Abstract. The present study aimed to identify differentially regulated genes between the peritumoral brain zone (PBZ) and tumor core (TC) of glioblastoma (GBM), to elucidate the underlying molecular mechanisms and provide a target for the treatment of tumors. The GSE13276 and GSE116520 datasets were downloaded from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) for the PBZ and TC were obtained using the GEO2R tool. The bioinformatics and evolutionary genomics online tool Venn was used to identify common DEGs between the two datasets. The Database for Annotation, Visualization, and Integrated Discovery online tool was used to analyze enriched pathways of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. The Search Tool for the Retrieval of Interacting Genes/Proteins online tool was used to construct a protein-protein interaction (PPI) network of DEGs. Hub genes were identified using Cytohubba, a plug-in for Cytoscape. The Gene Expression Profiling Interactive Analysis (GEPIA) database was utilized to perform survival analysis. In total, 75 DEGs, including 12 upregulated and 63 downregulated genes, were identified. In the GO term analysis, these DEGs were mainly enriched in ‘regulation of angiogenesis’ and ‘central nervous system development’. Furthermore, in the KEGG pathway analysis, the DEGs were mainly enriched in ‘bladder cancer’ and ‘endocytosis’. When filtering the results of the PPI network analysis using Cytohubba, a total of 10 hub genes, including proteolipid protein 1, myelin associated oligodendrocyte basic protein, contactin 2, myelin oligodendrocyte glycoprotein, myelin basic protein, myelin associated glycoprotein, SRY-box transcription factor 10, C-X-C motif chemokine ligand 8 (CXCL8), vascular endothelial growth factor A (VEGFA) and plasmolipin, were identified. These hub genes were further subjected to GO term and KEGG pathway analysis, and were revealed to be enriched in ‘central nervous system development’, ‘bladder cancer’ and ‘rheumatoid arthritis’. These hub genes were used to perform survival analysis using Cytohubba, and it was determined that VEGFA and CXCL8 were significantly associated with a reduction in the overall survival of patients with GBM. In conclusion, the results suggest that the recurrence of GBM is associated with high gene expression levels VEGFA and CXCL8, and the development of the central nervous system.

Introduction

Originating from adult brain tissue, glioblastoma (GBM) is a highly malignant primary tumor with numerous characteristics, including complicated pathological diagnosis, poor prognosis and easy relapse (1). Tumor cells have a strong ability for proliferation and invasion, and can invade the central nervous system (2). In 2018, patients with GBM had a poor prognosis, and the median survival time of patients was ~17 months (3). In 2019, following standard treatment, the
6-month progression-free survival rate was 25.2% in patients with recurrent glioblastoma (rGBM) (4). Traditional treatment for GBM includes surgical resection, radiation and chemotheraphy with temozolomide (5). Following surgical resection of GBM, substantial invading tumor cells are located in the near resection edge of the tumor or within 2-3 cm from the resection cavity, and these are the most common recurrence sites (6).

In previous years, with the development of medicine and bioinformatics, gene expression information has been generated and stored in the Gene Expression Omnibus (GEO) database. Bioinformatics analysis allows an improved understanding of the alterations in essential genes in the process of tumor development. Furthermore, it can provide a basis for target treatment and tumor diagnosis (7). A previous study identified that both the peritumoral brain zone (PBZ) and tumor core (TC) are heterogeneous in the same patients, and tumor cells in the PBZ exhibit increased invasiveness compared with cells in the TC (8). Van Meter et al (9) demonstrated that there were a number of different gene expression products between the PBZ and TC. Even in the absence of tumor cells in the PBZ, it is possible to detect the expression of genes associated with invasion and neo-angiogenesis (10). Therefore, identifying differentially expressed genes (DEGs) between the PBZ and TC may reveal the causes of GBM recurrence, and may provide a target for tumor treatment.

