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Research article

Predictive value of procollagen c-protease enhancer protein on the prognosis of glioma patients

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ABSTRACT

Procollagen c-protease enhancer protein (PCOLCE) performs an essential action in improving the recreation of procollagen c-protease and promoting the reconstruction of extracellular matrix. High PCOLCE expression was associated with a negative prognosis of stomach cancer, ovarian cancer, and osteosarcoma. The goal of this work is to investigate the function of PCOLCE in glioma. Multiple bioinformatics techniques have been employed to investigate the roles of PCOLCE in glioma, consisting of the correlation between PCOLCE and prognosis, immune checkpoints, immune cell infiltrates, and tumor microenvironment (TME). The gene ontology (GO) annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were used to assess the potential function of PCOLCE in glioma. PCOLCE was found to be increased in glioma. We revealed that PCOLCE was a potential prognostic factor and related to tumor grade. Up-regulated PCOLCE was related to poor prognosis in lower-grade glioma (LGG), glioblastoma multiforme (GBM), and recurrent glioma. PCOLCE was correlated with immune cell infiltration, particularly B cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells (DCs) in LGG, and DCs infiltration in GBM. PCOLCE was co-expressed with many genes related to the immune and the immune checkpoint. In addition, glioma patients with low expression of PCOLCE had a higher response to the immunological checkpoint blockade (ICB). Additionally, PCOLCE may exert its roles via several immune-related biological processes or pathways, such as leukocyte migration, activation of T cells, adaptive immune response, neutrophil-mediated immunity, NF-κB, and TNF signaling pathways. In conclusion, PCOLCE may be a new immune-related gene and regulate tumor development through immunological pathways.

1. Introduction

According to research, gliomas make up over 80% of all malignant brain tumors [1,2]. Numerous studies have shown that using a combination of histology classification and gene expression-based classification to predict the effects of therapy and prognosis is more accurate than using histological classification alone [3,4]. Molecular biomarkers, such as isocitrate dehydrogenase (IDH) mutation and 1p/19q codeletion are categorized by WHO as the two important indicators for glioma categorization [5]. In addition, IDH mutation and 1p/19q codeletion can also be used for the choice of treatment, and assessment the prognosis of glioma patients [6]. In other

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studies, many potential diagnostic and prognostic molecular biomarkers for glioma were identified, such as PIMREG [7], ZBTB7A [8], and CCN family [9]. However, many of the molecular targets identified were not used in clinical trials or failed in clinical trials [10]. Additionally, due to the exceptional molecular properties of gliomas, it is crucial to identify new biomarkers and molecular targets for glioma diagnosis, prognosis, and treatment advancement.

Procollagen c-protease enhancer protein (PCOLCE), was first identified as an enhancer of catalysis procollagens. PCOLCE increases collagen precursor maturation and the activity of bone morphogenetic protein-1 (BMP1) by binding to the c-propeptide of procollagen III and heparan sulfate through its CUB and NTR domains, respectively [11,12]. PCOLCE plays a crucial role in improving procollagen C-performance protease's and promoting the extracellular matrix's repair [13–15]. Previous studies have illustrated that PCOLCE dysregulation is associated with an increased incidence of several diseases. For instance, a PCOLCE deficit contributes to inadequate corneal repair, PCOLCE expression is positively correlated with the fibrosis of muscle and liver [16], and the overexpression of PCOLCE enhances the migration of osteosarcoma [17], gastric malignancies [18] and gastric cancer [19]. However, the role of PCOLCE in gliomas has not been fully studied.

Immunotherapies have recently been used to treat glioma patients, which has altered the paradigm of treatment for this disease [20]. It is known that tumor infiltrating immune cells (TIICs) have an impact on the immune system, interpreting unusual biological behavior in a complex way and playing a key role in the response to immunotherapies [21]. Immunotherapy for glioma, such as immune checkpoint inhibitors like LAG-3 [22], anti-PD-1 [23], or CTLA4 [24], has demonstrated anticancer effects [25]. Additionally, the TIICs have an impact on the prognosis of glioma patients [21,26], however, the response rate of GBM to ICB is very low, leading to the failure of clinical trials [27]. Therefore, it is important to identify immune interaction biomarkers with glioblastoma and identify new immune-related treatment goals in glioma.

