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INVESTIGATING GENE EXPRESSION CHANGES AND HUB GENES PLAYING A ROLE IN THE PROGRESSION OF GLIAL BRAIN TUMORS

GLİAL BEYİN TÜMÖRLERİNİN İLERLEMESİNDE ROL OYNAYAN GEN İFADE DEĞİŞİKLİKLERİ VE MERKEZİ GENLERİN İNCELENMESİ

Nurhan Külcü Sarıkaya[1*], Deniz Sünnetçi Akkoyunlu[1]

[1]Kocaeli University, Faculty of Medicine, Department of Medical Genetics, Kocaeli, Türkiye.

ABSTRACT

Objective: Glioblastoma, a highly aggressive form of glioma, is the most common malignant brain tumor, accounting for 80% of all malignant brain tumors and 55% of all gliomas. The median survival for patients with glioblastoma is just over one year, despite treatment. This study aims to understand impact of gene expression differences and pathways in the glioma development and progression.

Methods: 150 glioma samples and 15 normal brain tissues were included in the study from the Gene Expression Omnibus (GEO) database. GeneSpring Software was used to obtain the differentially expressed genes (DEGs) comparing tumor and normal. The DEGs were analyzed using the DAVID interface for GO and KEGG pathway analysis. Most connected 15 genes were selected as hub genes for each grade using the STRING application in Cytoscape software.

Results: DEGs were found associated with neuroactive ligand-receptor interaction, systemic lupus erythematosus, complement and coagulation cascades, and GABAergic synapse. A total of 21 genes (ALB, CXCL8, EGF, EGFR, FN1, GAPDH, GNG13, GNG7, GNGT1, IL10, IL6, INS, KNG1, MAPK1, MYC, NOTCH1, SRC, STAT3, TNF, TP53, VEGFA) were identified as hub genes. Among them, INS was found having the highest level of connections in all grades. IL6, were found associated with poor survival and is implicated in high-grade gliomas. STAT3 and poor survival-related EGF were detected at grade IV. The human cytomegalovirus (HCMV) infection was revealed to be the most significant pathway in high-grade gliomas according to KEGG analysis of hub genes.

Conclusion: Sonuç 8p, CalibriFindings of the current study suggest potential prognostic and therapeutic targets for the gliomas.

Keywords: Glioma, gene expression, hub genes, integrated analysis.

ÖZ

Amaç: Gliomanın oldukça agresif bir formu olan glioblastoma, tüm malign beyin tümörlerinin %80'ini ve tüm gliomaların %55'ini oluşturan en yaygın malign beyin tümörüdür. Tedaviye rağmen glioblastomalı hastalar için medyan sağkalım bir yıldan biraz fazladır. Bu çalışma, gen ekspresyon farklılıklarının ve yollarının glioma gelişimi ve ilerlemesindeki etkisini anlamayı amaçlamaktadır.

Yöntem: Çalışmaya Gene Expression Omnibus (GEO) veritabanından 150 glioma örneği ve 15 normal beyin doku örneği dahil edildi. Normal doku ile derece II, III ve IV glioma dokuları arasında diferansiyel olarak eksprese edilen genleri (DEG'ler) elde etmek için GeneSpring yazılımı kullanıldı. DEG'ler, GO ve KEGG yolak analizi için DAVID arayüzü kullanılarak analiz edildi. Cytoscape yazılımındaki STRING uygulaması kullanılarak her derecede en çok bağlantıya sahip 15 gen merkez gen olarak seçildi.

Bulgular: DEG'ler nöroaktif ligand-reseptör etkileşimi, sistemik lupus eritematozus, komplement ve pıhtılaşma kaskadları ve GABAerjik sinaps ile ilişkilendirildi. Toplam 21 gen (ALB, CXCL8, EGF, EGFR, FN1, GAPDH, GNG13, GNG7, GNGT1, IL10, IL6, INS, KNG1, MAPK1, MYC, NOTCH1, SRC, STAT3, TNF, TP53 ve VEGFA) merkezi gen olarak belirlendi. Merkezi genler arasında INS geni, bütün derecelerde en yüksek bağlantı düzeyine sahip olduğu bulundu. IL6, düşük sağkalım ile ilişkilendirildi ve yüksek dereceli gliomalarda rol oynadığı düşünüldü. STAT3 ve düşük sağkalımla ilişkili EGF, derece IV'de tespit edildi. İnsan sitomegalovirüsü (HCMV) enfeksiyonu, merkez genlerin KEGG analizine göre yüksek dereceli gliomalarda en anlamlı yol olarak ortaya çıktı.

Sonuç: Bu çalışmanın bulguları, gliomalar için potansiyel prognostik ve terapötik hedefleri işaret etmektedir.