In the present study, the gene expression profiles of the PBZ were compared with the gene expression profiles of the TC to identify DEGs. The DEGs of the PBZ and TC were screened by GEO2R online analysis, and the Database for Annotation, Visualization, and Integrated Discovery (DAVID) was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. A protein-protein interaction (PPI) network was constructed using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) and Cytoscape software tools, and the hub genes were obtained using the Cytoscape plug-in Cytohubba. Furthermore, the Gene Expression Profiling Interactive Analysis (GEPIA) database was used to analyze the survival rate of patients with aberrant expression levels of the hub genes.

Materials and methods

Microarray data analysis. GEO (https://www.ncbi.nlm.nih.gov/geo/) is a database that stores a large amount of genetic information. For analysis, ‘glioblastoma’ was selected as a key word, the tissue type was set as ‘Homo sapiens’ and the study type was set as ‘expression profiling by array’. The screening criteria were: i) Samples of peritumoral brain area and core of GBM; and ii) both samples should be obtained from the same patient. The two datasets that met the criteria were GSE13276 and GSE116520. The GSE13276 dataset, which was submitted by Mangiola et al (2), is based on the GPL96 platform and contains 5 cases of GBM core samples and 5 cases of GBM surrounding tissue (no replicate samples included). The GSE116520 dataset, which was submitted by Kruthika et al (11), is based on the GPL10558 platform and contains 17 cases of GBM core samples and 17 cases of GBM surrounding tissue (Table I).

Screening of DEGs. DEGs were screened using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/), which allowed a comparison of the differences between two different sets of samples in a series. In the GSE13276 dataset, log fold change (FC) ≥2 and P<0.05 were defined as statistically significant for the DEGs. Similarly, in the GSE116520 dataset, log FC ≥1 (and P<0.05 were defined as statistically significant for the DEGs). If the same criteria in the two datasets were selected, the resulting genes would be limited, therefore in order to obtain a larger number of target genes different criteria were selected. Excel software (Office 2016; Microsoft Corporation) was used to delete all genes without gene symbols and duplicate genes corresponding to multiple probe IDs in the GSE13276 and GSE116520 datasets. A Venn diagram of the DEGs obtained from the bioinformatics and evolutionary genomics online tool ‘Calculate and draw custom Venn diagrams’ (http://bioinformatics.psb.ugent.be/webtools/Venn/) was constructed, and the common DEGs were identified.

GO and KEGG enrichment analysis. GO (http://www.geneontology.org) is a database used to describe the characteristics of genes and gene products. KEGG (https://www.kegg.jp/) is a database containing information on genome, chemistry and system functions (12). DAVID (version 6.8; http://david.ncifcrf.gov/) is an online database, and was used to perform GO term and KEGG pathway enrichment analyses (13,14). P<0.05 was considered to indicate a statistically significant difference.

Interaction network analysis and hub gene identification. The STRING (https://string-db.org/) database is a tool for analyzing the interrelationships between genes, including direct (physical) and indirect (functional) links, and constructing PPI networks. STRING version 11.0 contains 24,584,628 proteins from 5,090 organisms (15). The PPI network association among DEGs was analyzed using the STRING database and the default minimum required interaction score was set as >0.4. Subsequently, the Cytoscape (version 3.7.1; http://www.cytoscape.org/) plug-in Cytohubba (version was 0.1; http://apps.cytoscape.org/apps/cytohubba) was used to screen the PPI network, and the top10 genes, which were the hub genes in the PBZ, were obtained using the Degree algorithm.

Survival analysis. GEPIA (http://geopia.cancer-pku.cn/detail.php) is a database used to analyze RNA sequencing expression data, and data of the tumors (GBM) and normal samples included in The Cancer Genome Atlas (TCGA: https://portal.gdc.cancer.gov/) and the Genotype-Tissue Expression (GTEx: https://gtexportal.org/home/) databases were analyzed (16). All the parameters were set to the default value, and the cut-off value was median=50%. The GBM sample was selected as the dataset and the hazard ratio (HR) was calculated based on the Cox Proportional-Hazards Model. The 95% CI was not calculated in the present study. For the HR, P<0.05 was considered to indicate a statistically significant difference.

