

Four Key Genes are Biomarkers Associated with Immunity in Neuroglioma

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Abstract

Background: Glioma is the most common intracranial tumor, with glioblastoma being the most malignant. However, its treatment is very few, and targeted therapy is an important breakthrough in treatment.

Methods: Numerous genes are differentially expressed during the progression of glioma, some of which may play a key role. To find key genes, we analyzed three multi-sample microarrays (GSE4290, GSE54004, and GSE29796) in the GEO database to obtain intersection differential genes among them. We entered all DEGs into the STRING database and characterized the protein interactions of these DEGs as visual PPI networks by Cytoscape software. Also, we used the GEPIA2 and CGGAdatabase to predict the relationship between key genes and the prognosis of glioma patients.

Results: A total of 222 up-regulated genes and 127 down-regulated genes were identified. Four genes(FN1, LAMB1, FAM20C, and COL6A1) were significantly negatively correlated with malignant glioma survival. Expression levels of four genes increased with the glioma grade. All gene expression is more common in IDH wild glioma and are enriched in the Mesenchymal subtype(AUC>0.8). In addition,they can be defined as hazard factors for glioma. We found that these genes were co-expressed and jointly involved in the infiltration of immune cells in tumors.

Conclusion: In conclusion, FN1, LAMB1, FAM20C, and COL6A1 is associated with poor prognosis in glioma patients. These genes might be clinical targets of glioma immunotherapy.

Background

Glioblastoma (GBM) is the most lethal primary malignant brain tumor in adults with poor survival because of acquired therapeutic resistance and rapid recurrence[1]. At present, temozolomide is the first-line drug, which has a good therapeutic effect for patients with MGMT positive. Differential correlation analysis of glioblastoma shows that immune cell interaction can predict patient survival rate, and there have been some advances in existing immunotherapy [2,3]. Besides, physical therapy and tumor electric field therapy have also entered the research stage [4]. With the advent of the big data era, gene sequencing has provided us with a wealth of gene chips. Through specific research methods, people can use the genetic data in them for bioinformatics analysis, and can study the key genetic changes and epigenetic characteristics of glioma at the molecular level [5,6]. Moreover, the treatment of high-grade glioma is currently a difficult problem to overcome, mainly due to its strong heterogeneity and invasion and metastasis instinct. Therefore, to find genes that may play a key role in tumor progression, we attempt to analyze the differential genes between LGG and GBM.

Materials And Methods

Data filtering

Three data set chips (GSE4290, GSE454004 ,and GSE29796) were screened from the GEO database(<https://www.ncbi.nlm.nih.gov/geo/>). Differential genes (DEGs) were determined using adjusted P-values < 0.05 and $|\log_{2}FC| > 1$ as screening criteria. Finally, $\log_{2}FC$ greater than 0 was considered an up-regulated genes, and $\log_{2}FC$ less than 0 was considered a down-regulated genes. The intersection of down-regulated genes on the three microarrays was identified by the Venn web tool.

Protein interaction network construction and hub gene screening

STRING(<https://string-db.org/>) is a database of 2031 protein interactions, including 964,376,3 proteins and 138,838,440 interactions in total. By inputting all the overlapped DEGs, and extracting a PPI with a score of 0.7, a differential gene protein network that can be used to evaluate potential protein interactions is obtained. Subsequently, we used Cytoscape software and MCODE plug-in to demonstrate the protein interaction network.

Survival cure, Boxplots and Co-expression of key genes analyzed by GEPIA

The GEPIA (<http://gepia2.cancer-pku.cn/#index>) network tool has been running for two years and has processed approximately 280,000 analysis requests from approximately 110,000 users in 42 countries. GEPIA2 is an updated version of GEPIA and contains 9,736 tumor samples and 8,587 normal samples from TCGA and GTEx projects. The calculation of hazard ratios was based on Cox PH (Proportional Hazards) Model, with 95% CI added as dashed lines and axis units as months. A P-value < 0.05 is regarded as statistically significant. The parameters were set as $|\log_{2}FC| > 1$ and P-value < 0.01. Tumor tissue was shown in red and normal tissue in black.

CGGA database

The CGGA(<http://www.cgga.org.cn/>) database involves nearly 2000 cases of primary and recurrent glioma genome chips which including different histopathological classifications and different WHO grades. All data in the CGGA database are freely available to researchers around the world. We used R (4.0.2) to verify the prognosis and expression of key glioma genes above and analyzed the correlation of these genes with IDH gene variation and the expression of four genes in different pathological phenotypes the amount.

GO and KEGG

We analyzed target genes on CC, MF and BP, and found out the genes related to them. The KEGG(<https://www.kegg.jp/kegg/pathway.html>) database is a database based on the various pathways involved in genes. Using R(4.0.2)to conduct enrichment analysis of key genes to determine the functions and pathways enriched by key genes.

TIMER

TIMER(<https://cistrome.shinyapps.io/timer/>) contains 10,897 tumors out of 32 types of cancer. It provides six major analysis modules, allowing users to interactively explore links between immune infiltration and a wide range of factors, including gene expression, clinical outcomes, somatic mutations, and somatic copy number changes. We inquired about the correlation between key genes and immune cells. And we assessed the major risk factors. TIMER outputs the Cox regression results including hazard ratios and statistical significance automatically.

Results

Screening of DEGs

Three gene data chips (GSE4290, GSE54004 and GSE29796) were downloaded from the GEO database. We analyzed and identified the co-expressed DEGs in the chips of GSE4290, GSE54004 and GSE29796 using the tool Venn. 1633 (907 up-regulated and 726 down-regulated), 1290 (576 up-regulated and 714 down-regulated) and 3440 (2445 up-regulated and 995 down-regulated) DEGs were identified. A total of 349 overlapping DEGs were identified, of which 222 were up-regulated and 127 were down-regulated (Fig. 1a).

Protein interaction network construction and key gene screening

Using the STRING web tool to explore the interactions between the proteins encoded by DEGs. 348 nodes and 834 edges of the PPI network were illustrated by Cytoscape software (Fig. 1b). Cluster 2 was chosen as the research object and then sorted by Cytohubba (Fig. 1c, Table 2).

Table 1
Details of three datasets

Dataset	WHO □	WHO □	Total number	Platform
GSE4290	45	77	122	GPL570[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array
GSE54004	12	98	110	GPL18281Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip [gene symbol version]
GSE29796	26	17	43	GPL570[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array

Table 2
Details of 21 genes.

