized that the genes in the center of the network may be hub genes in the pathological process of high grade LGG.

Functional analysis. The present study identified the top eight GO biological processes of the five gene modules (Table III), and performed KEGG analysis (Table IV). The pathway
enrichment analysis combined several physiological and pathological processes of the nervous system. The genes of the turquoise module were downregulated in glioma; in addition, GO and KEGG analysis predicted that these genes were involved in several important physiological processes in the central nervous system. However, the brown module included genes, which were upregulated in glioma, and GO and KEGG

| ID         | log2foldchange | pval | padj |
|------------|----------------|------|------|
| hsa-miR-137 | -3.52          | <0.01| <0.01|
| hsa-miR-876 | -3.18          | <0.01| <0.01|
| hsa-miR-433 | -2.92          | <0.01| <0.01|
| hsa-miR-218-2 | -2.85 | <0.01| <0.01|
| hsa-miR-485 | -2.83          | <0.01| <0.01|
| hsa-miR-873 | -2.81          | <0.01|  0.02|
| hsa-miR-448 | -2.67          | <0.01|  0.01|
| hsa-miR-770 | -2.67          | <0.01|  0.01|
| hsa-miR-329-2 | -2.66 | <0.01|  0.03|
| hsa-miR-329-1 | -2.63 | <0.01|  0.03|
| hsa-miR-495 | -2.60          | <0.01| < 0.01|
| hsa-miR-656 | -2.60          | <0.01|  0.02|
| hsa-miR-412 | -2.58          | <0.01|  0.01|
| hsa-miR-668 | -2.48          | <0.01|  0.03|
| hsa-miR-138-2 | -2.46 | <0.01| < 0.01|
| hsa-miR-7-3  | -2.45          | <0.01|  0.02|
| hsa-miR-139 | -2.43          | <0.01| < 0.01|
| hsa-miR-129-2 | -2.43 | <0.01| < 0.01|
| hsa-miR-487b | -2.39          | <0.01|  0.04|
| hsa-miR-129-1 | -2.39 | <0.01| < 0.01|
| hsa-miR-1298 | -2.38          | <0.01|  0.03|
| hsa-miR-1224 | -2.37          | <0.01|  0.02|
| hsa-miR-380 | -2.36          | <0.01|  0.04|
| hsa-miR-889 | -2.33          | <0.01|  0.01|
| hsa-miR-432 | -2.27          | <0.01|  0.01|
| hsa-miR-490 | -2.26          | <0.01|  0.03|
| hsa-miR-1258 | -2.25          | <0.01|  0.05|
| hsa-miR-543 | -2.22          | <0.01|  0.05|
| hsa-miR-323 | -2.18          | <0.01|  0.01|
| hsa-miR-431 | -2.16          | <0.01| < 0.01|
| hsa-miR-138-1 | -2.03 | <0.01|  0.03|
| hsa-miR-410 | -2.00          | <0.01|  0.05|
| hsa-miR-10b |  8.59          | <0.01| < 0.01|
| hsa-miR-891b |  5.35          | <0.01|  0.05|
| hsa-miR-181a-2 |  2.49 | <0.01| < 0.01|
| hsa-miR-92b |  2.13          | <0.01|  0.03|
| hsa-miR-27a |  2.05          | <0.01|  0.05|
| hsa-miR-23a |  2.015         | <0.01|  0.05|
| hsa-miR-374a |  1.91          | <0.01|  0.05|
| hsa-miR-25  |  1.68          | <0.01|  0.05|

miR, microRNA.