Anahtar Kelimeler: Glioma, gen ekspresyonu, merkezi genler, entegre analiz

Corresponding author/İletişim kurulacak yazar: Nurhan Külcü Sarıkaya; Kocaeli University, Faculty of Medicine, Department of Medical Genetics, Kocaeli, Türkiye.
Phone /Telefon: +90 (262) 303 78 52 **e-mail /e-posta** nurhan.kulcu@kocaeli.edu.tr
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Introduction

Glial cells are Central Nervous System supporting cells. When these supportive glial cells (or neuronal stem cells) become malignant, they form gliomas, or, in other words, glial brain tumors. Gliomas comprise approximately 30% of all brain tumors and 80% of all malignant brain tumors.[1] In approximately 55% of cases, glioma presents as the highly aggressive tumor known as grade IV astrocytoma (also known as glioblastoma or GBM.[2] Despite advances in treatments that include surgical resection, chemotherapy, and radiation therapy, median survival and prognosis, particularly for GBM patients, continue to be poor. The median overall survival time for GBM is approximately 1.25 years, and for Low-grade glioma (LGG), it is 6.5-8 years.[3] As a result, it is critical to shed light on glioma survival events that may aid in the diagnosis and prognosis of glioma patients.

In several studies, it has been found that genes such as *PDGF*, *EGFR, GFAP*, *MDM2*, *PTPRN*, *RGS14*, *G6PC3*, *IGFBP2*, and *TIMP4* are differentially expressed in glioma tissues compared to normal brain tissues.[4–9] Illuminating genetic expression differences in glial brain tumors using the microarray method can be used for diagnostic, prognostic, or therapeutic purposes.

The aim of this study is to analyze genetic expression differences and hub genes in gliomas and investigate the effect of related genes and pathways on the formation and/or progression of gliomas using microarray data from grade II, III, and IV glioma patients.

Methods

Microarray Data

Gene expression profiles from GSE52009, GSE109857, GSE83300 and GSE52604 were selected from the GEO (Gene Expression Omnibus) database. All gene expression data sets were based on the Agilent GPL6480 (014850 Whole Human Genome Microarray 4x44K G4112F) platform.

In total, 150 patient samples and 15 control samples were included in the study by downloading them from GEO database, which consists of 58 glioma samples (25 astrocytomas, 23 anaplastic oligodendrogliomas, 10 glioblastomas) from GSE66354 dataset, 72 glioma samples (25 astrocytomas, 10 anaplastic oligodendrogliomas, 8 anaplastic astrocytomas, 9 anaplastic oligoastrocytomas, 20 glioblastomas) and 5 normal brain tissue samples from GSE109857 dataset, 20 glioma samples (20 glioblastomas) from GSE83300 dataset, and 10 normal brain tissue samples from GSE52604 dataset. The samples consist of 75 patients (50%) female, 75 patients (50%) male. The age of the patients varied between 18 and 66, with an average age of 40.2.

Differential Gene Expression Analysis

GeneSpring Software version 14.9_gx_pa (GeneSpring GX software, Agilent, Santa Clara, United States) was used to obtain the differentially expressed genes (DEGs) between tumor tissues and normal tissues, and between different grades of tumor tissues. The DEGs were identified using one-way ANOVA statistical analysis between tumor tissues and normal tissues, and with a threshold of P-value <0.05 and a fold change of expression >2.0. The Benjamini-Hochberg correction method was used to reduce the false positive rate.

Enrichment Analysis of DEGs

In this study, the online tool of DAVID was used to perform Gene Ontology enrichment and KEGG (Kyoto Encyclopedia of Gens and Genomes) pathway analysis of DEGs. The GO analysis included biological process (BP), cellular component (CC) and molecular function (MF) categories. Pathway analysis involves a functional examination that charts the relationships between genes and KEGG pathways. The cutoff criterion was set as $p<0.05$.

Protein-Protein Interaction Network Analysis

To explore the potential relationship between DEGs at different grades, the Cytoscape 8.3.2 software (Cytoscape, University of California, San Diego, California, United States) was used with the STRING application. The first 15 genes with high connections were selected as hub genes based on Protein–protein interaction (PPI) information. The hub genes were added to STRING, and KEGG pathway analyses were performed on DAVID to determine potential information.

Comparison of Hub Gene Expression Levels

The expression levels of hub genes in glioma tissues and normal tissues were revealed on GEPIA (Gene expression profiling and interactive analyses). Box plots were generated using GEPIA based on TCGA (The Cancer Genome Atlas) and GTEx (Genotype-Tissue Expression) gene expression data, utilizing disease status (tumor or normal) as a variable for calculating differential expression. This was performed using a one-tailed, unpaired Student's t-test.

The expression of these hub genes was confirmed by obtaining immunohistochemical data from patients with or without gliomas based on Human Protein Atlas (HPA). Additionally, survival percentages were obtained from the Pathology section of the HPA, which includes Kaplan-Meier plots showing the correlation between mRNA expression of each human protein gene and cancer patient survival.

Analysis of Hub Gene Survival

Recurrent and overall survival information was analyzed using GEPIA, a web-based tool based on TCGA and GTEx data.[10] The 95% confidence interval hazard ratio (HR) and log rank p-value were calculated and displayed on the graph.

Results

Identifying DEGs

Differentially expressed genes were identified by comparing the expression ratio change of samples from tumor tissues with normal tissues in GeneSpring (GeneSpring GX software, Agilent, Santa Clara, United States), using a threshold of > 2.0. In grades II, III, and IV, the number of increasing genes was 6970, 6143, and 6107, respectively, while the number of decreasing genes was 2179, 1823, and 1460, respectively.

