PDIA4 correlates with poor prognosis and is a potential biomarker in glioma

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Abstract

Abstract Background: Gliomas, characterized by aggressiveness and invasiveness, remain incurable after conventional therapies. The molecular mechanisms driving the progression and maintenance of glioma are still poorly understood. Methods: PDIA4 expression was analyzed via Gene Expression Profiling Interactive Analysis (GEPIA) which data were from TCGA and GTEx databases. We estimated the prognostic value of PDIA4 using Kaplan–Meier survival analysis and the Cox proportional hazard model. The functional enrichment analysis was done by using cluster Profiler package in R language, including gene ontology (GO) analysis comprised of cellular component (CC), molecular function (MF), and biological process (BP), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. In addition, correlation between PDIA4 and immunity were analyzed by Protein-protein interaction (PPI) analysis, RNA extraction and Real-time RT-PCR. Results: In this study, we identified PDIA4 was highly expressed in gliomas and closely correlated with poor prognosis. The association with IDH1 and different patterns of gliomas also indicates the potential biological processes that PDIA4 involves in the development of tumor. Mechanistically, PDIA4 interacts with multiple immunological components to promote an immunosuppressive tumor microenvironment (TME). Conclusions: Our results confirm PDIA4 is an efficient biomarker of gliomas, with implications for prognosis and therapeutic strategies. Keywords: PDIA4, glioma, prognosis, biomarker, immune cells

Background

Gliomas are one of the most common malignant tumors in the central nervous system (CNS) and account for nearly 75% primary tumors in adults[1]. According to the histopathological features and prognostic factors, World Health Organization (WHO) classified gliomas into four grades (I-IV), from which the glioblastomas (GBMs) are categorized as the most malignant subtype (grade IV)[2]. The tradition multimodal therapeutic strategies against gliomas, which include advanced neurosurgery, radiation and chemotherapy, cannot dramatically improve the prognosis in glioma patients. Patients with GBM still have dismal prognosis, with median overall survival time less than 17 months[3]. The tolerance against multiple treatments and invariably relapse of gliomas are extensively studied as consequences of molecular or chromosomal subtypes, oncogenic activations and distinct metabolic immunosuppressive tumor microenvironment (TME)[3-6]. Pursuing better understanding of molecular landscape in gliomas, novel markers are successively detected with important clinical significance. Various discoveries, such as promoter mutations in TERT, mutations in IDH1/IDH2, co-deletion of chromosome arms 1p/19q and H2K27M-mutant are clearly associated with improved homogeneity in clinical outcomes and are referred as critical predictors in clinical practice[3,7-9]. Further strengthening the knowledge of such molecular alterations will definitely benefit our perception of gliomas from different perspectives. In this regard, investigate novel molecular biomarkers or driver genes will facilitate the establishment of comprehensive understanding about tumor promotion and the development of better therapeutic strategies to cure this disease.

The protein disulfide isomerases (PDIs) were originally discovered to enrich in endoplasmic reticulum (ER)

and participate into the procedures of protein folding[10]. Encoded by P4HB gene, PDI is a 57-kDa redox-dependent protein with multi-domain structure[11]. Performing as critical ER enzymes, PDIs majorly involve in the oxidoreductase and chaperone activities which mediate the redox state and maintain the proper folding and function of proteins[11,12]. The biological functions of PDIs are identified as reductase, oxidase and chaperone in ER which have been associated with abundant physiopathologic mechanisms, such as infection, coagulation, cellular viability, neurodegeneration and immunization[10,13-16]. PDIA4, one of the largest PDI members, comprises 645 amino acids and three classical CGHC active motifs. Similar to other PDI members, PDIA4 initiates coagulation and enhances formation of thrombus via series cascades reaction[17]. Besides the classic biological functions of PDIA4, emerging evidence indicate the potential association between PDIA4 and the development of tumor[17]. The upregulated expression of PDIA4 was detected in a variety of tumor cell lines as well as human lung adenocarcinoma tissue, the expression of PDIA4 mediates the inhibition of mitochondrial apoptosis-induced tumor death[18]. Further study revealed that PDIA4 promotes tumor progression through the reduction of caspases 3/7[12]. The ectopic expression and function of PDIA4 had also been reported in ovarian cancer. In ovarian carcinoma, PDIA4 was found to take part in the drug-resistance phenotype and can serve as a critical prognostic marker[19,20]. Moreover, in pancreatic carcinoma, hepatocellular carcinoma and esophageal squamous cell carcinoma, the increased expression of PDIA4 was respectively observed and associated with tumor development[21-25]. In our previous studies, we have already described PDIA4 as one of the prognostic markers in lower-grade gliomas and the potential association between PDIA4 and immunosuppressive TME[26]. From this perspective, we conduct further experiments to study the molecular mechanisms and behaviors of PDIA4 in gliomas.

Methods

2.1 Data sets

The Patient clinical annotation and gene expression data used in this study were obtained from publicly available databases. The TCGA lower grade glioma and glioblastoma (GBMLGG) dataset, which included genomic data and phenotypic data, was obtained from the University of California, Santa Cruz, Xena browser (https://xenabrowser.net/). Another cohort of glioma patients (LGG and GBM) was obtained from Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn/) and the mRNA sequencing data (RSEM) and clinical data were downloaded.