Table II. Differential expression of mRNAs between the glioma and normal groups.

| ID        | log2FC | pval  | padj  |
|-----------|--------|-------|-------|
| INS       | -7.90  | <0.01 |  0.05|
| LOC100129935 | -5.91  | <0.01 |  0.01|
| TRIM43    | -5.21  | <0.01 |  0.04|
| FAM153B   | -5.13  | <0.01 |  0.01|
| LOC440896 | -5.09  | <0.01 |  0.01|
| MSLNL     | -5.04  | <0.01 |  0.01|
| FAM153C   | -4.99  | <0.01 |  0.01|
| FAM153A   | -4.94  | <0.01 |  0.01|
| C6orf127  | -4.87  | <0.01 |  0.01|
| KRT77     | -4.79  | <0.01 |  0.02|
| LOC728276 | -4.69  | <0.01 |  0.01|
| EVPPL     | -4.67  | <0.01 |  0.05|
| LOC100132354 | -4.61 | <0.01 |  0.01|
| KRTAP17-1 | -4.58  | <0.01 |  0.03|
| ANXA8     | -4.38  | <0.01 |  0.05|
| MYH13     | -4.31  | <0.01 |  0.01|
| CRYGN     | -4.29  | <0.01 |  0.03|
| CRHR2     | -4.22  | <0.01 |  0.01|
| KIF12     | -4.21  | <0.01 |  0.01|
| GPR150    | -4.19  | <0.01 |  0.01|
| KRT33B    | -4.17  | <0.01 |  0.01|
| ADRB3     | -4.14  | <0.01 |  0.01|
| SLC22A10  | -4.13  | <0.01 |  0.01|
| KRT3      | -4.10  | <0.01 |  0.02|
| FSHB      | -4.09  | <0.01 |  0.02|
| MYL2      | -4.07  | <0.01 |  0.01|
| HOXD9     |  9.01  | <0.01 |  0.04|
| TLX1      |  8.91  | <0.01 |  0.04|
| TBX5      |  8.14  | <0.01 |  0.01|
| HOXD8     |  7.67  | <0.01 |  0.01|
| PAX1      |  6.94  | <0.01 |  0.01|
| TOP2A     |  6.81  | <0.01 |  0.01|
| VEPH1     |  6.71  | <0.01 |  0.01|
| C5orf38   |  6.71  | <0.01 |  0.01|
| DLGAP5    |  6.57  | <0.01 |  0.01|
| MYBL2     |  6.46  | <0.01 |  0.01|
| GSC       |  6.38  | <0.01 |  0.03|
| PBK       |  6.28  | <0.01 |  0.01|
| UBE2C     |  6.19  | <0.01 |  0.01|
| CDC45     |  6.13  | <0.01 |  0.01|
| NDC80     |  6.04  | <0.01 |  0.01|
| MELK      |  5.98  | <0.01 |  0.01|
| AURKB     |  5.94  | <0.01 |  0.01|
| ZNF560    |  5.92  | <0.01 |  0.04|
| RRM2      |  5.84  | <0.01 |  0.01|
| FAM64A    |  5.82  | <0.01 |  0.01|
| IRX1      |  5.79  | <0.01 |  0.01|
| CCNB2     |  5.76  | <0.01 |  0.01|
| MKI67     |  5.69  | <0.01 |  0.01|
| TSHR      |  5.68  | <0.01 |  0.04|
| KIF20A    |  5.66  | <0.01 |  0.01|
| NCAPG     |  5.51  | <0.01 |  0.01|
analysis showed these were involved in important pathways, including cell cycle and tumorigenesis.

Clinical biomarkers. Finally, the present study identified the gene symbols associated with clinical outcome (Table V). A total of eight prognostic RNA signatures were found (Fig. 5). C1orf115, CACS5, CDC45, DLL3, EPR1, HOXD9, KIF20, KIF4A, KIF14, KLK7, MELK, NCAPG, PBK, RASL1, SGOL1, SNAP25, SULT4A1, TMEM130, TSHR and VEPH1 were significantly associated with clinical survival rates (P<0.05). In addition, certain genes were associated with LGG patient prognosis (0.05<P<0.1), including A2BP, AURKB, CRHR2, HIPK4, HJURP, MIK67, MYBL2, RRM2, SPARC, TOP2A and VSNL1. When the miRNAseq data and clinical information of samples were combined, it was found that has-miR-10b, has-miR-27a, has-miR-138-2, has-miR-138-1, has-miR-329-1, has-miR-412, has-miR-431, has-miR-495 and has-miR-656 were also closely associated with LGG patient outcome and may be prognostic miRNA signatures (Table VI). The survival curves of has-miR-10b, has-miR-27a, has-miR-138-2 and has-miR-329-1 are shown in Fig. 6.