In this study, the PCOLCE expression in LGG and GBM was analyzed. The PCOLCE expression in different grades and histology was also explored. We also analyzed the cell type in which PCOLCE was expressed by single-cell RNA sequencing (scRNA-seq) analysis. The spatial location of the PCOLCE expression was analyzed by spatial transcriptomics analysis. The correlation of PCOLCE with TIIC infiltration, TME, and sensitivity of ICB were assessed to identify the immunological role of PCOLCE and to provide a novel therapy target in glioma.

2. Methods

2.1. Expression level analysis

Clinical data of LGG and GBM patients as well as PCOLCE gene expression information were collected from the TCGA database (https://portal.gdc.cancer.gov/). We performed log2(FPKM+1) transformation on RNA-seq data from TCGA glioma datasets. The differential expression of PCOLCE in normal and glioma tissue, as well as in different WHO grades, was identified by analyzing the expression data from the TCGA. The publicly available database GEPIA was created by Peking University, China (http://gepia.cancer-pku.cn/index.html), which is an online tool for extracting RNA sequencing data from TCGA and GTEx databases [28]. We also utilized GEPIA to corroborate our conclusions and produced boxplots in order to confirm the differential PCOLCE expressions in LGG, GBM, and common tissues. We also used the CGGA datasets (http://www.cgga.org.cn/index.jsp) to assess the expression and survival value of PCOLCE in various WHO grades [29].

2.2. Immunohistochemical (IHC) analysis

In normal, LGG, and GBM tissues, IHC images of PCOLCE protein expression, and evaluation of the differential PCOLCE expression at the protein level, have been carried out in HPA (http://www.proteinatlas.org/). The IHC results based on the antibody against PCOLCE (CAB017623) in normal brain tissues were obtained from the tissue section of the HPA database, while the data of LGG and GBM tumor tissues were downloaded from the pathology section. We downloaded three IHC images of the normal, LGG, and GBM, respectively. PCOLCE protein staining intensity in LGG and GBM tissues was quantified as a fold of normal brain tissues using ImageJ (NIH, Bethesda, MD, USA).

2.3. scRNA-seq analysis

Tumor Immune Single-cell Hub 2 (TISCH2, http://tisch.comp-genomics.org/) is a scRNA-seq database focusing on TME. By using 17 glioma scRNA-seq datasets from the TISCH2 database, we analyzed the cell type in which PCOLCE was expressed.

2.4. Spatial transcriptomics analysis

We analyzed a public glioblastoma dataset generated from the 10x Visium platform with 4326 genes per spot, 43 million transcripts in total, and median UMI counts per spot: 11,596. The STOmics data (https://db.cngb.org/stomics/) was applied to analyze the spatial location of the PCOLCE expression.

2.5. Prognostic value evaluation of PCOLCE in glioma

Age, gender, race, grade, PCOLCE, and other clinical factors were investigated to formulate a nomogram by the results of univariate