2.2 Differential expression analysis

Gene Expression Profiling Interactive Analysis (GEPIA) is an interactive web platform for gene expression analysis, which includes 9,736 tumors and 8,587 normal samples from TCGA and GTEx databases and its gene expression data have been re-computed from raw RNA-Seq data based on the UCSC Xena project and a uniform pipeline for solving the imbalance between tumor and normal data[27]. The differential expression analysis of PDIA4 between gliomas and normal brain tissues was performed using GEPIA.

2.3 Survival analysis

Kaplan–Meier survival analysis and the Cox proportional hazard model were used to estimate the prognostic value of PDIA4 based on TCGA and CGGA datasets using R language packages (survival and survminer).

2.4 Gene ontology (GO) enrichment analysis

The functional enrichment analysis, including gene ontology (GO) analysis comprised of cellular component (CC), molecular function (MF), and biological process (BP), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, were performed via the cluster Profiler package in R language[28]. Enriched ontological terms with adjusted P value < 0.05 were regarded as statistical significance.

2.5 Analysis of stromal and immune infiltration

Analysis of stromal and immune infiltration was performed as described in our previous article[29]. The scores, calculated by the ESTIMATE algorithm[30], were downloaded from https://bioinformatics.mdanderson.org/estimate/.

The pre-calculated TCGA data based on xCell[31] was downloaded from http://xcell.ucsf.edu/. Then the correlation between PDIA4 expression and ESTIMATE scores and 64 cell types from the TCGA glioma dataset were analyzed using R language.

2.6 Protein-protein interaction (PPI) analysis

The Search Tool for the Retrieval of Interacting Genes[32], an online database, was used to identify proteins that can interact with PDIA4 and construct PPI networks.

2.7 Cell lines and culture

The human glioma cell lines (U87, U251, and T98G) and the normal glial cell line HEB were cultured in DMEM with 10% FBS and antibiotics (100 μg/ml penicillin and 100 μg/ml streptomycin), and maintained in standard culture condition.

2.8 RNA extraction and Real-time RT-PCR

Total RNA was extracted from cell lines or human tissues by Trizol reagent (Invitrogen) according to the manufacturer's protocol. Then, total RNA was quantified and 1 μg of RNA was reverse-transcribed with the Reverse Transcription Kit (Thermo Fisher Scientific). Q-PCR was performed using SYBR Premix Ex Taq II (Takara Bio). β-Actin mRNA was used to normalize the expression of genes. The primers used were showed as follows: PDIA4: F: 5'- GGCAGGCTGTAGACTACGAG-3'and R: 5'- TTGGTCAACACAAGCGTGACT-3' GAPDH: F: 5'-GGGAGCCAAAAGGGTCAT-3' and R: 5'-GTCCTTCCACGATACCAA-3'.

2.9 Statistical analysis

Statistical computations and the creation of figures were performed with several packages (ggplot2, survival, survminer, corrplot) in the statistical software environment R, version 3.5.3 (http://www.r-project.org).

Results

Association of PDIA4 with clinicopathological characters in gliomas

According to the hypothesis that PDIA4 plays a critical role in glioma aggressiveness, we first measured the expression of PDIA4 in glioma tissues compared with normal brain tissues. The remarkably increased expression of PDIA4 was observed in both GBM samples and Low-Grade glioma (LGG) samples (Figure 1A, p¡0.001). To further validate this result, we performed Q-PCR in glioma cell lines. The results documented that the mRNA expression of PDIA4 was elevated in glioma cell lines when compared with normal glial cell line (Figure 1B). Moreover, PDIA4 expression was positively correlated with glioma histological grade in both TCGA and CGGA cohort (Figure 1C). It's widely recognized mutations in isocitrate dehydrogenase genes (IDH1 and IDH2) have strong connections with tumor behaviors in gliomas. Patients with IDH-mutant (IDH-Mut) histology exhibited better prognosis than IDH-wildtype (IDH-Wt). Intriguingly, we also observed the elevated expression level of PDIA4 in IDH-Wt subtype of glioma when compared with IDH-Mut tumors (Figure 1D). Additionally, we evaluated the expression of PDIA4 in different patterns of glioma. The results showed higher expression of PDIA4 in mesenchymal and classical subtypes rather than neural and proneural subtypes (Figure 1E).

The expression of PDIA4 is correlated with prognosis in glioma patients

Based on the findings that PDIA4 was aberrantly expressed in gliomas and showed strong relationships with histological grade and specific molecular subtype, we further studied the prognostic value of PDIA4 by Kaplan-Meier survival analysis with data obtained from TCGA and CGGA datasets. We found a marked correlation in the expression of PDIA4 and the poor prognosis of glioma patients (Figure 2A, B, p¡0.001).Moreover, we validated this correlation in both GBM cohort and LGG cohort. Results indicated the high expression of PDIA4 was consistently correlated with poor patient outcomes in both the GBM group (Figure 2C, D) and LGG group (Figure 2E, F). To study whether PDIA4 is an independent prognostic factor in glioma, we also performed the Cox regression analysis with data obtained from TCGA and CGGA. In multivariate analysis, after adjusting many clinical factors, such as patient age, patient gender, WHO grade

and IDH status, the results suggested the expression of PDIA4 was a strong predictor in patients with glioma (Table 1, Table 2).