Name	Degree Score	Gene description
FN1	14	fibronectin 1
LAMB1	12	laminin subunit beta 1
COL4A1	11	collagen type IV alpha 1 chain
COL4A2	10	collagen type IV alpha 2 chain
TIMP1	10	TIMP metalloproteinase inhibitor 1
SCG3	10	secretogranin III
LGALS1	10	galectin 1
CALU	10	calumenin
TNC	10	tenascin C
IGFBP3	10	insulin like growth factor binding protein 3
CYR61	10	cysteine rich angiogenic inducer 61
FAM20C	10	FAM20C, golgi associated secretory pathway kinase
FAM20A	10	FAM20A, golgi associated secretory pathway pseudokinase
COL5A1	10	collagen type V alpha 1 chain
COL6A3	9	collagen type VI alpha 3 chain
COL5A2	9	collagen type V alpha 2 chain
COL6A1	8	collagen type VI alpha 1 chain
ITGA1	7	integrin subunit alpha 1
COL8A1	7	collagen type VIII alpha 1 chain
LUM	7	lumican
CASP8	6	caspase 8

Table 3

Independent prognostic factors for OS(overall survival) of glioma. Factors were identified as independent prognostic factor, including age, IDH mutation and Grade. And four genes were independent prognostic factor. $P < 0.05$ was considered statistically significant

Clinical factors	univariate analysis			p	multivariate analysis			p
	HR	95%CI			HR	95%CI		
		Lower	Upper			Lower	Upper	
Age	1.03	1.017	1.044	4.00E-06	1.006	0.993	1.02	0.343718
Gender	0.922	0.699	1.216	0.565609	NA	NA	NA	NA
IDHmutation	0.389	0.294	0.515	0	0.932	0.644	1.35	0.709765
Grade	4.338	3.235	5.816	0	2.833	1.971	4.072	0
FN1	2.897	2.321	3.616	0	1.801	1.346	2.409	7.40E-05
LAMB1	3.629	2.745	4.797	0	2.065	1.45	2.941	5.90E-05
FAM20C	4.006	2.983	5.379	0	2.282	1.504	3.462	1.05E-04
COL6A1	3.859	2.791	5.335	0	1.837	1.239	2.723	0.002454

Survival analysis of DEGs

Our results revealed that 4 of 21 genes are significantly related to the prognosis of patients. To explore the prognosis of the four genes in LGG and GBM patients, we used the GEPIA2 online survival analysis tool to draw the overall survival curve of key genes (Fig. 2). The survival analysis results of FN1, LAMB1, FAM20C and COL6A1 showed significant statistical differences (Log-rank $p < 0.05$).

Analysis of gene expression by GEPIA

GEPIA was used to further analysis of the expression of each gene in LGG and GBM. In LGG, FN1 is highly expressed. LAMB1 expression is lower than normal tissues. FAM20C and COL6A1 have no significant difference in expression with normal tissues. In GBM, FN1, FAM20C, and COL6A1 are all expressed higher than normal tissues; LAMB1 has no differential expression in normal tissues. In general, the expression of these four genes increased during the progression from LGG to GBM (Fig. 3).

Key genes verification analysis

We validated the key roles of FN1, LAMB1, FAM20C and COL6A1 in glioma using the CGGA database. These four genes resulted in shorter survival in glioma patients ($p < 0.05$) (Fig. 4a). More importantly, they were significantly associated with poor prognosis in GBM patients ($p < 0.05$) (Fig. 4b). With the increase

of the WHO grade of glioma, gene expression also increased(Fig. 5a). Four genotypes were more common in wild-type IDH gliomas compared to mutant IDH (Fig. 5b).AUC(Area Under Curve) in the CGGA database predicting Mesenchymal subtype was greater than 0.8(Fig. 5c and d).

Enrichment analysis of four key genes

Four genes were mainly enriched in neutrophil degranulation, neutrophil activation involved in immune response and neutrophil activation of BP and receptor interaction of MF. Key genes in CC were mainly enriched in the collagen-containing extracellular matrix, focal adhesion,cell-substrate junction, and cytokine-cytokine, etc. Besides, DEGs in the KEGG pathway analysis were predominantly enriched in the Cytokine-cytokine receptor interaction signaling pathway(Fig. 8, Table 4).

Table 4
GO and KEGG.

Category	ID	Description	pvalue	Count
BP	GO:0043312	neutrophil degranulation	5.06E-23	43
BP	GO:0002283	neutrophil activation involved in immune response	6.45E-23	43
BP	GO:0042119	neutrophil activation	1.44E-22	43
BP	GO:0002446	neutrophil mediated immunity	1.55E-22	43
BP	GO:0030198	extracellular matrix organization	8.01E-20	35
BP	GO:0043062	extracellular structure organization	8.75E-20	35
BP	GO:0050900	leukocyte migration	4.40E-14	33
BP	GO:0042110	T cell activation	3.71E-11	28
BP	GO:0032496	response to lipopolysaccharide	1.38E-10	23
BP	GO:0050727	regulation of inflammatory response	2.92E-10	24
CC	GO:0062023	regulation of inflammatory response	1.45E-14	30
CC	GO:0005925	focal adhesion	5.95E-13	28
CC	GO:0030055	cell-substrate junction	9.03E-13	28
CC	GO:0030667	secretory granule membrane	1.15E-12	24
CC	GO:0005788	endoplasmic reticulum lumen	2.51E-12	24
CC	GO:0034774	secretory granule lumen	2.40E-10	22
CC	GO:0060205	cytoplasmic vesicle lumen	3.03E-10	22
CC	GO:0031983	vesicle lumen	3.41E-10	22

Category	ID	Description	pvalue	Count
MF	GO:0050839	cell adhesion molecule binding	1.60E-06	22
KEGG pathway	hsa04060	Cytokine-cytokine receptor interaction	7.86E-10	25

Co-expression and immune cell infiltration

The co-expression analysis showed the relationship between different genes. Through the CGGA database, GEPIA and TIMER, we analyzed the co-expression between FN1, LAMB1, FAM20C and COL6A1. These four genes were closely related to each other (Fig. 7). GO and KEGG result revealed that they are related to immunity. The immune infiltration of key genes in LGG and GBM was analyzed by TIMER (Fig. 9a and b). In LGG, hazard factors CD8 + Tcell, Macrophage, FAM20C, and COL6A1 are defined as hazard factors (Table 5, $P < 0.05$). FAM20C and COL6A1 are associated with poor prognosis in patients (Fig. 9c). In GBM, Dendritic, CD4 + Tcell were defined as hazard factors (Table 5, $P < 0.05$). COL6A1 are associated with poor prognosis in patients (Fig. 9d).

Table 5

Cox Proportional Hazard Model. In LGG, CD8_Tcell, Macrophage, FAM20C and COL6A1 were defined as hazard factors. In GBM Dendritic, CD4_Tcell and age were defined as hazard factors. $P < 0.05$ was considered statistically significant.

Type	Items	coef	HR	95%CI_l	95%CI_u	p.value	sig
LGG	B_cell	1.929	6.880	0.020	2367.542	0.518	
	CD8_Tcell	7.881	2645.986	3.121	2243379.629	0.022	*
	CD4_Tcell	-1.151	0.316	0.000	535.404	0.762	
	Macrophage	6.055	426.351	7.799	7.799	0.003	**
	Neutrophil	-6.592	0.001	0.000	2.072	0.078	.
	Dendritic	0.153	1.165	0.025	54.225	0.938	
	FN1	-0.143	0.867	0.687	1.093	0.227	
	LAMB1	0.026	1.026	0.797	1.322	0.840	
	FAM20C	0.377	1.457	1.039	2.043	0.029	*
	COL6A1	0.454	1.575	1.185	2.094	0.002	**
GBM	B_cell	-0.631	0.532	0.097	2.919	0.468	
	CD8_Tcell	0.620	1.858	1.858	5.979	0.299	
	CD4_Tcell	2.980	19.687	2.077	186.577	0.009	**
	Macrophage	0.016	1.016	0.093	11.095	0.990	
	Neutrophil	-1.661	0.190	0.011	3.349	0.257	
	Dendritic	1.042	2.835	1.041	7.717	0.041	*
	FN1	0.004	1.004	0.716	1.409	0.981	
	LAMB1	0.039	1.039	0.774	1.396	0.798	
	FAM20C	0.120	1.127	0.856	1.485	0.394	
	COL6A1	0.009	1.009	0.762	1.334	0.952	
P < 0.05, **P < 0.01, ***P < 0.001.							