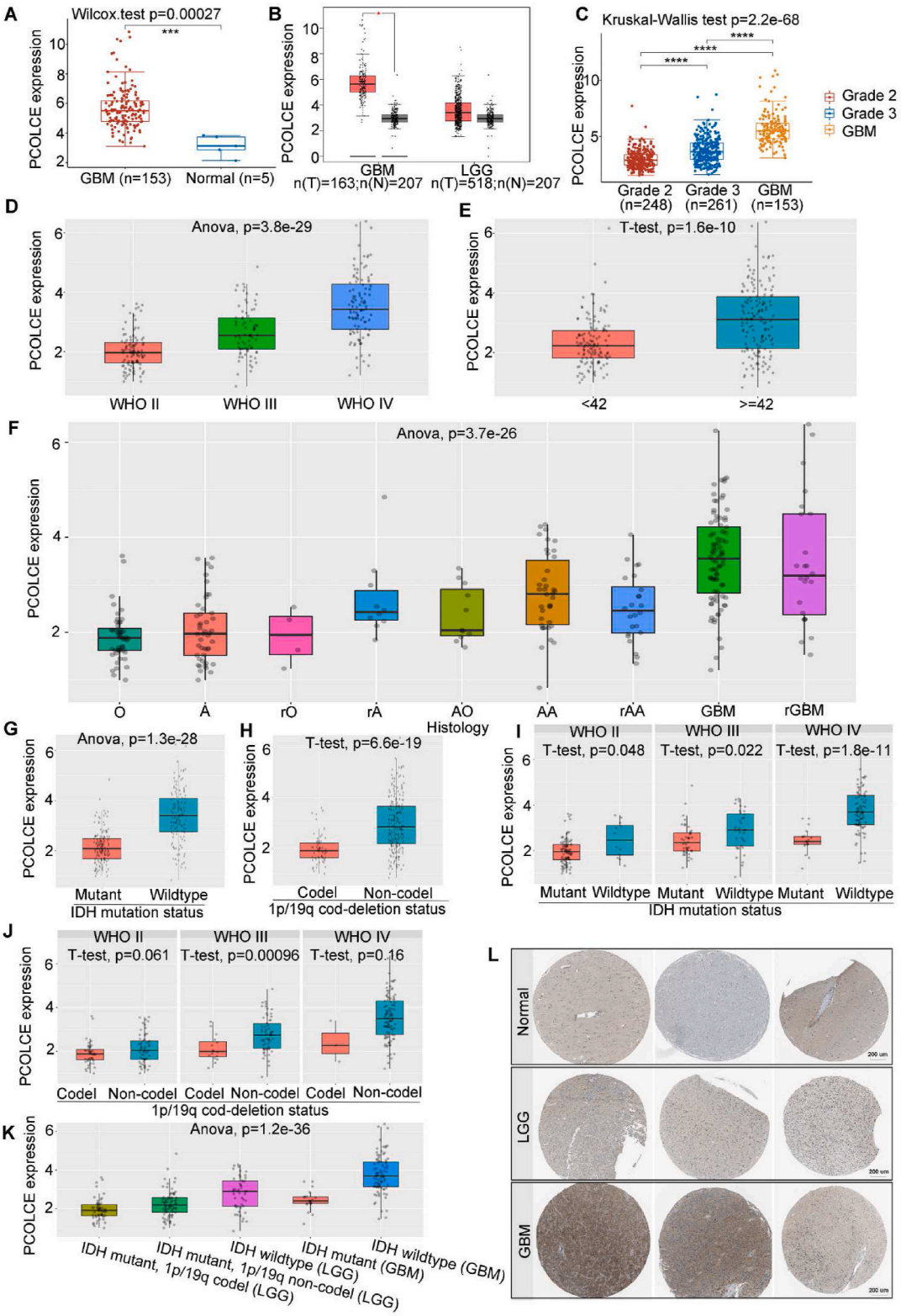

Fig. 1. Different expressions in normal and glioma tissues, and association between PCOLCE and clinical features of glioma. (**A**) Different expression levels of PCOLCE in GBM and normal tissues. (**B**) The expression of PCOLCE in GBM, LGG, and normal tissues in GEPIA. (**C**) The expression of PCOLCE in grades II, III, and IV of glioma. (**D**) The expression of PCOCLE in WHO II, III, and IV grade of glioma in CGGA. (**E**) Expression of PCOLCE in glioma patients of different ages. (**F**) Gene expression of PCOLCE in different histological gliomas. O: oligodendroglioma; A, astrocytoma; rO,

recurrent oligodendroglioma; rA, recurrent astrocytoma; AO, anaplastic oligodendroglioma; AA, anaplastic astrocytoma; rAA, recurrent anaplastic astrocytoma; rGBM, recurrent glioblastoma. (**G-K**) Association of PCOLCE with IDH mutation and 1p/19q co-deletion. (**L**) IHC photographs of normal tissues (upper channel), LGG (middle channel), and GBM (lower channel) in HPA. Scale bar = 200 μm. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

and multivariate Cox regression analysis to predict the 1-year, 3-year, and 5-year overall recurrence. The predictive accuracy of PCOLCE and risk score was once assessed using Time ROC analysis. Then, information on overall survival (OS) and disease-free survival (DFS) for patients with LGG and GBM was gathered using the GEPIA dataset. The relationship between PCOLCE and survival probability for primary and recurrent gliomas at different WHO grades was examined using the CGGA dataset. We defined the high or low expression by the cut-off at the median expression.