Results

Screening of DEGs. For the GSE13276 dataset, 429 DEGs were identified, including 279 upregulated (UP) genes and
150 downregulated (DOWN) genes. For the GSE116520 dataset, 204 DEGs were identified, including 32 UP genes and 172 DOWN genes. The bioinformatics and evolutionary genomics webpage tool, Venn, was used to construct the Venn diagram of the two groups of DEGs, and through the Venn diagram, 75 common DEGs were identified (Table II), including 12 common UP and 63 common DOWN genes (Fig. 1).

**GO term enrichment analysis.** The DAVID online tool was used to analyze the GO terms and KEGG pathways of the DEGs. The GO term enrichment analysis results demonstrated that the UP genes were significantly enriched in ‘regulation of angiogenesis’ (P<0.01; Fig. 2A). DOWN genes were significantly enriched in ‘central nervous system development’ (P<0.01; Fig. 2B).

**KEGG pathway analysis.** By analyzing the DEGs carefully, the most significantly enriched KEGG pathways were identified (Table III). The UP genes were mainly enriched in ‘bladder cancer’ and the DOWN genes were mainly enriched in ‘endocytosis’ (Table III).

**PPI network and hub gene analysis.** STRING online software was used to analyze the DEGs and then a PPI network was constructed to predict the hub genes. Following analysis of the PPI network, it was identified that 52 nodes and 101 interactions were involved in the PPI network. The top 10 hub genes were calculated using the Degree algorithm of the Cytohubba plug-in, as shown in Fig. 3. The 10 hub genes included proteolipid protein 1 (PLP1), myelin associated oligodendrocyte basic protein (MOBP), contactin 2 (CNTN2), C-X-C motif chemokine ligand 8 (CXCL8), myelin oligodendrocyte glycoprotein (MOG), vascular endothelial growth factor A (VEGFA), myelin basic protein (MBP), and proteolipid protein 1 (PLP1).
VEGFA, vascular endothelial growth factor A; CXCL8, C-X-C motif chemokine ligand 8; SH3GL3, SH3 domain containing GRB2 like 3, endophilin A3; HSPA2, heat shock protein family A (Hsp70) member 2; BIN1, bridging integrator 1; MVB12B, multivesicular body subunit 12B.

Survival analysis. The prognostic value of the 10 hub genes was evaluated using the GEPIA online tool and survival curves were obtained. The GEPIA online tool is based on the TCGA and GTEx databases. Survival analysis of the 10 hub genes revealed that PLP1, MOBP, CNTN2, MOG, MBP, MAG, SOX10 and PLLP were not associated with the overall survival rate of patients with GBM. However, CXCL8 and VEGFA were significantly associated with a reduction in the overall survival rate of patients with GBM (Fig. 4B and D). The expression levels of the 10 hub genes in the normal and tumor groups in the GEPIA database were also compared. The results of the present study demonstrated that the VEGFA and CXCL8 genes expression were differentially expressed in tumor tissues and normal tissues (Fig. 4A and C), and both genes were highly expressed in the PBZ region.

Discussion

In the present study, to investigate the causes of GBM recurrence, the DEGs between the PBZ and TC were evaluated by bioinformatics analysis. The GSE13276 and GSE116520 datasets were analyzed and 75 common DEGs were identified,
including 12 common UP genes and 63 common DOWN genes. DAVID was used to perform a functional annotation analysis of the 75 DEGs. In the GO analysis, the UP genes were enriched in ‘regulation of angiogenesis’. The DOWN genes were enriched in ‘central nervous system development’. In the KEGG pathway analysis, UP genes were enriched in ‘bladder cancer’ and DOWN genes were enriched in ‘endothytosis’. Based on this, Cytohubba, a plug-in of Cytoscape, was used to analyze the DEGs, and a total of 10 hub genes were identified, including PLP1, MOBP, CNTN2, MOG, MBP, MAG, SOX10, CXCL8, VEGFA and PLLP. Only CXCL8 and VEGFA were UP genes, and the other hub genes were DOWN genes. In addition, GEPIA was used to assess the prognostic value of the 10 hub genes. It was determined that the PLP1, MOBP, CNTN2, MOG, MBP, MAG, myelin associated glycoprotein; SOX10, SRY-box transcription factor 10; PLLP, plasmolipin.