The DEG's pathway analysis

The most significant molecular pathways that were identified through the pathway analysis of DEGs in tumor tissues were the neuroactive ligand-receptor interaction for increased DEGs in grades II, III, and IV, and the systemic lupus erythematosus, complement, and coagulation cascades, and GABAergic synapse for decreased DEGs in grades II, III, and IV, respectively. The top 5 enriched KEGG pathways for the increased and decreased DEGs according to grades are shown in Table 1.

Table 1. The KEGG pathway analysis of differentially expressed genes associated with glioma according to grades.

		Pathways
Grade II	Up'd (6970 DEG)	Neuroactive ligand-receptor interaction Cytokine-cytokine receptor interaction Cell adhesion molecules (CAMs) Inflammatory bowel disease (IBD) Taste transduction
	Down'd (2179 DEG)	Systemic lupus erythematosus Alcoholism Cardiac muscle contraction Biosynthesis of antibiotics Biosynthesis of amino acids
Grade III	Up'd (6143 DEG)	Neuroactive ligand-receptor interaction Cytokine-cytokine receptor interaction Allograft rejection Cell adhesion molecules (CAMs) Staphylococcus aureus infection
	Down'd (1823 DEG)	Complement and coagulation cascades Focal adhesion Proteoglycans in cancer Thyroid hormone synthesis Aldosterone-regulated sodium reabsorption
Grade IV	Up'd (6107 DEG)	Neuroactive ligand-receptor interaction Cytokine-cytokine receptor interaction Inflammatory bowel disease (IBD) Staphylococcus aureus enfeksiyonu Graft-versus-host hastalığı
	Down'd (1460 DEG)	GABAergic synapse Morphine addiction Alcoholism Carbon metabolism Glycolysis / Gluconeogenesis

The GO analysis of DEGs

As a result of the GO analysis, the biological processes, cell components, and molecular functions of genes that are over- and under-expressed in different grades of glioma were determined. In all grades, DEGs with increased expression were most closely associated with the G protein-coupled receptor signaling pathway, cell-to-cell signaling, and inflammatory response in terms of biological process; the plasma membrane integral component, cell membrane, cell outer region, and extracellular space in terms of cell component; and G protein-coupled receptor activity, cytokine activity, and calcium ion binding in terms of molecular function. DEGs with decreased expression in all grades were most closely associated with response to drugs in terms of biological process; and extracellular space and extracellular exosomes in terms of cell component (Table 1, Table 2).

Protein-Protein Interaction Network Analysis

Based on information from publicly available databases such as STRING, protein-protein interaction networks were constructed for DEGs in each grade, and the first 15 genes with the highest level of connectivity were identified as hub genes. The distributions of these hub genes according to grades, their degrees of association with other genes, and the differential gene expression levels compared to normal tissues (upregulated-downregulated) are shown in Table 3.

KEGG Analysis of Hub Genes

The KEGG pathway analysis of the hub genes for each grade have been performed. The hub genes were associated with the breast cancer pathway in Grade II and with the Human cytomegalovirus infection pathway in Grades III and IV, according to the KEGG pathway analysis results (Table 4).

Survival Analysis of Hub Genes

The prognostic information of 15 hub genes was analyzed on the GEPIA web server among genes with increased and decreased expression in all grades (http://gepia.cancer-pku.cn/detail.php).

In LGG, among the hub genes, expression of *VEGFA* (HR 2.4) was found to be associated with worse overall survival for LGG patients, and this was followed by *GAPDH* (HR 2.2), *FN1* (HR 1.8), *TP53* (HR 1.6), and *IL10* (HR 1.6). Additionally, *ALB* (HR 0.61) was found to be associated with better overall survival for LGG patients (Table.5, Figure 1).

In GBM, among the hub genes, the expression of *IL6* (HR 1.4) and *VEGFA* (HR 1.4) was found to be associated with worse overall survival for GBM patients, followed by *ALB* (HR 1.2, p = 0.41), *EGF* (HR 1.2), *SRC* (HR 1.2), *IL10* (HR 1.2), and *STAT3* (HR 1.2); also, *NOTCH1* (HR 0.91) was found to be associated with better overall survival for GBM patients (Table 5, Figure 1).

Table 2. The Gene Ontology analysis of differentially expressed genes associated with glioma according to grades.