Functional enrichment of PDIA4 in glioma

To understand the mechanism of PDIA4 promoting tumor growth and illustrate the key signaling regulated by PDIA4, we performed GO functional enrichment analysis.

Data from both TCGA and CGGA were analyzed by Pearson correlation analysis and genes with |R|¿0.6 were collected for functional enrichment. As a consequence, 408 terms of biological process (BP), 110 terms of cellular component (CC), 40 terms of molecular function (MF) were identified from TCGA database, and 140 terms of BP, 56 terms of CC, 15 terms of MF were identified from CGGA database respectively (Supplementary Table 1, 2). The top 10 terms of BP mainly enriched functions of neutrophil mediated immune function (Figure 3A, B). The MF enrichment indicated functions predominantly involved in transferase activities, cell adhesion and molecule binding (Figure 3C, D). Meanwhile, genes from CC terms showed significant association with focal adhesion, cell-substrate junctions and endoplasmic reticulum lumen (Figure 3E, F). Besides, we also conducted KEGG pathway analysis with selected genes. The results revealed strong correlation between PDIA4 related genes and important biological signalings, such as protein processing in endoplasmic reticulum, human immunodeficiency virus 1 infection and apoptosis (Figure 3G, H).

The correlation between PDIA4 and immunity.

Considering the results from GO and KEGG pathway analysis which elucidated strong connections between PDIA4 and immunological functions, we next performed examinations to confirm this phenomenon. Firstly, we examined the association between expression of PDIA4 and immune scores. The results showed that the PDIA4 expression had relatively lower correlation with both stromal score and immune score in GBM patients (Figure 4A). However, in LGG samples, we could find strong correlation between PDIA4 and stromal or immune scores (Figure 4B). Moreover, we studied the correlation between PDIA4 and 64 non-cancerous cell types to determine the critical cellular components involved in PDIA4 associated immunological process. The results revealed there were 46 cell types were correlated with PDIA4, among which 33 types were positively related whereas 13 types were negatively related (Figure 4C, Table 3). Notably, the cellular components which exhibited dramatic correlation with PDIA4, such as astrocyte, M1 macrophages, $CD4^+$ memory T cells, $CD8^+$ T cells, Tregs and eosinophils have already been demonstrated to play critical roles in the glioma TME. We further validate the correlation between PDIA4 and immune properties via classic immunological markers. The results suggested that PDIA4 was closely related to several immunosuppressive factors, especially the dendritic cell, M2 macrophage, monocyte and T cell exhaustion markers (Table 4). Besides, we subjected PDIA4 to Protein Interaction Analysis (PPI) to study the regulatory network of this protein. Based on the results, we found that PDIA4 could interact with several heat shock proteins (Figure 4D). And the functional study revealed these genes were closely related to stress-reduced responses and endoplasmic reticulum which was consistent with the data described above. Meanwhile, the majority of PDIA4-related genes, such as PDIA6, ERO1LB, ERO1L, HSPA5, HSP90B1 and HYOU1, were found to be tightly involved in the tumor-promoting phenotype.

Discussion

Despite the current multi-therapeutic strategies against glioma, including modern neurosurgery, radiotherapy, chemotherapy and immunotherapy, the prognosis of glioma patients remains poor due to the aggressive features of this type of cancer. Novel efficient management for glioma requires comprehensive understanding of the biological nature of this disease. The illustration of potential critical factors which overexpress and play essential role in glioma progression is of great importance to increase our knowledge of this malignant disease. Our present study first identified PDIA4 is a novel molecular marker which shows close relationship with the clinicopathological characters and immunological surveillance of glioma, and provides alternative strategies for the subsequent treatment of this disease.

PDIA4 was originally described to present in various biological processes, including coagulation[33], thrombo-

sis formation[34,35]and injury reaction[36]. Recently, mounting evidence reported the aberrant expression and the potential mechanisms of PDIA4 participates in the development of multiple types of cancer[12,17,18,21]. Moreover, our recent study documented that PDIA4 involved in the prognostic model in LGG, subsequently participating in the immunosuppressive TME[26]. Based on these findings, the present study identified PDIA4 was not only overexpressed in glioma tissues, but also significantly consistent with WHO grade. Mechanistically, PDIA4 was significantly associated with the IDH status and different subtypes of glioma. Also, our study revealed increased mRNA expression of PDIA4 in glioma cell lines. Furthermore, we examined the clinical importance of PDIA4 and found PDIA4 was an independent prognostic marker whose expression was negatively correlated with outcomes of patients with glioma. To elucidate the critical functions of PDIA4 in glioma, we conducted GO function and KEGG pathway analysis in both TCGA and CGGA datasets. As a result, the PDIA4-related biological functions were mainly enriched in transferase activities, endoplasmic reticulum responses and immunities.