Table IV. GO enrichment analysis of 10 hub genes.

| Term                                      | Count, n | P-value     | Genes                        |
|-------------------------------------------|----------|-------------|------------------------------|
| GO:0048469~cell maturation                | 3        | 1.59x10⁻⁴   | SOX10, PLP1, VEGFA           |
| GO:0021762~substantia nigra development   | 3        | 2.96x10⁻⁴   | MAG, PLP1, MBP               |
| GO:0007417~central nervous system development | 3        | 0.001764    | CNTN2, MOG, MBP              |
| GO:0008366~axon ensheathment              | 2        | 0.003746    | PLP1, MBP                    |
| GO:0022010~central nervous system myelination | 2        | 0.003746    | PLP1, CNTN2                  |
| GO:0050930~induction of positive chemotaxis | 2        | 0.008013    | VEGFA, CXCL8                 |
| GO:0002052~positive regulation of neuroblast proliferation | 2        | 0.010671    | SOX10, VEGFA                 |
| GO:0031623~receptor internalization       | 2        | 0.022817    | CNTN2, CXCL8                 |
| GO:0007155~cell adhesion                  | 3        | 0.023633    | MAG, CNTN2, MOG              |
| GO:0042552~myelination                    | 2        | 0.024392    | PLLP, MBP                    |

B, GOTERM_CC_DIRECT

| Term                                      | Count, n | P-value     | Genes                        |
|-------------------------------------------|----------|-------------|------------------------------|
| GO:0043218~compact myelin                 | 2        | 0.001974    | PLLP, MBP                    |
| GO:0043209~myelin sheath                  | 3        | 0.002394    | PLP1, MOBP, CNTN2            |

C, GOTERM_MF_DIRECT

| Term                                      | Count, n | P-value     | Genes                        |
|-------------------------------------------|----------|-------------|------------------------------|
| GO:0019911~structural constituent of myelin sheath | 4        | 1.26x10⁻⁸   | PLP1, MOBP, PLLP, MBP        |

GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function; PLP1, proteolipid protein 1; MOBP, myelin associated oligodendrocyte basic protein; CNTN2, contactin 2; CXCL8, C-X-C motif chemokine ligand 8; MOG, myelin oligodendrocyte glycoprotein; VEGFA, vascular endothelial growth factor A; MBP, myelin basic protein; MAG, myelin associated glycoprotein; SOX10, SRY-box transcription factor 10; PLLP, plasmolipin.

Figure 3. Top 10 hub genes obtained from the analysis of the protein-protein interaction networks, constructed using Cytohubba, a plug-in of Cytoscape. Different colors represent the different importance of the hub genes, in terms of their degree of connectivity. The redder the color block is, the more important it is to other genes. PLP1, proteolipid protein 1; MOBP, myelin associated oligodendrocyte basic protein; CNTN2, contactin 2; CXCL8, C-X-C motif chemokine ligand 8; MOG, myelin oligodendrocyte glycoprotein; VEGFA, vascular endothelial growth factor A; MBP, myelin basic protein; MAG, myelin associated glycoprotein; SOX10, SRY-box transcription factor 10; PLLP, plasmolipin.

including 12 common UP genes and 63 common DOWN genes. DAVID was used to perform a functional annotation analysis of the 75 DEGs. In the GO analysis, the UP genes were enriched in ‘regulation of angiogenesis’. The DOWN genes were enriched in ‘central nervous system development’. In the KEGG pathway analysis, UP genes were enriched in ‘bladder cancer’ and DOWN genes were enriched in ‘endothytosis’. Based on this, Cytohubba, a plug-in of Cytoscape, was used to analyze the DEGs, and a total of 10 hub genes were identified, including PLP1, MOBP, CNTN2, MOG, MBP, MAG, SOX10, CXCL8, VEGFA and PLLP. Only CXCL8 and VEGFA were UP genes, and the other hub genes were DOWN genes. In addition, GEPIA was used to assess the prognostic value of the 10 hub genes. It was determined that the PLP1, MOBP, CNTN2, MOG, MBP, MAG, SOX10 and PLLP genes did not reduce the overall survival rate of patients with GBM. However, it was demonstrated that CXCL8 and VEGFA were significantly associated with a reduction in the overall survival rate of patients with GBM.