		Biological Process	**Cellular Component**	**Molecular Function**
Grade II	**Up'd (6970 DEG)**	G-protein coupled receptor signaling pathway Chemical synaptic transmission Cell-cell signaling Cell surface receptor signaling pathway Inflammatory response	Integral component of plasma membrane Plasma membrane Integral component of membrane Extracellular region Extracellular space	G-protein coupled receptor activity Cytokine activity Bitter taste receptor activity Calcium ion binding Voltage-gated potassium channel activity
	Down'd (2179 DEG)	Response to drug Cell-cell adhesion Sister chromatid cohesion. Cell division Mitotic sister chromatid segregation	Extracellular exosome Cytosol Extracellular space Cytoplasm Nucleosome	Protein binding Cadherin binding involved in cell-cell adhesion Protein heterodimerization activity Integrin binding Cuprous ion binding
Grade III	**Up'd (6143 DEG)**	G-protein coupled receptor signaling pathway Cell-cell signaling Immune response Inflammatory response Chemical synaptic transmission	Integral component of plasma membrane Plasma membrane Integral component of membrane Extracellular region Extracellular space	G-protein coupled receptor activity Calcium ion binding Cytokine activity Hormone activity Bitter taste receptor activity
	Down'd (1823 DEG)	Response to drug Negative regulation of endopeptidase activity Triglyceride catabolic process Response to estrogen Retinoid metabolic process	Extracellular space Extracellular exosome Cytoplasm Endoplasmic reticulum lumen Basolateral plasma membrane	Protein binding Endopeptidase inhibitor activity Serine-type endopeptidase inhibitor activity ATP binding Cuprous ion binding
Grade IV	**Up'd (6107 DEG)**	Immune response G-protein coupled receptor signaling pathway Cell-cell signaling Inflammatory response Cytokine-mediated signaling pathway	Integral component of plasma membrane Plasma membrane Extracellular region Integral component of membrane Extracellular space	G-protein coupled receptor activity Cytokine activity Receptor activity Calcium ion binding Growth factor activity
	Down'd (1460 DEG)	Response to drug Retinoid metabolic process Triglyceride catabolic process Long-term synaptic potentiation Gluconeogenesis	Extracellular exosome Extracellular space Integral component of plasma membrane Plasma membrane Cell junction	Serine-type endopeptidase inhibitor activity GABA-gated chloride ion channel activity GABA-A receptor activity Structural constituent of myelin sheath Protein heterodimerization activity