The orchestrated immunological interactions within glioma TME have received increased focus and harnessing the immune system is becoming a hotspot in the field of oncology. Various components of glioma TME, such as immune cells, cytokines and markers are coordinately interact with each other to establish the immunosuppressive phenotype and promote the development of glioma[5]. From this perspective, advanced clinical practices by targeting specific immunotherapies have already showed profound outcomes compared to conventional therapy against glioma. As PDIA4 was previously revealed to participate in the immunological TME in LGG, we further detected its correlation with multiple immune factors. Consistent with our further study, we found PDIA4 was tightly related to both the immune and stromal scores in LGG. The relationship between PDIA4 and immune scores was relatively lower in GBM, which suggested the potential heterogeneities between different grades of glioma. After checking the association between PDIA4 and 64 non-cancerous cells, we found significant linkage between 46 types of cell and PDIA4. Furthermore, we studied the connections between PDIA4 and classic genes and markers of immune cells. Interestingly, the dada showed close relationship with several infiltrating immune cells, such as monocytes, tumor-associated macrophage and neutrophils, which are widely considered as immunosuppressive components in glioma TME. Association had also been detected among markers of dendritic cells and PDIA4, which suggested us the potential functions of PDIA4 in the process of antigen presentation and immune surveillance. Consistently, our PPI analysis of PDIA4 indicated that the major proteins related to PDIA4 are members of heat shock proteins or endoplasmic reticulum proteins which had also been reported as tumor promoting in various cancers[21,37-40].

Conclusions

Based on our findings, our study is the first time to describe the novel function of PDIA4 and we propose a new linkage between PDIA4 and various immune components in glioma. PDIA4 is highly expressed in glioma and significantly related with the clinical outcomes. The tumor promoting character of PDIA4 is potentially mediated by the immune system because of the certain connections with multiple immune factors in the glioma TME. The detailed molecular mechanism of PDIA4 and the development of glioma need to be further illustrated. To this end, our study provides novel possibilities for future discoveries to find new therapeutic approaches by targeting PDIA4 for immunotherapy in glioma.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions:

H.L. performed the experiments and wrote the manuscript. K.X. performed the Q-PCR experiments. Q.L. revised the manuscript. Q.M. and J.S. designed the experiments, interpreted the data, wrote the manuscript, and provided supervision. All authors read and approved the final manuscript.

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Figure 1. PDIA4 is highly expressed in gliomas and significantly associated with tumor aggressiveness.

1. Differential expression of LCTL in brain lower grade glioma (LGG) and glioblastoma (GBM) compared to levels in normal brain tissues.
2. The relative mRNA expression level of PDIA4 in the normal glial cell line HEB and glioma cell lines.
3. The PDIA4 expression in glioma of WHO grade II-IV based on both TCGA and CGGA datasets.
4. The expression of PDIA4 in IDH subtypes of gliomas based on both the TCGA and CGGA datasets.
5. PDIA4 expression pattern in different molecular subtypes of glioma (classical, mesenchymal, neural, proneural) in the TCGA dataset.

Figure 2. PDIA4 is a prognostic factor for glioma patients.

(A-B) Kaplan–Meier survival analysis showing that high PDIA4 expression predicts poor prognosis for glioma patients based on both the TCGA and CGGA datasets.

(C-D) Kaplan–Meier survival analysis showing that high PDIA4 expression predicts poor prognosis for glioblastoma multiform (GBM) patients in both the TCGA and CGGA datasets.

(E-F) Kaplan–Meier survival analysis showing that high PDIA4 expression predicts poor prognosis for lower grade glioma (LGG) patients in both the TCGA and CGGA datasets.

Figure 3. Functional enrichment analysis of PDIA4 in TCGA and CGGA cohorts

(A-B) The top10 biological process terms of GO enrichment analysis based on TCGA and CGGA datasets respectively.

(C-D) The top10 molecular function terms of GO enrichment analysis based on TCGA and CGGA datasets respectively.

(E-F) The top10 cellular component terms of GO enrichment analysis based on TCGA and CGGA datasets respectively.

(G-H) KEGG pathway analysis based on TCGA and CGGA datasets and the top 10 terms were visualized respectively.

Figure 4. PDIA4 correlated with ESTIMATE algorithm/xcells scores in glioma and the PPI network

(A) PDIA4 expression was positively correlated with immune score and stromal score in glioblastoma multiform (GBM) patients.

(B) PDIA4 expression was positively correlated with immune score and stromal score in lower grade glioma (LGG) patients.

(C) PDIA4 expression was significantly correlated with 46 cell types, as calculated by xcells in glioma.

(D) Protein-protein interaction (PPI) network of PDIA4.

Table 1 Univariate and multivariate analysis based on the TCGA Dataset.

Variable	Univariate analysis	Univariate analysis	Multivariate analysis	Multivariate analysis
	HR (95% CI)	P	HR (95% CI)	P
PDIA4	1.618 (1.477-1.773)	<0.001	1.271 (1.149-1.406)	<0.001
Gender	1.012 (0.826-1.241)	>0.05	0.977 (0.793-1.205)	>0.05
Age	1.027 (1.018-1.035)	<0.001	1.011 (1.002-1.019)	<0.05
WHO grade	3.979 (3.227-4.906)	<0.001	2.142 (1.621-2.831)	<0.001
IDH status	3.238 (2.616-4.009)	<0.001	1.846 (1.422-2.397)	<0.001

Table 2 Univariate and multivariate analysis based on the CCGA Dataset.