GO term analysis of the 10 hub genes demonstrated that these genes were significantly enriched in terms such as ‘cell maturation’, ‘substantia nigra development’ and ‘central nervous system development’. In particular, three key genes, CNTN2, MOG and MBP, were enriched in the ‘central nervous
system development' term. Rickman et al (17) reported that CNTN2 gene expression is increased in middle and high-grade gliomas. Abnormal CNTN2 gene expression is associated with glioma (17). Inhibiting the expression of CNTN2 can inhibit the migration of glioma (17,18). Using the UniProt website (https://www.uniprot.org/), it has been demonstrated that MOG gene expression was associated with cell adhesion (19). In 1994, Nakagawa et al (20) measured the expression level of myelin basic protein (MBP) in the cerebrospinal fluid of patients with various types of tumors, including glioblastoma.
using radioimmunoassay. It was reported that the expression level of MBP was >4.0 ng/ml, which was associated with active malignant tumors. In the pathways of ‘cell maturation’ and ‘substantia nigra development’, it was indicated that the SOX10, PLP1, and MAG genes were associated with GBM. In 2011, Serio et al (21) found that the SOX10 gene was a high-risk factor for GBM in different races, and Kong et al (22) found that the expression of the PLP1 gene was associated with the morphological classification of GBM. Ljubimova et al (23) found that the MAG gene cannot be expressed in normal brain tissue, but its expression level in GBM was high. In this study by analyzing these genes in ‘cell maturation’, ‘substantia nigra development’ and ‘central nervous system development’ pathways, it was determined that these genes are associated with GBM recurrence.

In the KEGG pathway analysis, the 10 hub genes were enriched in ‘bladder cancer’ and ‘rheumatoid arthritis’. Both pathways involved the two genes VEGFA and CXCL8. The two genes are involved in the angiogenesis of the pathway and promote the progression of these two diseases (24,25). A previous study demonstrated that the use of an anti-rheumatoid arthritis drug (auranofin) inhibits the invasiveness of GBM cells (26). Based on the consideration of these pathways, Lanzardo et al (27) revealed that αVβ3 is a target marker of rheumatoid arthritis. Additionally, it has been demonstrated that αVβ3 could be used as a marker of GBM molecular imaging (27). The enrichment of these two pathways suggested that the PBZ is more susceptible to the expression of these two genes, which leads to the formation of blood vessels and the possibility of GBM metastasis.

The VEGFA gene can encode angiogenic factors, and its products can alter the microenvironment of endothelial cells and promote angiogenesis (28). In numerous tumors, gene expression products of VEGFA not only stimulate tumor growth, metastasis and survival, but also stimulate hematopoietic cells, endothelial cells and neuronal cells (29-31). Desbailliet et al (32) reported that hypoxia stimulates VEGFA expression in tumor cells in the pseudopalisades region. Wong et al (33) demonstrated that GBM is a type of tumor that is rich in blood vessels and that VEGFA is upregulated in cancer, providing insight to develop anti-VEGF and other anti-angiogenic medicines. Gong et al (34) found that the growth and invasiveness of GBM are associated with the regulation of matrix metalloproteinase 2 (MMP2) by VEGFA. It has also been suggested that blocking MMP2 expression could inhibit angiogenesis when the anti-VEGFA gene is expressed (34). Egorova et al (35) have demonstrated that small interfering RNA can be used to target and knockout VEGFA gene expression in tumor and endothelial cells. Bevacizumab (an antibody that specifically binds to VEGFA) has been found to reduce vascular density and heterogeneity of tumors, but also increases the invasiveness of tumors and causes cognitive impairment in patients (36,37).