In the present study, miR-10b and miR-27a were expressed at high levels in glioma tissue, and the expression levels were associated with poor overall survival rates in patients with high grade gliomas. A number of downregulated miRNAs, including has-miR-138-2, has-miR-138-1, has-miR-139 and has-miR-329-1, were also associated with outcome in patients with glioma.

Discussion

Malignant gliomas are the most common and life-threatening type of primary intracranial tumor, which include anaplastic astrocytoma, anaplastic oligodendroglioma and GBM. Several efforts have been made to identify the key regulatory genes or molecules in these types of malignant tumor. However, to the best of our knowledge, few studies have been performed to predict the prognosis of grade III gliomas, and no reliable biomarkers for the detection and risk stratification of gliomas have been identified.

BUB1B/BubR1, a protein that monitors proper spindle microtubule attachment to the kinetochore, has been found to be a promising candidate for targeted therapies in GBM (12,13). In the present study, BUB1B was overexpressed in glioma tissues, and was associated with the clinical outcome.
of patients with glioma (P=0.06). Patients with a high expression of BUB1B had shorter survival rates. In the co-expression network, BUB1B was located centrally in the network, and the results suggested that BUB1B may be a potential target for high grade glioma.

The kinesin motor KIFC1 has been suggested as a potential chemotherapeutic target due to its importance in the clustering of multiple centrosomes found in cancer cells (14). However, the function of KIFC1 in high grade gliomas remain to be elucidated. The present study found that KIFC1 was upregulated in grade III glioma tissues and located centrally in the network. Further investigations are required to annotate its effect on brain tumors.

Topoisomerase 2A (TOP2A) is overexpressed in proliferating cells (15,16). The expression of TOP2A has been correlated with aggressive and highly proliferative types...
of cancer (17,18). In glioma, the levels of TOP2A have been reported as a proliferation marker in association with the Ki-67 index (19). The protein levels of TOP2A were correlated with survival rates in two previous studies, which noted that patients with improved survival rates had lower mean levels of TOP2A (20,21). The data revealed that temozolomide inhibited the expression of TOP2A. In the present study, TOP2A was associated with the clinical outcome of patients with glioma (P=0.08) and may be a hub gene in gliomagenesis.

As with BUB1B, BUB1 is a major mitotic spindle assembly checkpoint gene and significantly correlates with glioma grade and survival rates (22). In the present study, the