Variable	Univariate analysis	Univariate analysis	Multivariate analysis	Multivariate analysis
	HR (95% CI)	P	HR (95% CI)	P
PDIA4	1.618 (1.477-1.773)	<0.001	1.271 (1.149-1.406)	<0.001
Gender	1.012 (0.826-1.241)	>0.05	0.977 (0.793-1.205)	>0.05
Age	1.027 (1.018-1.035)	<0.001	1.011 (1.002-1.019)	<0.05
WHO grade	3.979 (3.227-4.906)	<0.001	2.142 (1.621-2.831)	<0.001
IDH status	3.238 (2.616-4.009)	<0.001	1.846 (1.422-2.397)	<0.001

Table 3 Correlation ship between *PDIA4* and 64 types of non-cancerous cells

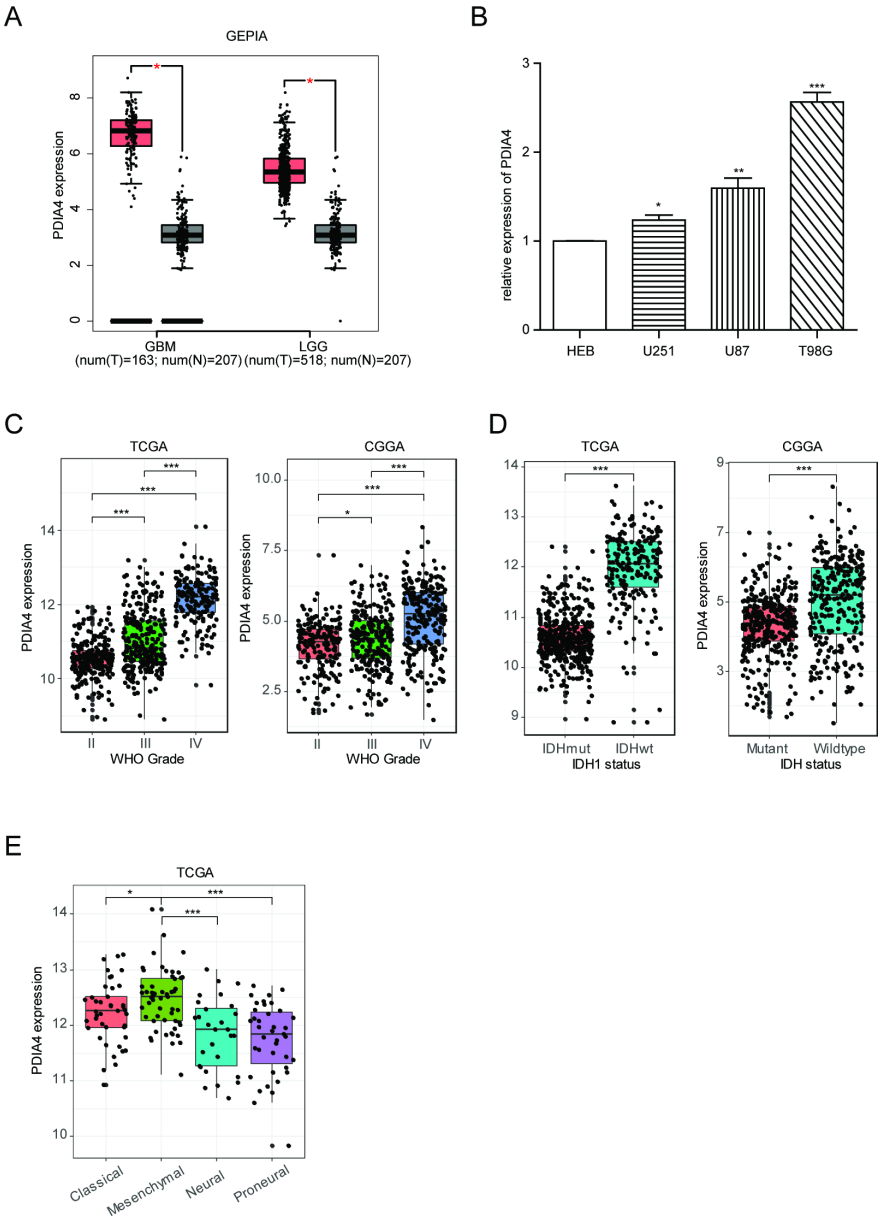
xcells	category	Pearson's r (95%CI)	adj.p
B cells	lymphoids	0.089(-0.042~0.217)	*
CD4+ memory T cells	lymphoids	0.541(0.442~0.627)	***
CD4+ naive T cells	lymphoids	0.175(0.045~0.298)	***
CD4+ T cells	lymphoids	-0.021(-0.151~0.109)	.
CD4+ Tcm	lymphoids	-0.091(-0.219~0.04)	*
CD4+Tem	lymphoids	0.054(-0.076~0.183)	.
CD8+ naive T cells	lymphoids	0.127(-0.004~0.253)	**
CD8+ Tcm	lymphoids	-0.413(-0.515~-0.299)	***
CD8+ Tem	lymphoids	0.055(-0.075~0.184)	.
CD8+T cells	lymphoids	0.062(-0.069~0.19)	.
Class switched memory B cells	lymphoids	-0.295(-0.409~-0.171)	***
Memory B cells	lymphoids	0.003(-0.127~0.133)	.
naive B cells	lymphoids	-0.002(-0.132~0.129)	.
NK cells	lymphoids	-0.094(-0.222~0.036)	*
Natural killer T cells (NKT)	lymphoids	0.043(-0.088~0.172)	.
Plasma cells	lymphoids	-0.359(-0.467~-0.24)	***
pro B cells	lymphoids	0.006(-0.124~0.136)	.
Tgd cells	lymphoids	0.038(-0.093~0.168)	.
Th1 cells	lymphoids	0.394(0.278~0.499)	***
Th2 cells	lymphoids	0.198(0.07~0.32)	***
Tregs	lymphoids	-0.627(-0.7~-0.541)	***
Activated dendritic cells (aDC)	myeloids	0.468(0.36~0.564)	***
Basophils	myeloids	-0.325(-0.437~-0.204)	***
Conventional dendritic cells (cDC)	myeloids	0.02(-0.111~0.149)	.
Denritic cells (DC)	myeloids	0.234(0.107~0.354)	***
Eosinophils	myeloids	-0.491(-0.584~-0.385)	***
Immature DC (iDC)	myeloids	0.202(0.074~0.324)	***
Macrophages y	myeloids	0.551(0.454~0.636)	***
Macrophages M1	myeloids	0.593(0.501~0.671)	***
Macrophages M2	myeloids	0.441(0.329~0.54)	***
Mast cells	myeloids	0.061(-0.07~0.189)	.
Monocytes	myeloids	0.401(0.286~0.505)	***
Neutrophils	myeloids	0.286(0.162~0.401)	***
Plasmacytoid dendritic cells (pDC)	myeloids	-0.093(-0.22~0.038)	*
Astrocytes	others	0.767(0.707~0.815)	***
Epithelial cells	others	0.517(0.415~0.607)	***
Hepatocytes	others	0.331(0.21~0.442)	***