Up'd: Up-regulated, Down'd: Down-regulated

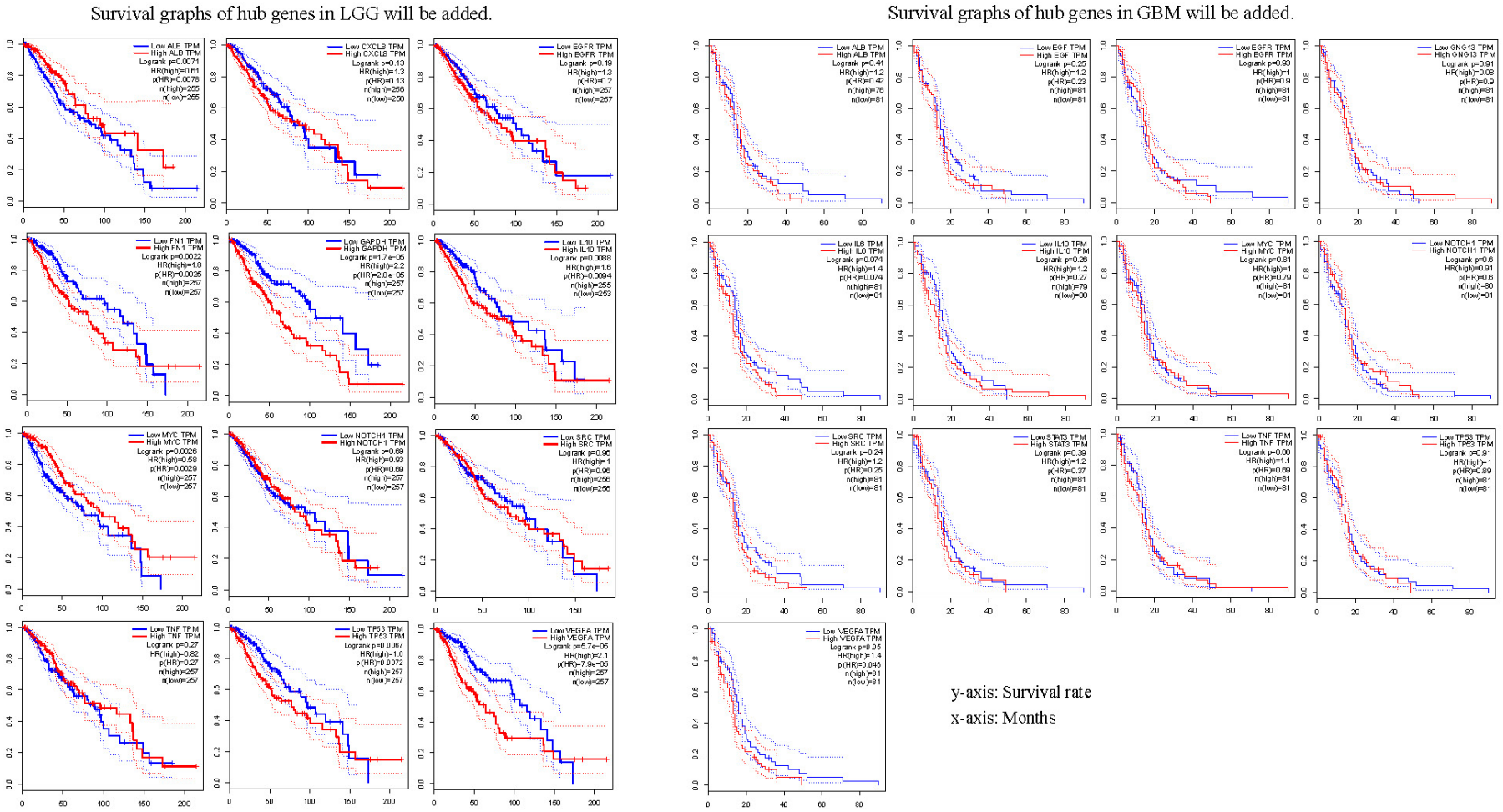

Figure 1. Survival graphs of hub genes in LGG and GBM

Table 3. List of hub genes according to grades.

Grade II			Grade III			Grade IV		
Gene	**CD**	**EL**	**Gene**	**CD**	**EL**	**Gene**	**CD**	**EL**
INS	761	↑	*INS*	791	↑	*INS*	759	↑
ALB	678	↓	*IL6*	704	↑	*IL6*	683	↑
TP53	677	↑	*ALB*	696	↓	*ALB*	675	↓
GAPDH	629	↓	*TP53*	678	↑	*TP53*	610	↑
TNF	568	↑	*TNF*	597	↑	*TNF*	579	↑
EGFR	526	↑	*EGFR*	536	↑	*EGFR*	513	↑
VEGFA	503	↓	*FN1*	512	↓	*EGF*	503	↑
MYC	501	↑	*VEGFA*	511	↓	*VEGFA*	492	↑
FN1	485	↓	*SRC*	497	↑	*SRC*	484	↑
SRC	480	↑	*MYC*	492	↑	*GNGT1*	463	↓
CXCL8	474	↓	*CXCL8*	476	↓	*MYC*	453	↑
GNGT1	470	↓	*GNGT1*	470	↓	*STAT3*	444	↑
NOTCH1	437	↑	*NOTCH1*	456	↑	*GNG13*	439	↑
IL10	409	↑	*MAPK1*	446	↓	*NOTCH1*	439	↑
KNG1	387	↑	*GNG7*	444	↑	*IL10*	425	↑

CD: Connection degree, EL: Gene expression level, ↑: Up-regulated, ↓: Down-regulated

Table 4. KEGG pathway analysis of hub genes according to grades

Grade	Pathways	P-Value
Grade II	Bladder cancer	4,00E-09
	Human cytomegalovirus infection	2,50E-08
	Proteoglycans in cancer	4,80E-07
	Pathways in cancer	4,90E-07
	Kaposi sarcoma-associated herpesvirus infection	1,00E-05
Grade III	Human cytomegalovirus infection	1,30E-13
	Bladder cancer	2,60E-11
	Kaposi sarcoma-associated herpesvirus infection	1,80E-10
	PI3K-Akt signaling pathway	5,90E-10
	Pathways in cancer	6,90E-10
Grade IV	Human cytomegalovirus infection	1,00E-11
	Bladder cancer	4,00E-09
	Kaposi sarcoma-associated herpesvirus infection	8,90E-09
	PI3K-Akt signaling pathway	2,10E-08
	Pathways in cancer	2,10E-08

Comparison of Hub Gene Expression Levels

Box plots were made using GEPIA to reveal the expression levels of hub genes in glioma tissues and normal tissues, and to visualize the associations. In LGG, it was found that the expression levels of *EGFR, IL10, MYC, NOTCH1, SRC, TNF*, and *TP53* were significantly increased in cancer tissue when compared to normal brain tissue, and that the expression level of *ALB* was significantly decreased (Figure 2).

In GBM, it was found that the expression levels of *EGF, EGFR, IL6, IL10, MYC, NOTCH1, SRC*, *STAT3, TNF, TP53*, and *VEGFA* were significantly increased in cancer tissue compared to normal brain tissue when correlated with the results obtained, and that the expression level of *ALB* was significantly decreased (Figure 2).

The expression levels of other hub genes in LGG (*CXCL8, FN1, GAPDH, GNGT1, IL6, INS, KNG1*, and *VEGFA*) and in GBM (*INS, GNG13*, and *GNGT1*) did not correlate with the results obtained from GEPIA.

The expression of these hub genes was confirmed using immunohistochemical data from glioma or non-glioma patients based on the Human Protein Atlas, and 3-year survival percentages were found based on the expression differences in glioma (Figure.3).

Table 5. The survival rates of the hub genes in LGG and GBM

Gene	LGG-HR	Gene	GBM-HR
INS	*	*INS*	*
ALB	0,61	*IL6*	1,4
TP53	1,6	*ALB*	1,2
GAPDH	2,2	*TP53*	1
TNF	0,82	*TNF*	1,1
EGFR	1,3	*EGFR*	1
VEGFA	2,4	*EGF*	1,2
MYC	0,58	*VEGFA*	1,4
FN1	1,8	*SRC*	1,2
SRC	1	*GNGT1*	*
CXCL8	1,3	*MYC*	1
GNGT1	*	*STAT3*	1,2
NOTCH1	0,93	*GNG13*	0,98
IL10	1,6	*NOTCH1*	0,91
KNG1	*	*IL10*	1,2
IL6	1,9		

HR: Hazard ratio, *: There is not enough research available.