Discussion

The mortality rate of glioma is very high, and the current status of treatment is very worrying. A large amount of genotype-oriented disease classification principles have been introduced, which have made medical treatment possess a broad research direction [7]. We obtained three microarrays of the GEO database based on a systematic analysis method, targeting important participating genes throughout the

development of glioma, and extracted four key genes. These genes play a vital role in the development of glioma. Immunotherapy is a new therapeutic method at present, which can inhibit the tumor during the treatment, and can specifically act on the tumor to achieve the effect of adjuvant therapy[8]. Part of the treatment of brain tumors has shifted to immunomodulatory intervention therapies. In gliomas, a variety of immune cell types are infiltrated, such as neutrophils, macrophages, and T cells, which are infiltrated[9,10]. Microglia and macrophages are enriched in the microenvironment, and there is a significant interaction between these cells to promote the malignant progression of gliomas[11]. At present, many recognized immune markers play an important immunoregulatory function in gliomas. IDH1 (R132H) is a neoantigen that triggers immune responses in IDH1 (R132H) mutant gliomas[12]. Programmed cell death protein and its ligands (PD-1 and PD-L1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) may be key factors for tumor cells to evade immunosuppression[13]. Unfortunately, the single-target immunotherapy effect is still not significant and patient survival is not significantly increased[14,15]. We hope to search for meaningful immune research targets and promote the progress of immunotherapy.

Fibronectin 1 (FN1) is a central component of the extracellular matrix (ECM), which constructs the tumor microenvironment (TME) and participates in the invasion, migration, immune infiltration, and metabolism of tumor cells [16,17]. By comparing the genetic differences between grade III and IV gliomas, it is found that the genes ELAV-like protein 1 (ELAVL1) and FN1 may participate in the growth of gliomas through the PI3K-Akt signaling pathway, and ECM can be found to promote tumor invasion [18]. Similar to our results, COL3A1, FN1, MMP9 and other genes can be considered to play an important role in glioblastoma, and these genes are also mainly present in ECM [19]. GBM tumor guanylate binding protein 2 (GBP2) is a large-scale GTPase induced by interferon, which can improve the immunity of microorganisms. Studies have found the role of GBP2/Stat3/FN1 signaling cascade in GBM invasion[8]. There are many genes in the TME of malignant gliomas that are related to the prognosis of the patient. These genes include LAMB1, FN1, ACTN1, TRIM, SERPINH1, CYBA, LAIR1, LILRB2[20]. Also, MIR-1 and MIR-1271 exert an inhibitory function on FN1, which can ultimately improve the effect of chemotherapy. Their low expression is all related to the poor prognosis of glioma patients[21,22].

In the process of tumor epithelialization and metastasis, LAMB1 (laminin β -1) is activated to promote the EMT process[23]. The level of protein phosphorylation in breast cancer has an obvious change, and the level of secreted phosphorylated protein group may reflect the progression and subtype of the disease. Among them, CD44, OPN, FSTL3, LAMB1, STC2 are of great significance[24]. In colorectal cancer, LAMA1, LAMA3, LAMB1 and LAMB4 are more abundant[25]. LAMB1 is superior to CEA (carcinoembryonic antigen) in distinguishing colorectal cancer patients from control groups. The combined measurement of LAMB1 and CEA may improve the accuracy of diagnosing colorectal cancer[26]. The silencing of LAMB1 and CACNA1D in prostate tissue can also reduce tumor cell infiltration[27]. These candidate genes may assist diagnosis and treatment, and predict the risk of tumor metastasis in the early stage of tumor development.

FAM20C protein is a new kinase that phosphorylates secreted proteins and proteoglycans. FAM20C phosphorylates hundreds of secreted proteins and is activated by the pseudokinase Fam20A, which is closely related to the metabolism of substances in the Golgi apparatus[28,29]. It phosphorylates many extracellular proteins, including the small integrin-binding ligand, N-linked glycoproteins [30]. Studies believe that the activator of Fam20C may be beneficial in cancer. In addition, the activator of G-CK/Fam20C may provide a new treatment tool for the field of biomineralization and low phosphate diseases [31,32]. In lung cancer, FAM20C, MYLIP, and COL7A1 have been identified as key hypoxia-related genes in the LUAD process, and are regulated by DNA methylation [33,34]. The triple-negative breast cancer (TNBC) cells that activate FAM20C exhibit a strong anti-proliferation effect, with increased apoptosis and decreased migration [35]. There are few studies on Fam20C in gliomas. In this study, we found for the first time that the expression of FAM20C was also up-regulated in GBM. We speculate that FAM20C may also play an anti-proliferative effect as an antagonist of glioma evolution. Perhaps, its gene expression is up-regulated with the up-regulation of tumor-promoting gene expression.

The COL6A1 (VI collagen $\alpha 1$) is located on chromosome 21 and can maintain the integrity of various tissues[36].COL6A1 gene expression is significantly different in normal glial cells compared with low-grade (grade I, II) astrocytoma and high-grade astrocytoma (grade III, IV). And the difference is more obvious in high-grade samples [37,38]. Many studies have used this protein as one of the markers of epithelial-mesenchymal transition, and play an important role in tumor ECM receptor interaction and lesion adhesion pathway[39,40].

Compared with differentiated glioblastoma cells(DGCs), the expression levels of 10 proteins that interact with ECM in cancer stem cells (CSCs) are increased(COL6A1, COL6A3, FN1, ITGA2, ITGA5, ITGAV, ITGB1, ITGB3, LAMB1, and LAMC1), indicating that CSC may be highly aggressive(12). Therefore, these three genes(FN1, LAMB1, and COL6A1)are also involved in tumor recurrence, which is one of the characteristics of glioma stem cells. Considering the close connections between these three genes, it is very meaningful to explore their specific interactions in glioma. Our research screened out genes related to the prognosis of GBM as potential therapeutic targets. Moreover, in the process of glioma evolution, glioma cells can evolve their suitable microenvironment, increasing their proliferation and invasion capabilities[41]. In general, the expression of 4 genes increased during the progression from LGG to GBM. If we can regulate key microenvironment genes FN1, LAMB1, COL6A1, and FAM20C expression at an early stage, a good therapeutic effect may be obtained. Moreover, from the results of enrichment analysis and co-expression, it is reasonable to think that these genes may also be involved in immune-related processes.While COL6A1,FAM20C may be risk factors for immune cell infiltration and resulted in a shorter survival time for glioma patients.

Conclusion

Our bioinformatics analysis was based on microarray screening of gene expression data from the GEO database looking for DEG between GBM samples and LGG brain tissues. Ultimately, 21 possible hub genes were screened. According to the final survival analysis, four genes including FN1, LAMB1, FAM20C,

COL6A1 overexpression were associated with a poorer prognosis in LGG and GBM patients. And these four genes are associated with the immune process of neuroglioma. Of course, further research is merited to explore the biological functions of these genes and the underlying mechanisms involved in the pathogenesis of glioma.