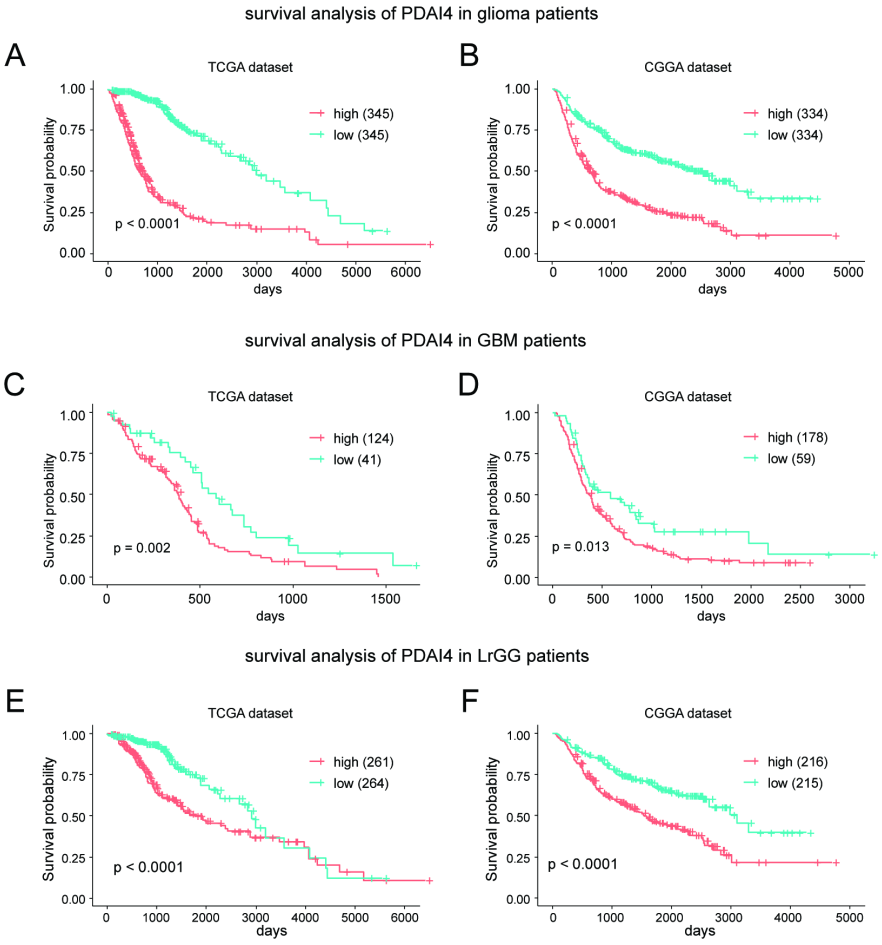
xcells	category	Pearson's r (95%CI)	adj.p
Keratinocytes	others	0.064(-0.067~0.193)	.
Melanocytes	others	0.002(-0.128~0.133)	.
Mesangial cells	others	0.505(0.401~0.596)	***
Myocytes	others	-0.163(-0.287~-0.033)	***
Neurons	others	-0.687(-0.75~-0.612)	***
Sebocytes	others	0.273(0.148~0.389)	***
Common lymphoid progenitors (CLP)	stem cells	0.565(0.47~0.648)	***
Common myeloid progenitors (CMP)	stem cells	0.007(-0.123~0.137)	.
Erythrocytes	stem cells	0.092(-0.038~0.22)	*
Granulocyte-macrophage progenitor(GMP)	stem cells	0.138(0.008~0.264)	***
Hematopoietic stem cells (HSC)	stem cells	0.283(0.158~0.398)	***
Megakaryocytes	stem cells	-0.01(-0.14~0.121)	.
Megakaryocyte-erythroid progenitors (MEP)	stem cells	0.273(0.148~0.39)	***
Multipotent rogenitors (MPP)	stem cells	-0.015(-0.145~0.116)	.
Platelets	stem cells	-0.399(-0.503~-0.283)	***
Adipocytes	stromal cells	-0.01(-0.141~0.12)	.
Chondrocytes	stromal cells	0.026(-0.104~0.156)	.
Endothelial cells	stromal cells	0.412(0.297~0.514)	***
Fibroblasts	stromal cells	0.376(0.259~0.483)	***
ly Endothelial cells	stromal cells	0.248(0.122~0.366)	***
Mesenchymal stem cells (MSC)	stromal cells	-0.196(-0.318~-0.067)	***
mv Endothelial cells	stromal cells	0.333(0.212~0.444)	***
Osteoblast	stromal cells	0.108(-0.022~0.235)	**
Pericytes	stromal cells	-0.171(-0.295~-0.042)	***
Preadipocytes	stromal cells	0.385(0.268~0.49)	***
Skeletal muscle	stromal cells	0.128(-0.002~0.254)	**
Smooth muscle	stromal cells	0.393(0.277~0.498)	***