The CXCL8 gene has a number of aliases, one of which is interleukin (IL)-8, and is a chemokine with 77 amino acids. Previous studies have demonstrated that it can promote the formation of blood vessels (38-41). Furthermore, Desbailliet al et al (32) identified IL-8 gene expression in necrotic cells surrounding GBM, and demonstrated that hypoxia and anoxia could promote IL-8 gene expression. Additionally, the deficiency of glucose and amino acids, and the increase of reactive oxygen species could promote IL-8 expression (42). Ahn et al (43) reported that necrotic cells induce NF-κB/activator protein-1, which could promote IL-8 expression in tumor cells. Previous studies have demonstrated that IL-8 promotes the growth and metastasis of GBM by stimulating its receptors C-X-C chemokine receptor type (CXCR1) and CXCR2 (44-46). A recent study has demonstrated that bradykinin can induce IL-8 expression through the B1 bradykinin receptor (B1R), whereas B1R promotes the transfer of STAT3 and transcription factor SP-1 to the nucleus, and promotes the metastasis of GBM cells (47). Hasan et al (48) analyzed 17 matched pairs of primary and rGBM samples; IL-8 expression was increased in 11 pairs of rGBM samples. Furthermore, blocking IL-8 gene expression may reduce the expression of key glioma-initiating cells genes, temozolomide (TMZ)-induced increases in transcription factors (Sox2 and C-myc), and enhance the efficacy of TMZ therapy to prolong survival time (48). In accordance with Desbailliet et al (32), this study also found that the IL-8 gene is highly expressed in the PBZ, which may be a signal of migration and proliferation for tumor cells. By targeting CXCR2 using the inhibitor SB225002 (49), knockout of cyclophilin A can reduce the activity of NF-κB, inhibit the expression of IL-8, and prevent the metastasis and growth of tumors (50).

CXCL8 and VEGFA genes serve an important role in the growth, proliferation and metastasis of tumors in GBM (29,38-41). However, the principle of using anti-VEGF agents to reduce tumor perfusion, and cause ischemia and hypoxia (51), may additionally promote the expression of vascular endothelial growth factor and IL-8 following hypoxia, and in turn stimulate the invasiveness of tumors. At present, bevacizumab is mainly used in the treatment of rGBM; however, it is not able to prolong overall survival (36), and the recurrence of GBM mainly depends on the surgery (52). Combined with the results of the present study, it may be suggested that both anti-VEGF and anti-IL-8 therapy may be used to improve the prognosis of patients. At present, it was determined that both the VEGFA and CXCL8 genes are highly expressed in the PBZ of GBM, which can cause peripheral angiogenesis, migration and proliferation of tumor cells (29,38-41). Additionally, it was determined that through data analysis, the high expression levels of both VEGFA and CXCL8 can reduce the overall survival rate of patients. Taking into consideration the results of Desbailliet et al (32) and this study, it is suggested that high expression of CXCL8 and VEGFA genes were associated with the recurrence of GBM. However, the association of GBM with recurrence requires further investigation. The gene-related pathways have become increasingly clear. However, further studies are required to understand these pathways and to develop appropriate drugs to improve the prognosis of patients.

In conclusion, through a comprehensive bioinformatics analysis, 10 hub DEGs between PBZ and TC were identified, including PLP1, MOBP, CNTN2, MOG, MBP, MAG, SOX10, VEGFA, CXCL8 and PLLP. The GO and KEGG enrichment analyses revealed that the hub genes were mainly enriched in "central nervous system development" and "rheumatoid
arthritus’. According to literature, the genes in these pathways have a specific association with tumor invasion, metastasis and angiogenesis (24,25), and pathway and term analyses can provide further insights for the development of future drugs and treatments. Through survival analysis of the 10 hub genes, it was demonstrated that high expression levels of the VEGFA and CXCL8 genes are associated with a reduced survival time of patients with GBM, and they were expressed differently in the tumor and normal samples. The present study could provide targets for future therapies and novel insights to block gene expression, which may reduce the recurrence rate of tumors.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the GEO repository (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSEI3276) and (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSEI16520).

Authors’ contributions

XLu, SX and FY designed the study. XLu, SX, YT, YX, XLi and BW performed the bioinformatics analysis and interpretation of the data. XLu, SX and TT wrote the manuscript. FY revised the manuscript and gave final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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