| Module | P-value | ID          | Ontology | Term name                                             |
|--------|---------|-------------|----------|------------------------------------------------------|
| Blue   | 0.001965| GO:0015631  | MF       | Tubulin binding                                      |
| Blue   | 0.003507| GO:0017034  | MF       | Rap guanyl-nucleotide exchange factor activity       |
| Blue   | 0.008008| GO:0010008  | CC       | Endosome membrane                                    |
| Blue   | 0.009040| GO:0005768  | CC       | Endosome                                             |
| Blue   | 0.010111| GO:0003376  | BP       | Sphingosine-1-phosphate signaling pathway            |
| Blue   | 0.010111| GO:0015693  | BP       | Magnesium ion transport                              |
| Blue   | 0.010111| GO:0018345  | BP       | Protein palmitoylation                              |
| Blue   | 0.010111| GO:0031365  | BP       | N-terminal protein amino acid modification           |
| Brown  | 4.57E-38 | GO:1903047  | BP       | Mitotic cell cycle process                           |
| Brown  | 3.76E-37 | GO:000278   | BP       | Mitotic cell cycle                                   |
| Brown  | 6.10E-37 | GO:0022402  | BP       | Cell cycle process                                   |
| Brown  | 1.17E-36 | GO:0007049  | BP       | Cell cycle                                           |
| Brown  | 6.96E-34 | GO:0007067  | BP       | Mitotic nuclear division                             |
| Brown  | 2.90E-33 | GO:0007059  | BP       | Chromosome segregation                               |
| Brown  | 1.24E-29 | GO:0051301  | BP       | Cell division                                        |
| Brown  | 4.40E-25 | GO:0005694  | CC       | Chromosome                                           |
| Grey   | 9.10E-10 | GO:0009888  | BP       | Tissue development                                   |
| Grey   | 8.98E-09 | GO:0009887  | BP       | Organ morphogenesis                                  |
| Grey   | 1.98E-08 | GO:0005578  | CC       | Proteinaceous extracellular matrix                   |
| Grey   | 2.65E-08 | GO:0048729  | BP       | Tissue morphogenesis                                 |
| Grey   | 4.74E-08 | GO:0031012  | CC       | Extracellular matrix                                 |
| Grey   | 6.47E-08 | GO:0007389  | BP       | Pattern specification process                        |
| Grey   | 8.30E-08 | GO:0001655  | BP       | Urogenital system development                        |
| Grey   | 1.05E-07 | GO:0060429  | BP       | Epithelium development                               |
| Turquoise | 5.13E-16 | GO:0007268  | BP       | Synaptic transmission                                |
| Turquoise | 4.20E-14 | GO:0045202  | CC       | Synapse                                              |
| Turquoise | 3.08E-12 | GO:0034220  | BP       | Ion transmembrane transport                          |
| Turquoise | 3.35E-12 | GO:0006811  | BP       | Ion transport                                        |
| Turquoise | 8.37E-11 | GO:0007267  | BP       | Cell-cell signaling                                 |
| Turquoise | 3.69E-10 | GO:0098655  | BP       | Cation transmembrane transport                       |
| Turquoise | 6.46E-10 | GO:0050858  | BP       | Transmembrane transport                              |
| Turquoise | 1.53E-09 | GO:0042995  | CC       | Cell projection                                      |
| Yellow | 7.08E-16 | GO:0000184  | BP       | Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay |
| Yellow | 7.60E-15 | GO:0000956  | BP       | Nuclear-transcribed mRNA catabolic process           |
| Yellow | 4.44E-14 | GO:0006402  | BP       | mRNA catabolic process                               |
| Yellow | 1.88E-13 | GO:0006401  | BP       | RNA catabolic process                                |
| Yellow | 2.09E-13 | GO:0006413  | BP       | Translational initiation                             |
| Yellow | 2.44E-13 | GO:0006613  | BP       | Cotranslational protein targeting to membrane        |
| Yellow | 2.44E-13 | GO:0006614  | BP       | SRP-dependent cotranslational protein targeting to membrane |
| Yellow | 8.38E-13 | GO:0006412  | BP       | Translation                                          |

Go, Gene ontology; MF, molecular function; CC, cellular component; BP, biological process.
patients with overexpression of the BUB1 gene had a shorter survival rate (P=0.1). In colorectal cancer, mutation of the BUB1 gene was found to be associated with lymph node metastasis and lower relapse-free survival rates following surgery (23). Further investigations may be required to identify whether BUB1 mutations are important in the glioma process. In addition, the expression of ESPL1 in human glioma and its possible correlations with histoclinical features remains to be fully elucidated, however, evidence suggests that ESPL1 is a candidate oncogene in breast cancer and lung cancer (24,25).