List Of Abbreviations

LGG, low grade glioma;GBM, glioblastoma;GEO, Gene Expression Omnibus; DEG, differentially expressed gene; CGGA, Chinese Glioma Genome Atlas;TCGA, The Cancer Genome Atlas;TIMER, Tumor IMMune Estimation Resource;GEPIA, Gene Expression Profiling Interactive Analysis; PPI, protein-protein interaction; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CC, cellular components; BP, biological processes; MF, molecular functions;GBP2, guanylate binding protein 2.

Declarations

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

The data and materials used to support the findings of this study are available from the corresponding author upon request.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Xin Yang , Jia-Qi Hao and Yu Zhang contributed to the entire project, from the design proposal, to the collection and collation of data, to the writing of the paper. Jia-Ying Shi and and Xiao-Lin Zhu participated in the revision of the manuscript. You-Chao Xiao and Hao Bai helped retrieve and organize the data. Chun-Yan Hao and Hu-Bin Duan are responsible for supervising and providing financial support.

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Figures

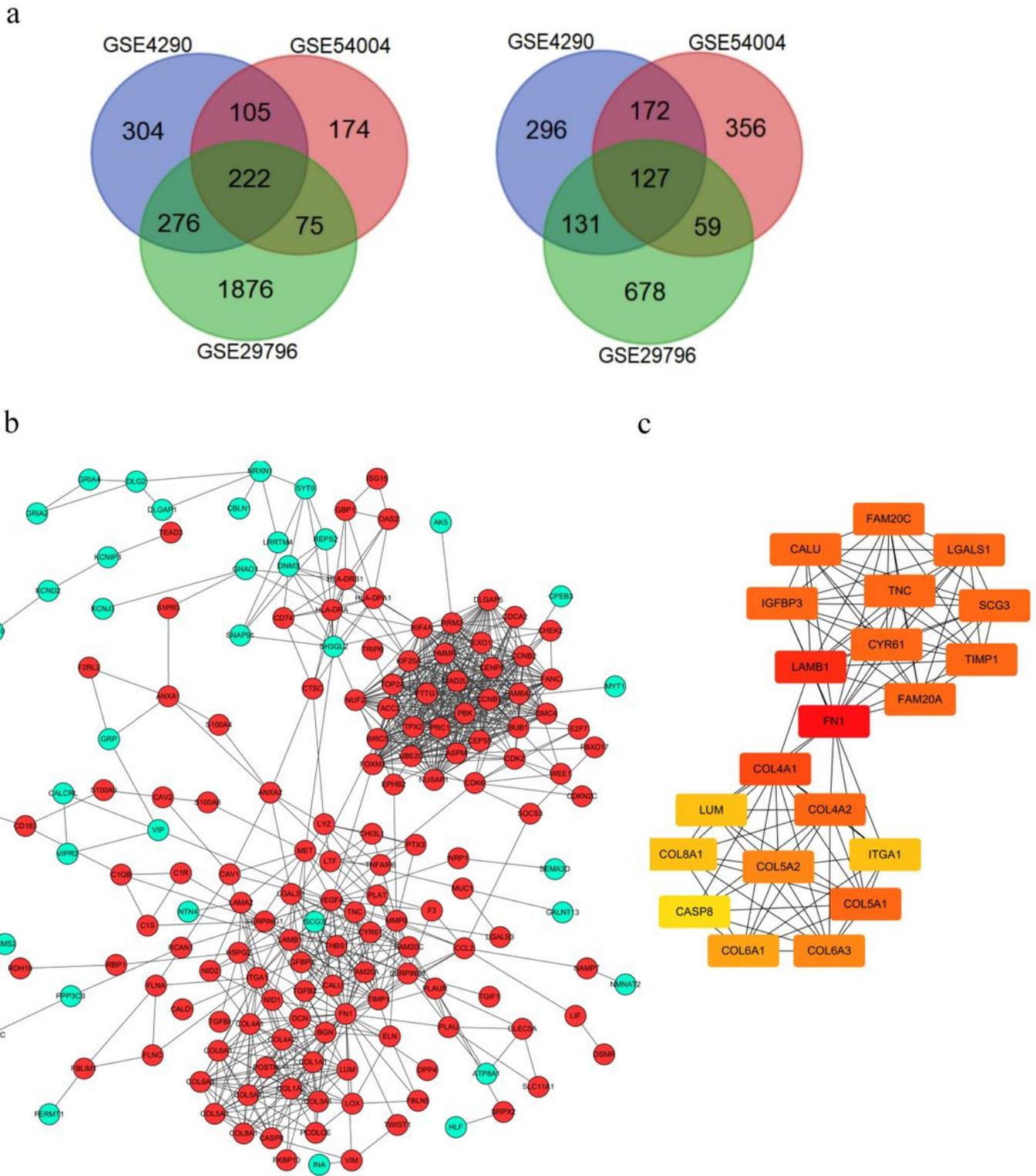


Figure 1

Extraction of differential genes. a 222 Up-regulated genes and 127 Down-regulated genes. b Protein Interaction Network of DEGs. Red dots indicate up-regulated genes, blue dots indicate down-regulated genes. c MCODE analysis of the protein diagram. A Darker color indicates high Degree score.