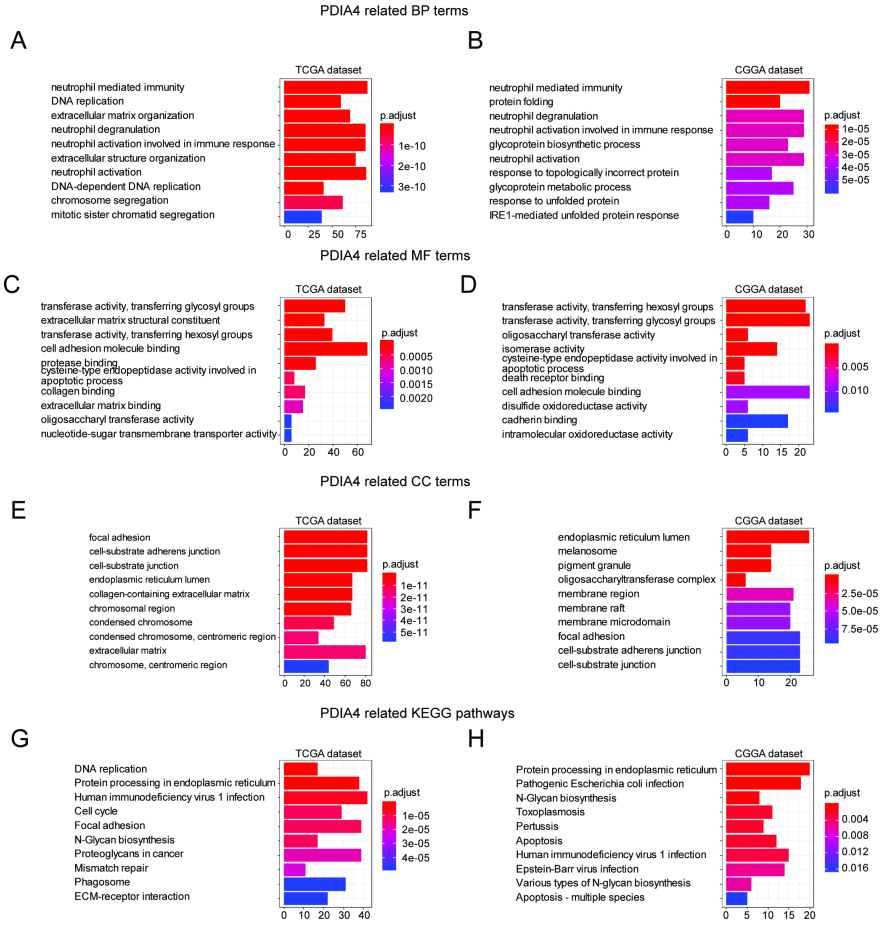
Table 4 Correlation analysis between LAYN and relate genes and markers of immune cells based on TCGA database

Description	Gene markers	Pearson's r(95%CI)	P
CD8+ T cell	CD8A	0.375 (-0.062-0.692)	**
	CD8B	0.322 (-0.123-0.659)	*
T cell (general)	CD3D	0.332 (-0.111-0.665)	*
	CD3E	0.433 (0.006-0.726)	**
	CD2	0.427 (-0.001-0.723)	**
B cell	CD19	0.201 (-0.248-0.579)	.
	CD79A	0.233 (-0.216-0.601)	.
Monocyte	CD86	0.616 (0.255-0.826)	***
	CD115 (CSF1R)	0.617 (0.257-0.826)	***
TAM	CCL2	0.569 (0.187-0.802)	***
	CD68	0.806 (0.577-0.917)	***
	IL10	0.355 (-0.086-0.68)	*
M1 Macrophage	INOS (NOS2)	0.386 (-0.05-0.698)	**
	IRF5	0.638 (0.289-0.837)	***
	COX2(PTGS2)	0.354 (-0.087-0.679)	*
M2 Macrophage	CD163	0.555 (0.167-0.794)	***
	VSIG4	0.651 (0.309-0.844)	***