SLC12A5 has been found to have an important oncogenic role in colorectal carcinogenesis; its overexpression can be an independent prognostic factor for patients, and the mutation frequency of SLC12A5 may have potential oncogenic effects in colon cancer (26,27). However, the functional characterization of SLC12A5 in brain tumors remains to be fully elucidated, and few investigations have been performed. The present study found that patients with a high expression of SLC12A5 showed improved prognosis (P=0.11). Further investigations are required to clarify the function of SLC12A5 in glioma and other brain tumors.

VSNL1 is a known tumor-suppressor gene regulating cell migration in several types of cancer. It is also downregulated in GBM (28). The data obtained in the present study suggested that the overexpression of VSNAL1 may be associated with

Table IV. Kyoto Encyclopedia of Genes and Genomes enrichment analysis of gene modules with the top eight significantly enriched biology terms.

| Module ID | ID        | Description                        | Gene ratio | P-value |
|-----------|-----------|------------------------------------|------------|---------|
| Brown     | hsa04110  | Cell cycle                         | 11/39      | <0.01   |
| Brown     | hsa04114  | Oocyte meiosis                      | 7/39       | <0.01   |
| Brown     | hsa04914  | Progesterone-mediated oocyte maturation | 6/39   | <0.01   |
| Brown     | hsa04115  | p53 signaling pathway              | 5/39       | <0.01   |
| Turquoise | hsa04080  | Synaptic vesicle cycle             | 59/558     | <0.01   |
| Turquoise | hsa04020  | Glutamatergic synapse              | 51/558     | <0.01   |
| Turquoise | hsa04921  | Retrograde endocannabinoid signaling | 47/558 | <0.01   |
| Turquoise | hsa04024  | Morphine addiction                 | 47/558     | <0.01   |
| Turquoise | hsa04010  | GABAergic synapse                   | 45/558     | <0.01   |
| Turquoise | hsa04724  | Calcium signaling pathway           | 43/558     | <0.01   |
| Turquoise | hsa04723  | Oxytocin signaling pathway          | 40/558     | <0.01   |
| Turquoise | hsa04728  | Circadian entrainment              | 38/558     | <0.01   |

Figure 5. Genes predicting the prognosis of patients with glioma. Downregulated genes KIF4A, NCAPG, SGOL1 and CASC5 predicted increased survival rate; upregulated genes C1orf115, KLK7, SULT4A1 and TSHR predicted increased survival rate. Exp, expression.
Increased survival rate (P=0.10). GO annotation revealed that VSNL1 is involved in several normal neuron physiological functions. Current data also indicate that VSNAL1 may be associated with schizophrenia and frontal cortical function (29).

SULT4A1 encoded protein is a brain-specific sulfotransferase, which is widely expressed in the majority of human brain compartments and may be involved in the metabolism of neurotransmitters (30). The SULT4A1 gene, located in the frequently deleted 22q13.3 chromosomal region, is downregulated in ependymoma (31,32). The present study showed that SULT4A1 was downregulated in the glioma group (log2foldchange=−3.15; P<0.05). In addition, a high expression of SULT4A1 was associated with increased survival rates, compared with a low expression. Therefore, SULT4A1 may be important in tumorigenesis and as a prognostic molecule in grade III gliomas.

Few studies have been performed on SNAP25 in glioma. SNAP-25 is a t-SNARE protein, which is encoded by the SNAP25 gene in humans (33). SNAP-25 is considered to account for the specificity of membrane fusion and to directly execute fusion by forming a tight complex, which brings the synaptic vesicle and plasma membranes together (34). In the present study, SNAP25 was a prognostic factor in patients with high grade glioma (P=0.03). The overexpression of SNAP25 predicted increased survival rates, compared with glioma patients with a lower expression of SNAP25.

A2BP1 serves to regulate the alternative splicing of TPM1 to promote cytoskeletal organization and terminal differentiation, and the loss of A2BP1 contributes to the tumorigenesis in GBM by causing compromised terminal differentiation (35). The present study found that A2BP1 was downregulated in grade III gliomas, and that a high expression level of A2BP1 was predictive of longer survival rates (P=0.05).