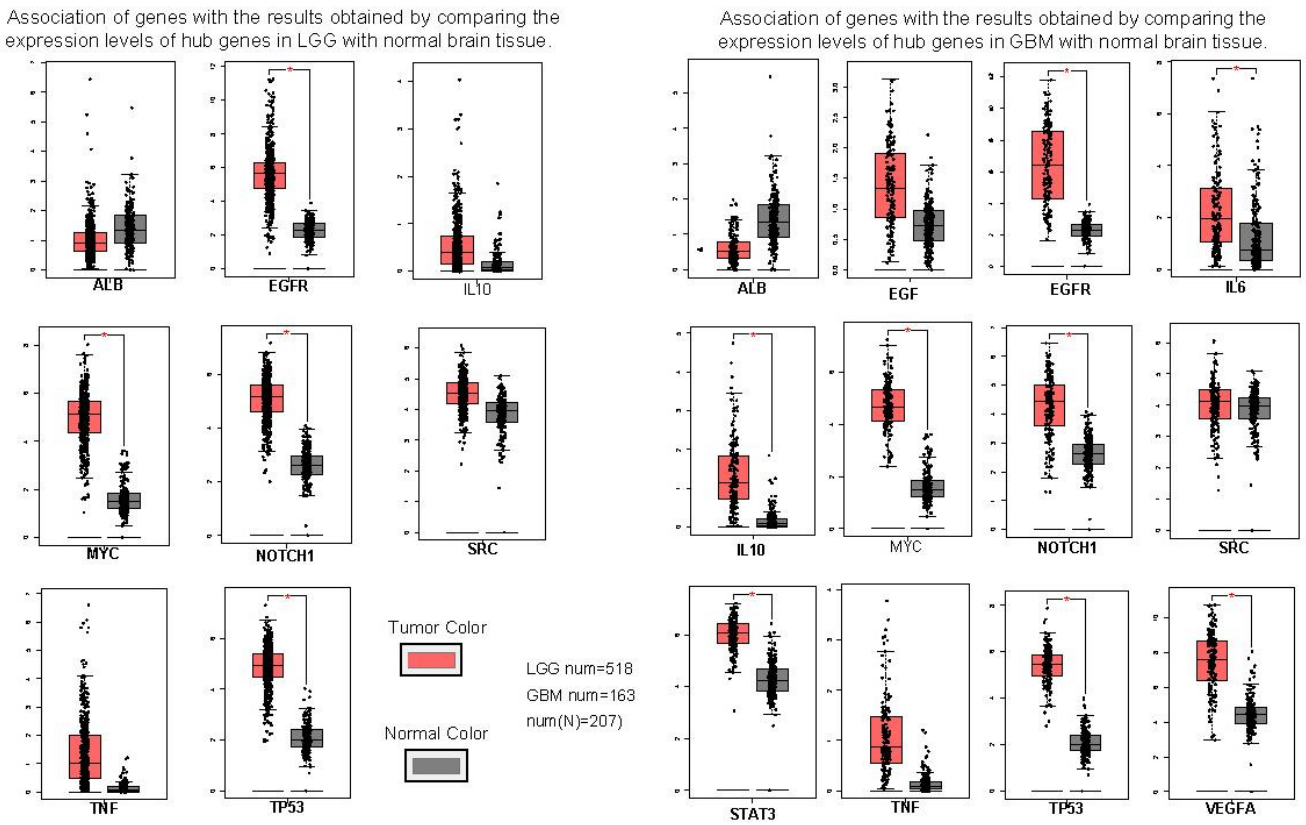

Figure 2. Association of genes with the results obtained by comparing the expression levels of hub genes in LGG and GBM with normal brain tissue.

Discussion

Patients with gliomas still have a poor prognosis despite therapies such extensive surgery, radiation, and chemotherapy. Determining potential molecular targets and actively investigating the pathophysiology of gliomas are so crucial. Despite the fact that, many of these targets have already been found, DEGs from various research often contradict. For the purpose of this study, we obtained datasets from GEO database to evaluate hub genes in 150 glioma samples in comparison to 15 normal tissues.

In study, DEGs were analyzed using DAVID Bioinformatics Database for KEGG pathways. It was found that DEGs with increased expression were mainly associated with neuroactive ligand-receptor interaction, while DEGs with decreased expression were most associated with systemic lupus erythematosus, complement and coagulation cascades, and GABAergic synapse at different grades of brain cancer. Recently, it has been reported that patients with GBM with a damaged neuroactive ligand-receptor interaction pathway had a poor prognosis.[11] These results suggest that abnormalities in the neuroactive ligand-receptor interaction pathway may play a role in the development and progression of brain cancer. The complement and coagulation cascades pathway, which is associated with protumorigenic phenotypes of immune cells, protection of tumor cells from immune attack, development and metastasis of the tumor, has been reported in gliomas before.[12–14] Involvement of distruption in this pathway in glioma is consistant with low immunity of the diasease nature. Malignant and invasive gliomas have been shown to have defects in the GABAergic synapse pathway, which is particularly apparent in grade 4 in our study, with decreased expressions.[15] This demonstrates the significance of this route for the CNS and suggests a potential target for slowing tumor growth. Further research is needed to understand the mechanisms involved and potential therapeutic interventions.