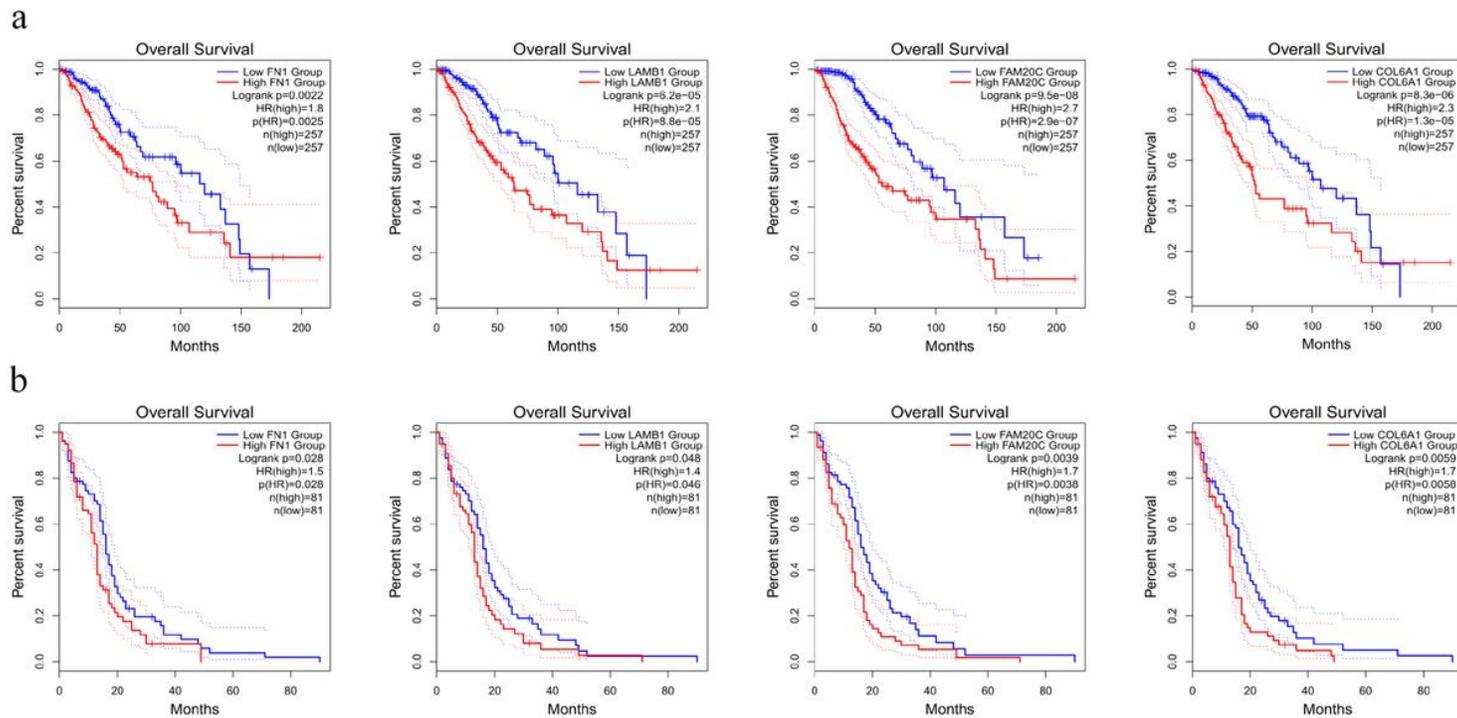


Figure 2

Overall survival curve of key genes in LGG and GBM patients mapped using GEPIA2. a LGG. b GBM.

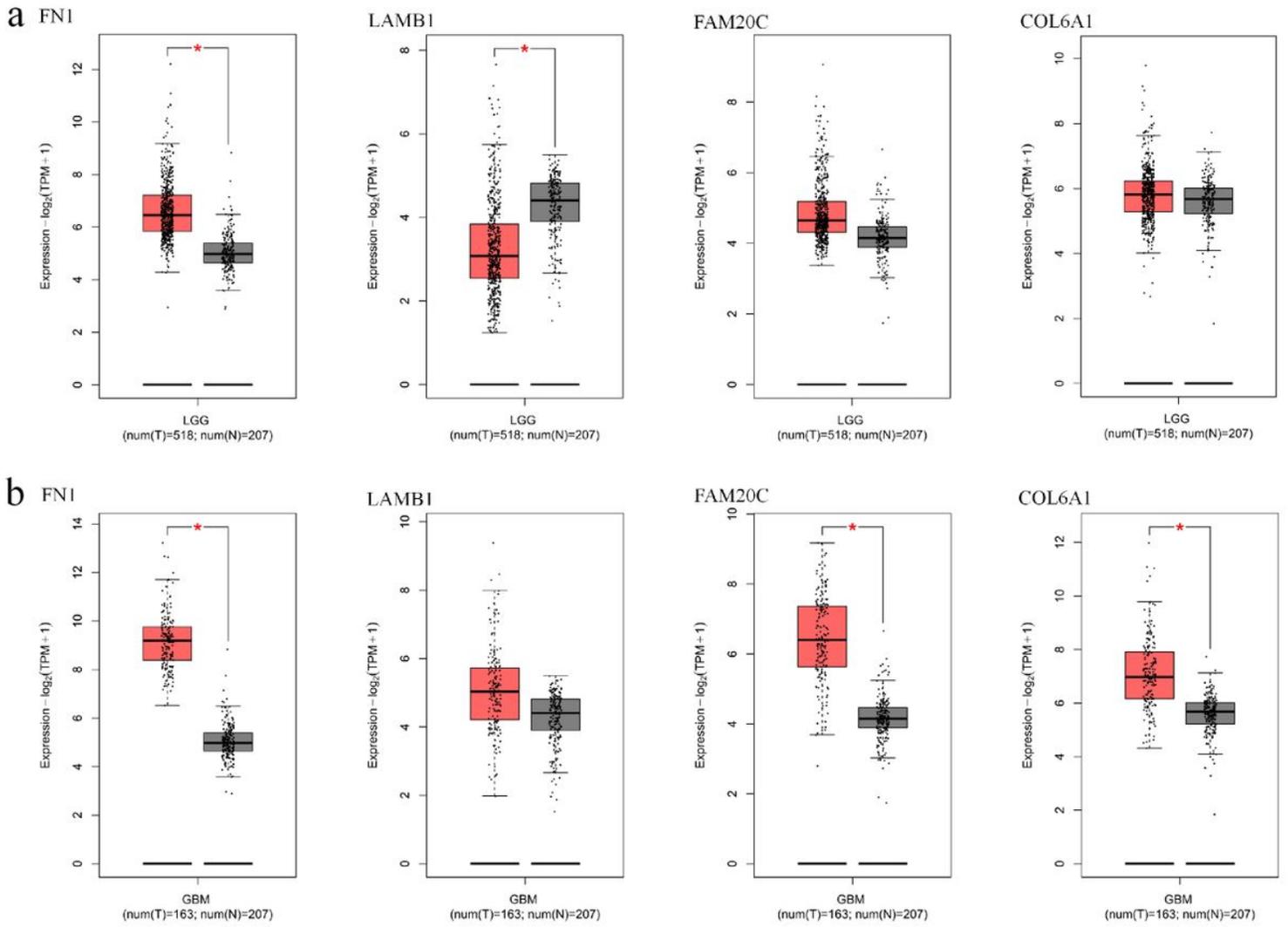


Figure 3

Gene expression of glioma specimens compared with normal specimens. a LGG. b GBM.