In the present study, CASC5 was identified as a prognostic factor in high grade glioma (P=0.02). CASC5 is a component of the kinetochore. It is involved in microtubule attachment to chromosome centromeres and in activation of the spindle checkpoint during mitosis. The CASC5 gene is upregulated in the regions of cell proliferation surrounding the ventricles during fetal brain development (36). In GBM, the expression level of CASC5 is higher, compared with that in the normal brain (37).

Current data suggests that the KLK7 protein offers potential as a prognostic marker of patient survival rates in GBM, with elevated expression levels of KLK7 associated with poor patient survival rates (38,39). By contrast, the present study found that KLK7 was downregulated in glioma (foldchange=−3.98;
The decline in the expression of KLK7 was associated with poor patient survival rates in high grade glioma (P<0.01). Previous evidence suggests that KLK7 is differentially regulated in a variety of tumors, and is important in the normal physiology of the skin, particularly in epidermal homeostasis. The majority of evidence indicates that overexpression of KLK7 is associated with poor patient survival rates or increased tumor cell proliferation (40). However, the present study revealed that the expression of KLK7 was downregulated in prostate cancer and that the low expression was closely correlated with advanced disease stage, predictive of a poor prognosis (41). Further investigations are required in the future to identify the role of KLK7 in brain glioma.

The present studies found that BMP2, DLL3 and HEY2 were overexpressed in glioma (42,43). In addition, the elevation of these neurogenesis-associated genes was associated with an increase survival rate in patients with high grade glioma. Current evidence suggests that neurogenesis-associated genes are expressed at high levels in patients with glioma, including BMP2 (43), DLL3 and HEY2, which are important in neurogenesis and may preferentially lead to the terminal differentiation of malignant cells (42).

The present study also observed that tumors with higher expression levels of HJURP were associated with poor prognosis. A previous study demonstrated that the overexpression of HJURP may be important in the maintenance of highly proliferative cells in glioma, and may be an independent prognostic factor, or a potential therapeutic target, for patients with high grade glioma (44).

According to the results of the present study, the expression of HOXD9 was markedly increased in high grade glioma, and the higher expression of HOXD9 was associated with poor survival rates in patients with glioma. HOXD9 was expressed at a low level in the normal brain, however, in glioma tissues and glioma cancer stem cells, expression was higher, compared with that in normal brain samples. Therefore, HOXD9 may be a novel marker of cell proliferation and survival rates in glioma, and a potential therapeutic target (45).

Consistent with the present study, the gene expression levels of KIF2C, KIF14, MELK and AURKB were higher in glioma samples, compared with those in normal brain tissues. The expression of these genes was associated with histopathological grades or invasiveness of glioma, and may be a candidate prognostic marker for human glioma (46-48).

It has been shown that the increased expression of miR-10b in glioma is associated with poorer prognosis (49). In the present study, has-miR-10b, has-miR-27a, has-miR-138-2, has-miR-138-1, has-miR-139, has-miR-329-1, has-miR-431, has-miR-329-2, has-miR-329-1, and has-miR-329-2 predicted increased survival rate; upregulated miR-329-1 and miR-138-2 predicted increased survival rate.
has-miR-495 and has-miR-656 were associated with the gliomagenesis of high grade gliomas. miR-128 and miR-129 were involved in the co-expression network, however, they were not associated with the hub genes in the network. miR-128 and miR-129 are important regulators of proliferation and can promote the apoptosis of glioma (50,51). The mechanisms underlying the changes of important miRNAs in grade III glioma require further investigation.

In conclusion, using bioinformatics analysis, the present study provided improved understanding of how to identify the mechanisms underlying the tumorigenesis of high grade gliomas. The results predicted that two factors involved in the glioma deterioration process, the downregulated genes and upregulated genes, are important. A number of these genes were found to be closely associated with clinical prognosis.

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