In our study, a total of 21 genes (*ALB, CXCL8, EGF, EGFR, FN1, GAPDH, GNG13, GNG7, GNGT1, IL10, IL6, INS, KNG1, MAPK1, MYC, NOTCH1, SRC, STAT3, TNF, TP53,* and *VEGFA*) were identified as hub genes, with the top 15 genes having the most connections at each grade. INS (Insulin) has been found to have the highest connection degree in each grade. *IL6* is implicated in high-grade gliomas, and this gene was found associated with poor survival. *STAT3* and poor survival-related *EGF* were detected with *IL6* at grade IV.

Insulin (*INS*) has known to promote cell growth in both healthy and cancerous cells. At physiological amounts insulin interacts with its own receptor (IR) and triggers a number of intracellular signaling pathways, promoting both metabolic and mitogenic effects.[16] IR plays a key role in metabolic regulation of glucose homeostasis.[17] This homeostasis plays a critical role in the development and progression of GBM. Detection of the insulin gene as the

hub gene in our study correlates with the observation of increased expression of insulin and insulin growth-like factor receptors in GBM tumor tissues in previous studies. This suggests that insulin and the insulin pathway are very important for glioma development and progression.

IL6 is a multifunctional cytokine that regulates cell growth, differentiation, and cellular functions in many cell lineages.[18] Recent evidence suggests that *IL-6* plays a crucial role in linking chronic inflammation to cancer by driving tumor initiation and subsequent growth and metastasis.[19] GBM samples were found to contain significantly higher IL6 protein levels compared to control brains, and higher *IL6* mRNA was linked to poor GBM patient survival.[20] The increased *IL6* expression detected in high-grade gliomas (grade III and IV) in our study is correlated with information in the literature. *STAT3* (signal transducer and activator of transcription 3), is an oncogenic transcription factor, is involved in various biological processes, including angiogenesis, cell proliferation, survival, differentiation and metastasis. *STAT3* activation has been reported in breast, melanoma, and thyroid cancers, as well as GBM before.[21,22] *STAT3* activation occures through growth factor receptors including epidermal growth factor receptor (*EGFR*) and platelet-derived growth factor receptors (*PDGFR*), and interleukin-6 receptor (*IL-6R*/gp130), Janus family kinases (*JAK*), Abl family kinases, and Src family kinases.[23] The epidermal growth factor (*EGF*) plays a critical role in the processes of brain tissue healing and regeneration.[24] *EGF* and *EGFR* play critical roles in the GBM in a matrix metalloproteinase-9 (*MMP-9*)–dependent manner. The stimulatory effects of *EGF* on the expression and activity of *MMP-9* in GBM are mediated by *EGFR*.[25] *EGFR* is crucial for Human Cytomegalovirus (HCMV) binding, signaling, and host cell entry, also.[26]