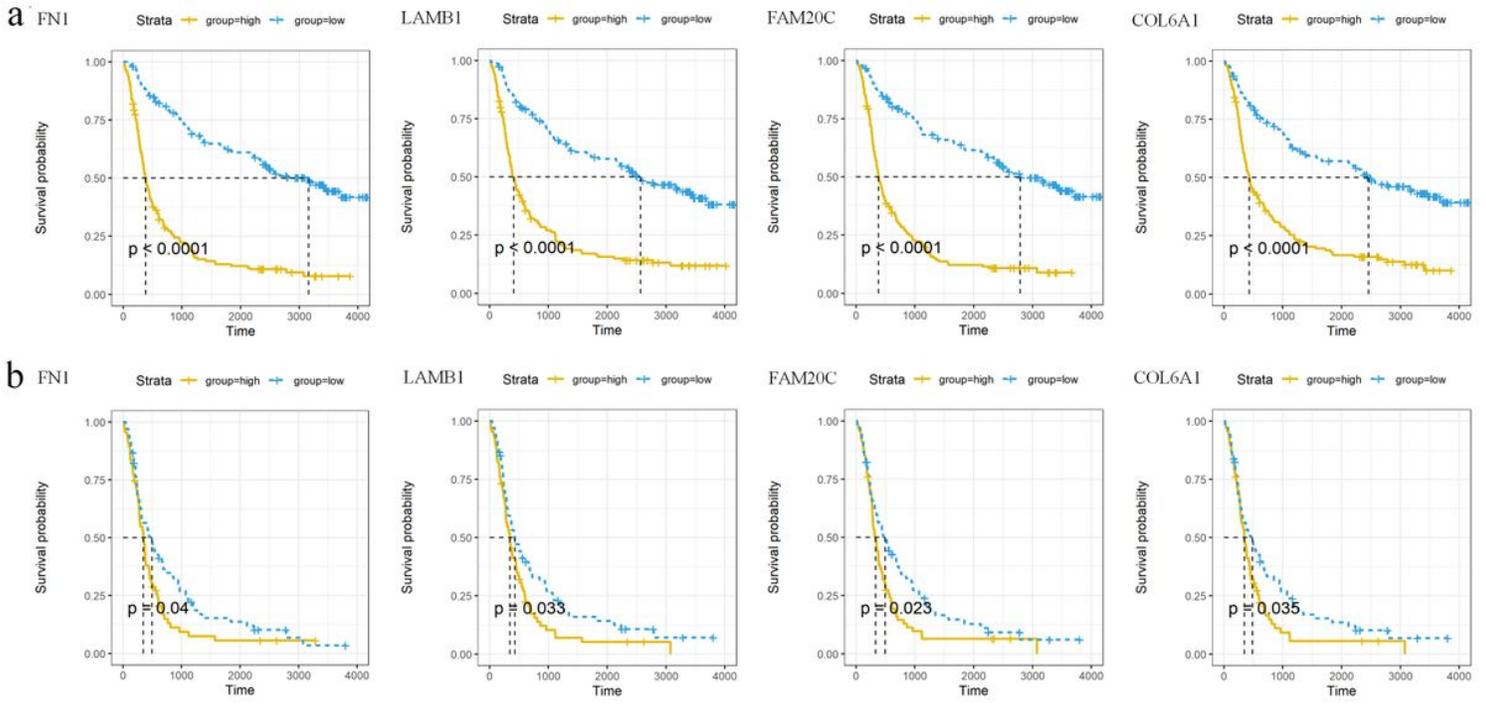


Figure 4

Validation of survival correlation of four genes in glioma. a All grade glioma. b \boxtimes grade glioma.

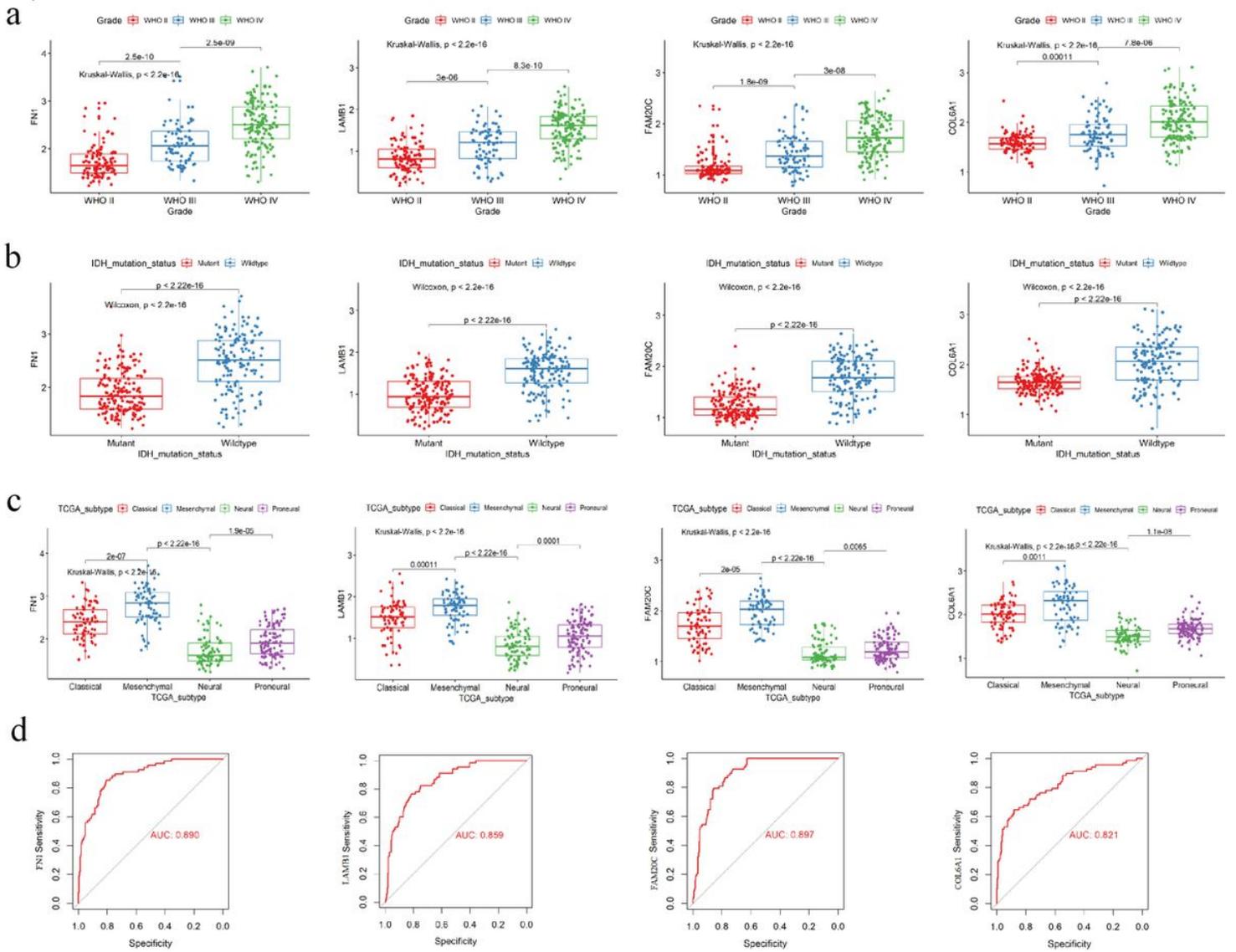


Figure 5

Expression levels of four genes increased with the glioma grade. a Gene expression in different grade glioma. b Gene expression in IDH mutant and IDH-wild glioma. c Gene expression in the four subtypes of glioma. d Four genes could serve as a biomarker to predict the mesenchymal subtype in CGGA databases.