Description	Gene markers	Pearson's r(95%CI)	P
	MS4A4A	0.66 (0.324-0.848)	***
Neutrophils	CD66b (CEACAM8)	0.121 (-0.323-0.522)	.
	CD11b (ITGAM)	0.553 (0.164-0.793)	***
	CCR7	0.34 (-0.102-0.67)	*
Natural killer cell	KIR2DL1	-0.039 (-0.459-0.395)	.
	KIR2DL3	-0.003 (-0.43-0.425)	.
	KIR2DL4	0.069 (-0.369-0.483)	.
	KIR3DL1	0.13 (-0.315-0.528)	.
	KIR3DL2	0.057 (-0.38-0.473)	.
	KIR3DL3	-0.374 (-0.691-0.064)	**
	KIR2DS4	0.051 (-0.385-0.468)	.
Dendritic cell	HLA-DPB1	0.644 (0.299-0.84)	***
	HLA-DQB1	0.549 (0.159-0.791)	***
	HLA-DRA	0.692 (0.376-0.864)	***
	HLA-DPA1	0.652 (0.311-0.844)	***
	BDCA-1(CD1C)	0.17 (-0.278-0.557)	.
	BDCA-4(NRP1)	0.876 (0.716-0.948)	***
	CD11c (ITGAX)	0.529 (0.131-0.78)	***
Th1	T-bet (TBX21)	0.372 (-0.066-0.69)	**
	STAT4	0.081 (-0.36-0.491)	.
	STAT1	0.822 (0.608-0.925)	***
	IFN-g (IFNG)	0.128 (-0.317-0.526)	.
	TNF-a (TNF)	0.158 (-0.289-0.549)	.
Th2	GATA3	0.389 (-0.047-0.7)	**
	STAT6	0.808 (0.582-0.918)	***
	STAT5A	0.735 (0.449-0.885)	***
	IL13	0.076 (-0.364-0.488)	.
Tfh	BCL6	0.703 (0.394-0.869)	***
	IL21	-0.154 (-0.546-0.293)	.
Th17	STAT3	0.896 (0.759-0.957)	***
	IL17A	-0.083 (-0.493-0.358)	.
Treg	FOXP3	0.316 (-0.129-0.655)	*
	CCR8	0.098 (-0.344-0.505)	.
	STAT5B	0.756 (0.485-0.895)	***
	TGFb (TGFB1)	0.8 (0.567-0.915)	***
T cell exhaustion	PD-1 (PDCD1)	0.37 (-0.068-0.689)	**
	CTLA4	0.257 (-0.192-0.617)	.
	LAG3	0.484 (0.071-0.756)	***
	TIM-3 (HAVCR2)	0.648 (0.305-0.842)	***
	GZMB	0.243 (-0.206-0.607)	.







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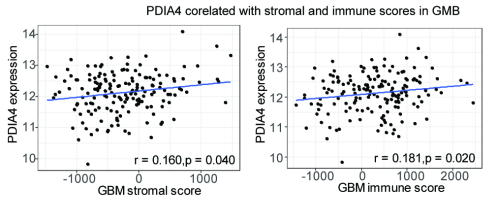

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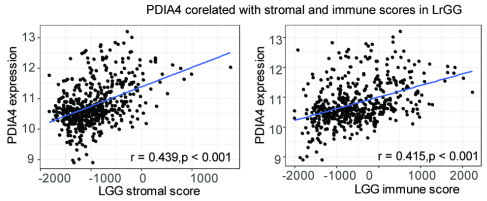

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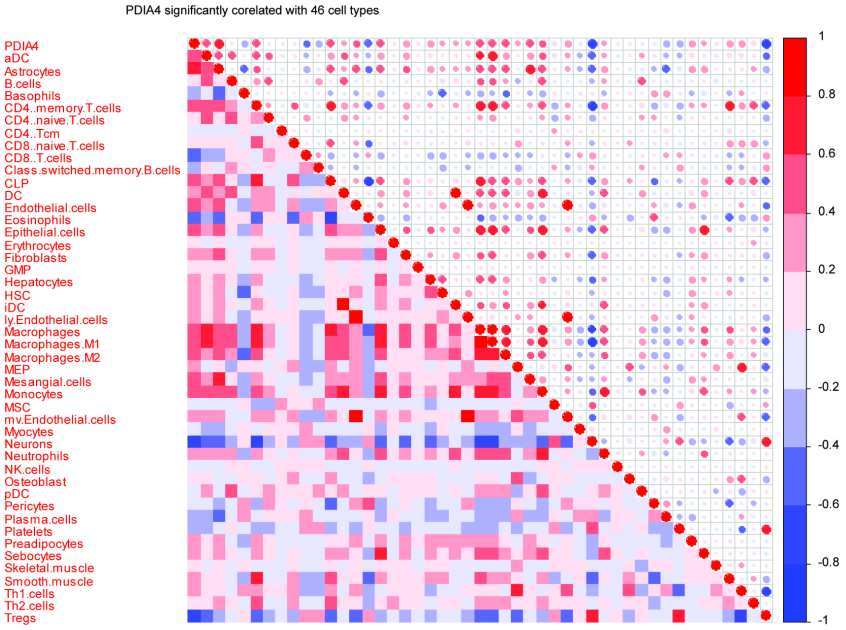

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