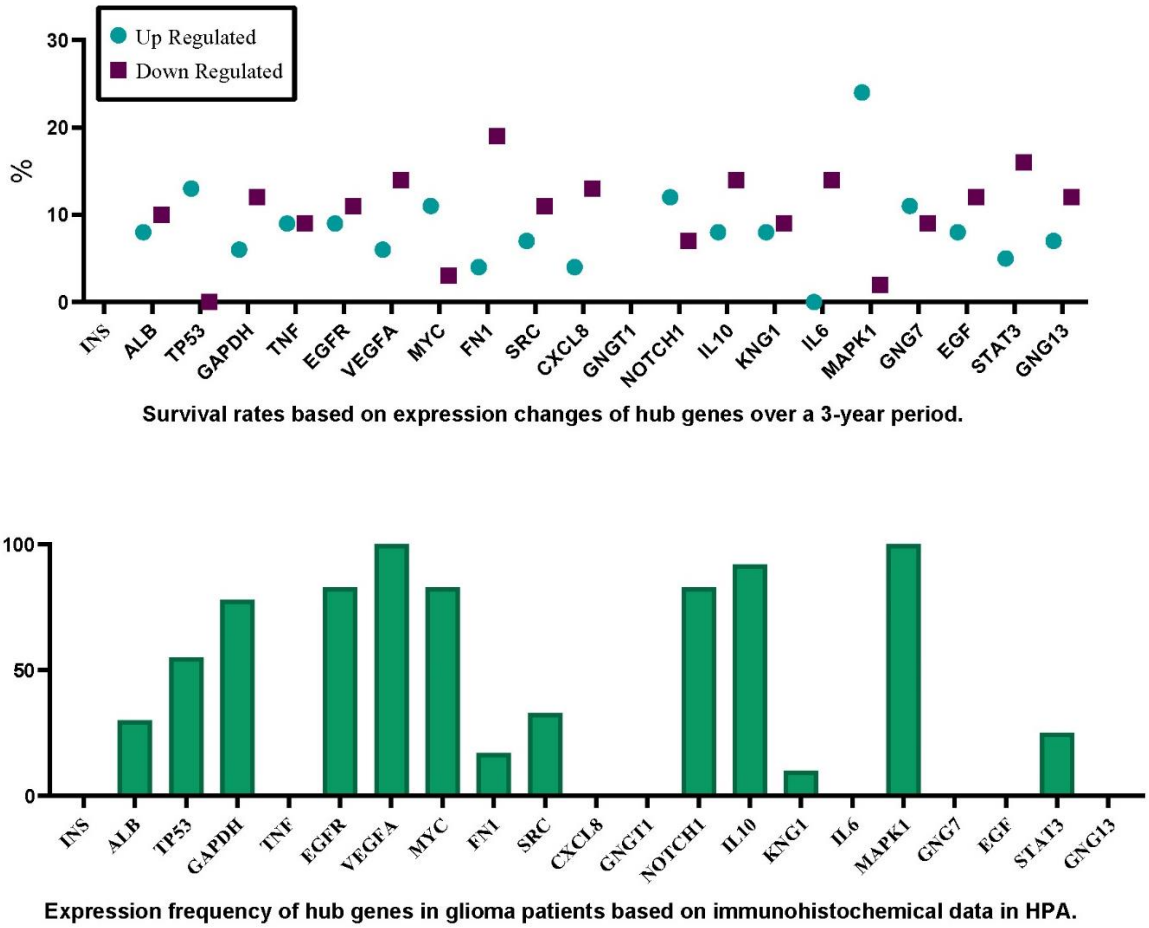

Figure 3. The protein levels of Hub genes in HPA data.

The human cytomegalovirus (HCMV) infection was revealed to be the most significant pathway in high-grade gliomas according to our KEGG analysis of hub genes. Human cytomegalovirus (HCMV) has been reported in medullablastoma, neuroblastoma, prostate, breast, colorectal and ovarian malignancies, as well as glioblastoma. More than 90d of malignant gliomas has been linked to HCMV infection, according to recent clinical epidemiological data.[27] But, it remains unclear how the HCMV leads to the development of glioblastoma. Studies conducted in vitro indicate that HCMV may influence the etiology of glioblastoma by onco-modulation of host cellular pathways.[28] Culturing studies has shown that long-term infection of glioma cells with HCMV upregulate

key signaling mediators such *SOX2, STAT3, BMX*, and *IL-6*.[29] Price and Chiocca developed a malignant glioma mouse model via CMV-infection using mut3 mice to study the role of virus in cancer development and progression. Their results showed that CMV-infected mut3 mice developed grade III and IV astrocytomas spontaneously. Also, they demonstrated that MCV-infected mut3 mice had decreased overall survival compared to mock-infected Mut3 mice as a control.[30] In line with all these data, we can say that the HCMV infection pathway detected significantly in high-grade gliomas (grade III and grade IV) in our study is compatible with the literature. The results of our study emphasizes that the HCMV can cause high-grade glioma and can be potantial immuno-therapeutic target for further treatment strategies developing for the malignant gliomas.

In conclusion, this study identified DEGs between glial brain tumors and normal brain tissues by analyzing four microarray datasets. A total of 21 genes (*ALB, CXCL8, EGF, EGFR, FN1, GAPDH, GNG13, GNG7, GNGT1, IL10, IL6, INS, KNG1, MAPK1, MYC, NOTCH1, SRC, STAT3, TNF, TP53*, and *VEGFA*) were identified as hub genes, with the top 15 genes having the most connections at each stage. The development of gliomas may be influenced by these genes. The identification of new biomarkers may improve the clinical outcomes of Glial brain tumor patients and provide a comprehensive approach. These hub genes are potentially considered new biomarkers for prognosis in Glial brain tumors. They may contribute to the identification of Glial brain tumor patients and potentially facilitate monitoring of patient outcomes. The human cytomegalovirus (HCMV) infection was revealed to be the most significant pathway in high-grade gliomas in this study. The findings are expected to shed light on the progression and differentiation of glial brain tumors and provide insight for new clinical, epidemiological, and experimental studies to develop new therapeutic approaches. However, this study has limitations due to the limited sample size derived from microarray datasets and the lack of survival analysis on sufficient clinical samples. In future prospective studies including large sample size are essential to evaluate the clinical significance of the identified hub genes as biomarkers for Glial brain tumors.

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This article was taken from the master's thesis entitled "investigation of gene expression changes and pathways that play a role in the progression of glial brain tumors" (2021).

Our research is based on open-source data and therefore does not require ethics committee approval for the study.

Conflict of Interest

The authors have no conflicts of interest to disclose.

Author contributions

Study idea/Hypothesis: DSA; Data preparation: NKS; Analysis: NKS, DSA; Literature review: NKS, DSA; Manuscript writing: NKS, DSA; Critical Review: DSA.

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