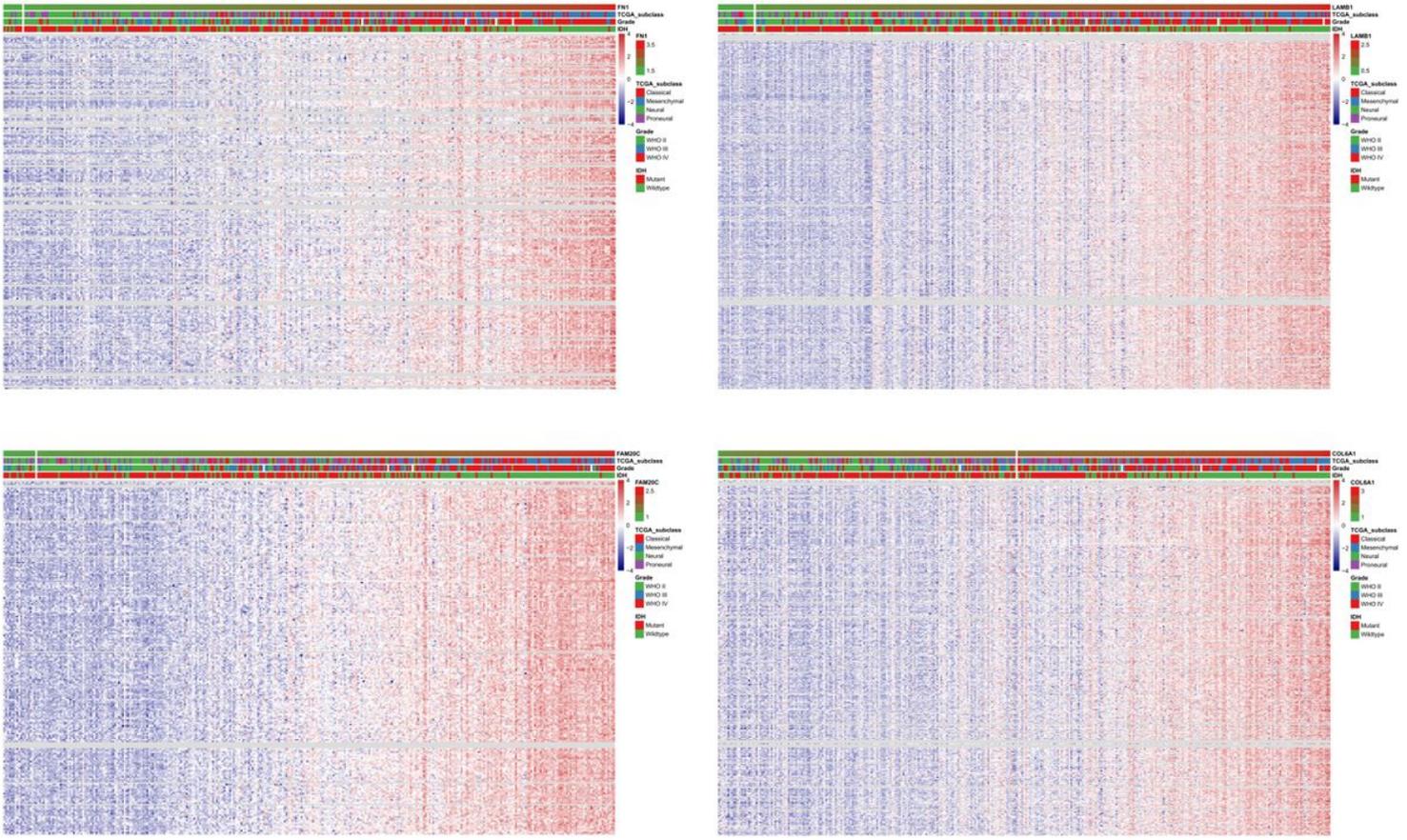


Figure 6

Using CGGA database to analyze the key genes, IDH mutation, tumor grade, and glioma subtypes. The pheatmap showed that the color depth distribution is obviously different under different factors.

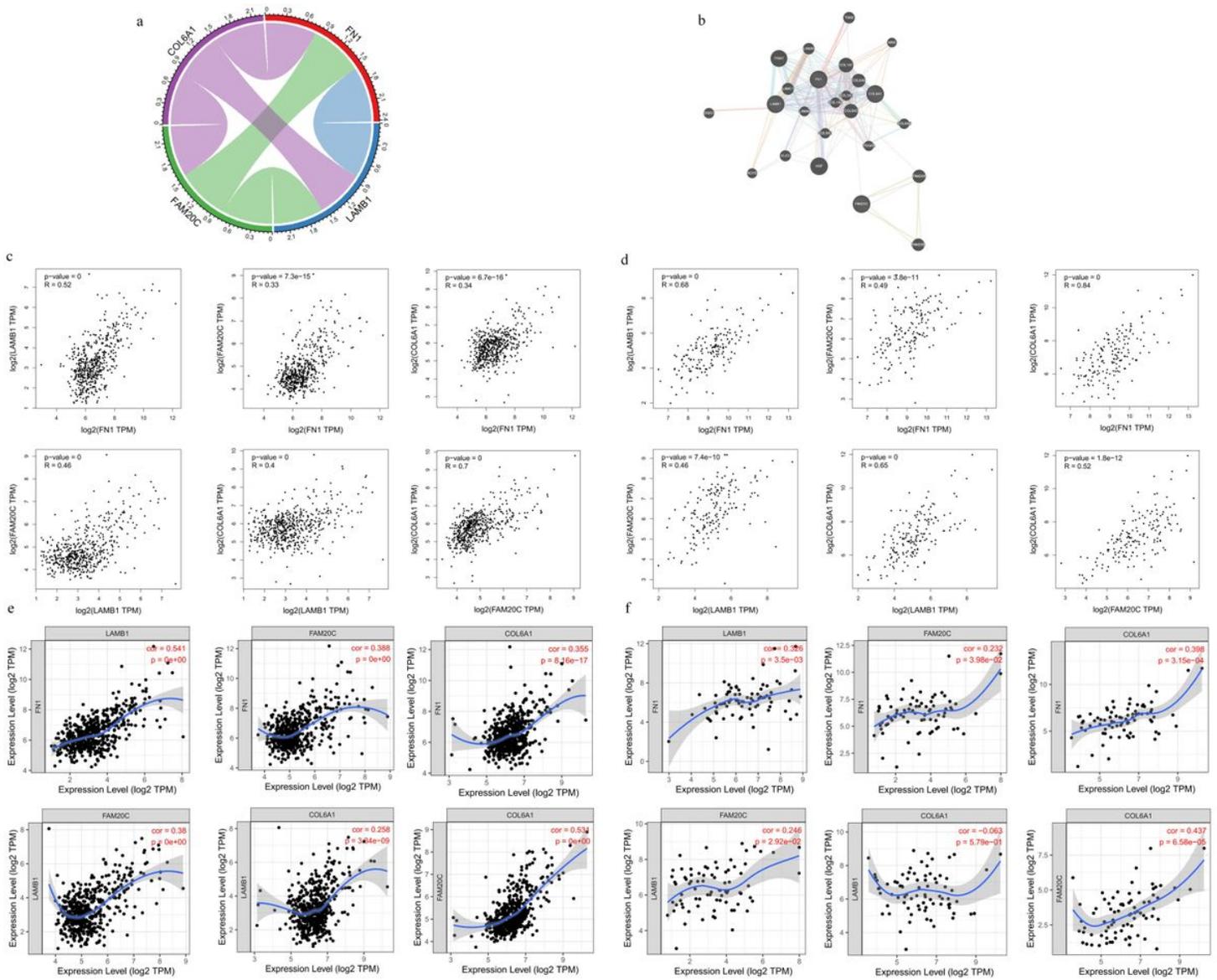


Figure 7

The co-expression state of four genes. **a** Co-expression between different genes in the CGGA database. **b** Using Genemania to showed the protein network diagram. **c,d** The co-expression diagram of each gene obtained from GEPIA. **e,f** The co-expression diagram of different genes obtained from TIMER.

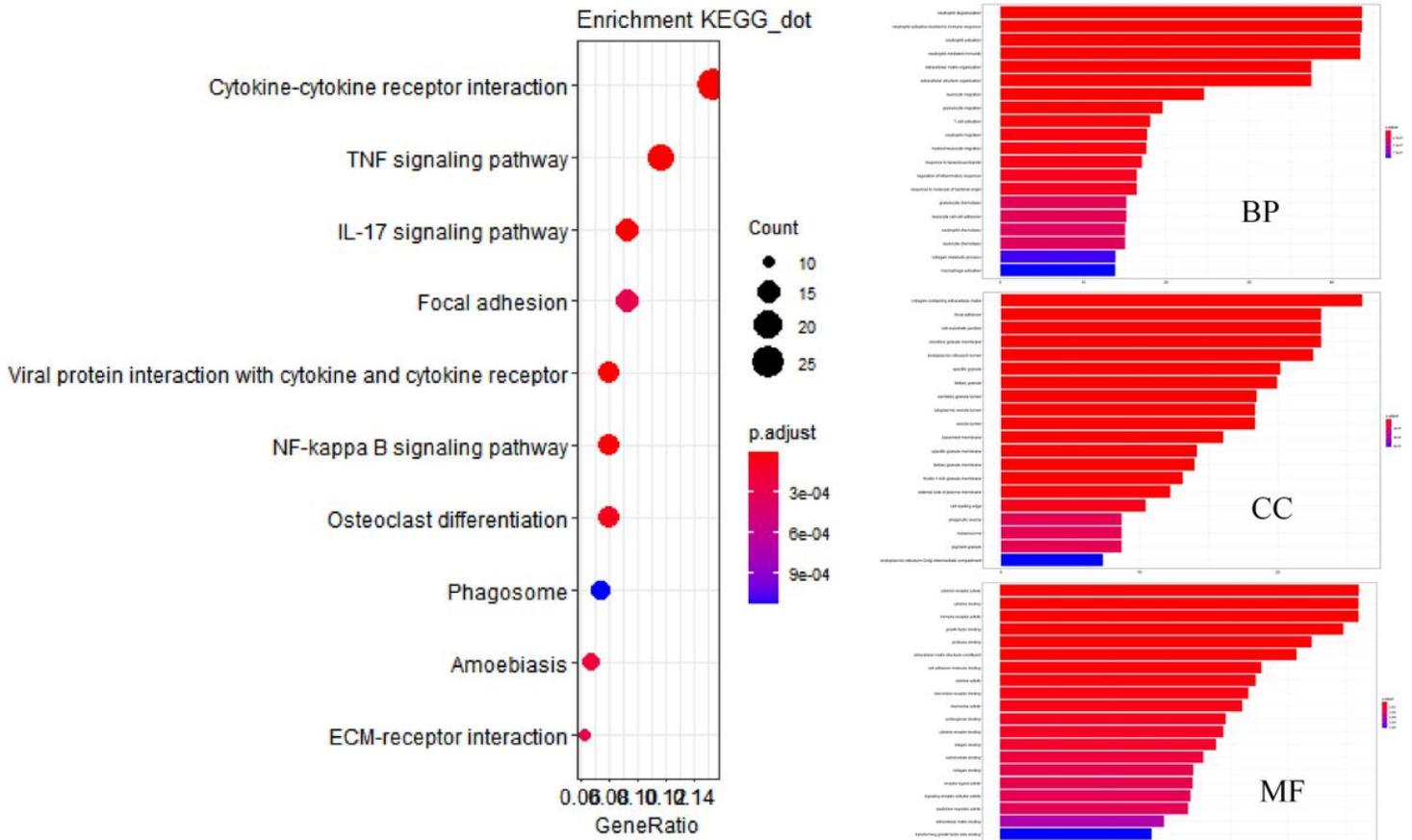


Figure 8

GO and KEGG. FN1, LAMB1, FAM20C and COL6A1 are mainly involved in neutrophil activation, collagen-containing extracellular matrix, cell adhesion molecule binding, Cytokine-cytokine receptor interaction signaling pathway and etc.

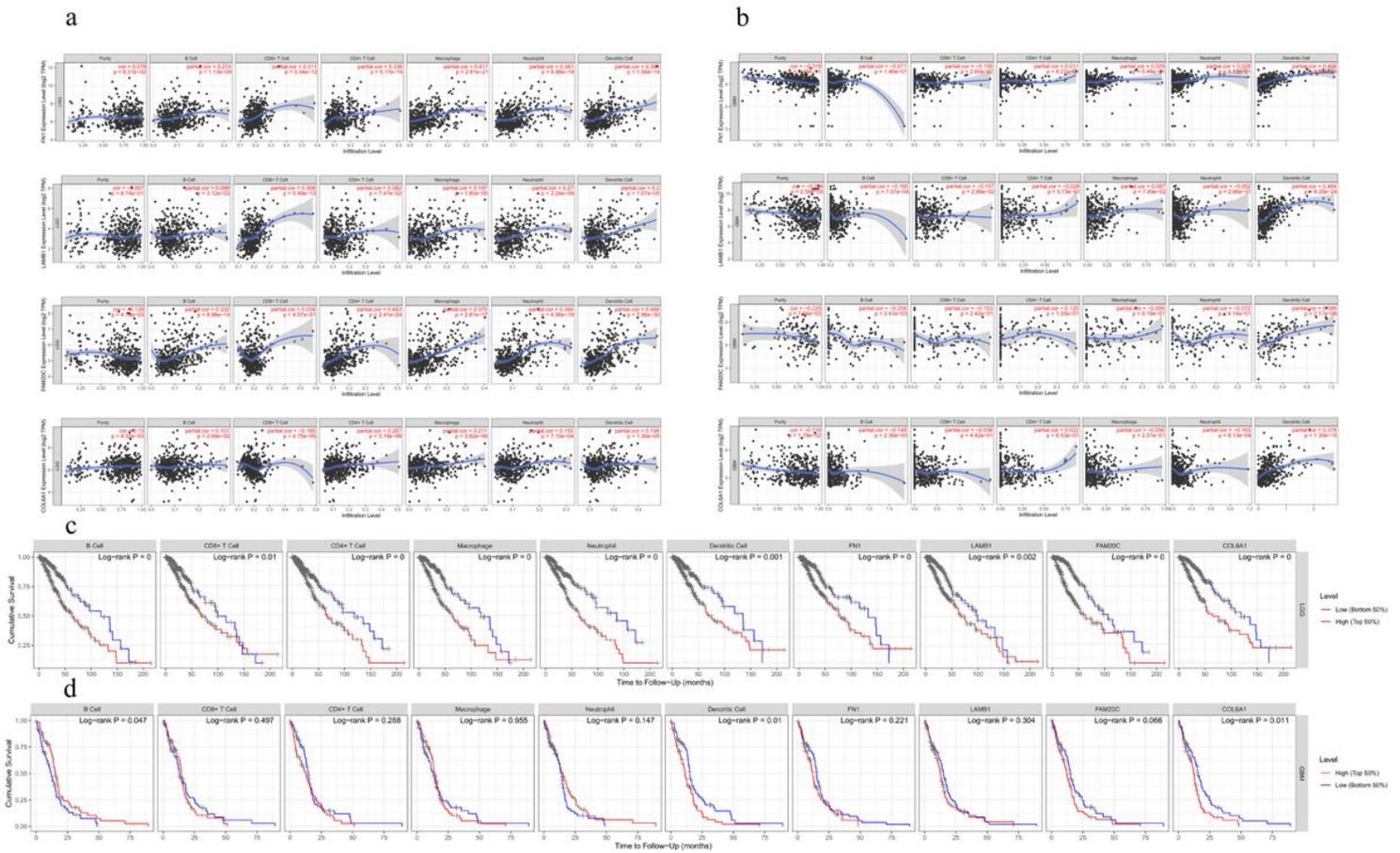


Figure 9

Immune infiltration in LGG and GBM. a Using TIMER to show the immune infiltrate in LGG. b Using TIMER to show the immune infiltrate in GBM. c Survival curve of hazard factors in LGG. d Survival curve of hazard factors in GBM.