2.6. Immune cells infiltration analysis of PCOLCE in glioma

We used the TIMER (https://cistrome.shinyapps.io/timer/) database to find the correlation between PCOLCE and TIICs. Then, we calculated the immune, stromal, and ESTIMATE scores using the ESTIMATE algorithm via the R package "estimate" and "limma". ESTIMATE was an algorithm used to estimate the immune and stromal cells in tumor tissues using expression data [30]. By using CIBERSORT, we examined tumor purity and the infiltration of stromal/immune cells in LGG and GBM tissues based on PCOLCE expression as we previously did. (cut-off: the value of $p < 0.01$). XCELL algorithm was also used to verify the correlation of PCOLCE with PCOLCE.

2.7. Immune-checkpoint analysis

The immune checkpoint related transcripts have been chosen, and the expression data in PCOLCE-high and PCOLCE-low groups have been retrieved. The expression of the immune checkpoints and the co-expression of PCOLCE with these immune-checkpoints were determined using the R packages "ggplot2," "pheatmap," and "immuneeconv." With the use of the TIDE algorithm, a potential immune checkpoint blockade response was predicted in PCLOCE-high and PCOLCE-low groups [31]. TIDE was reported as a computational method to model the mechanisms of tumor immune evasion and served as a biomarker for cancer immunotherapy response [32]. A high TIDE score represents poor ICB efficacy and short survival after receiving ICB treatment.

2.8. The correlation between PCOLCE and drug sensitivity

Based on the GDSC database (https://www.cancerrxgene.org/) we predicted the chemotherapeutic response for glioma samples. The "pRRophetic" R package was used to make the prediction. With the removal of the batch effects of "combat" and tissue type "allSoldTumours," all parameters were set through default values, and we averaged duplicate gene expression [33].

Co-expression, protein-protein interaction (PPI), and pathways enrichment analyses of PCOLCE in glioma.

As previously noted, the R packages "limma," "reshape2," and "RColorBrewer" were used to carry out the co-expression studies [34]. The PPI network had been built using the STING (https://cn.string-db.org/) and GeneMANIA (https://genemania.org/) datasets. LinkedOmics (http://www.linkedomics.org/login.php) has been used to analyze the KEGG enrichment pathways and GO meaningful annotations of PCOLCE. Additionally, the top 50 genes that were correlated with PCOLCE were obtained from LinkedOmics.

2.9. Statistical analysis

The log2(FPKM+1)-transformation was used to standardize the data of gene expression. A two-group *t*-test or Wilcoxon test has been used to contrast normal and malignant tissues. The Kruskal-Wallis one-way ANOVA followed by multiple comparisons was employed to compare data between groups more than or equal to three. For all survival analyses, the Cox proportional hazards model, KM analyses, and log-rank test were employed. The correlation between two variables was examined using Spearman's or Pearson's tests, with a *p-value* < 0.05 being considered significant. For the statistical analysis, R software (version 4.0.2) was utilized.

3. Results

3.1. PCOLCE expression levels in gliomas and normal tissues

From the TCGA dataset, we extracted the information on PCOLCE's mRNA expression in GBM. We discovered that GBM patients had higher levels of PCOLCE expression than normal tissues ($p = 0.00027$) (Fig. 1A). We also evaluated the expression of PCOLCE in GBM, LGG, and normal tissues by matching the data from GTEx using the GEPIA web tool because the TCGA dataset lacked the data for PCOLCE expression in normal brain samples. The outcomes also showed that GBM had higher PCOLCE expression than normal tissues (Fig. 1B). PCOLCE expression level was higher in grade 3 glioma than in grade 2, and PCOLCE expression level was higher in GBM than in both grade 2 and grade 3 gliomas (Fig. 1C). We further explored the PCOLCE expression in the CGGA dataset, we found that it was higher in the WHO 3 grade than the WHO 2 grade and highest in the WHO 4 grade (Fig. 1D). Patients with gliomas under 42 years old had lower levels of PCOCLE expression than those over 42 years old (Fig. 1E). The PCOLCE expression in different glioma histology is

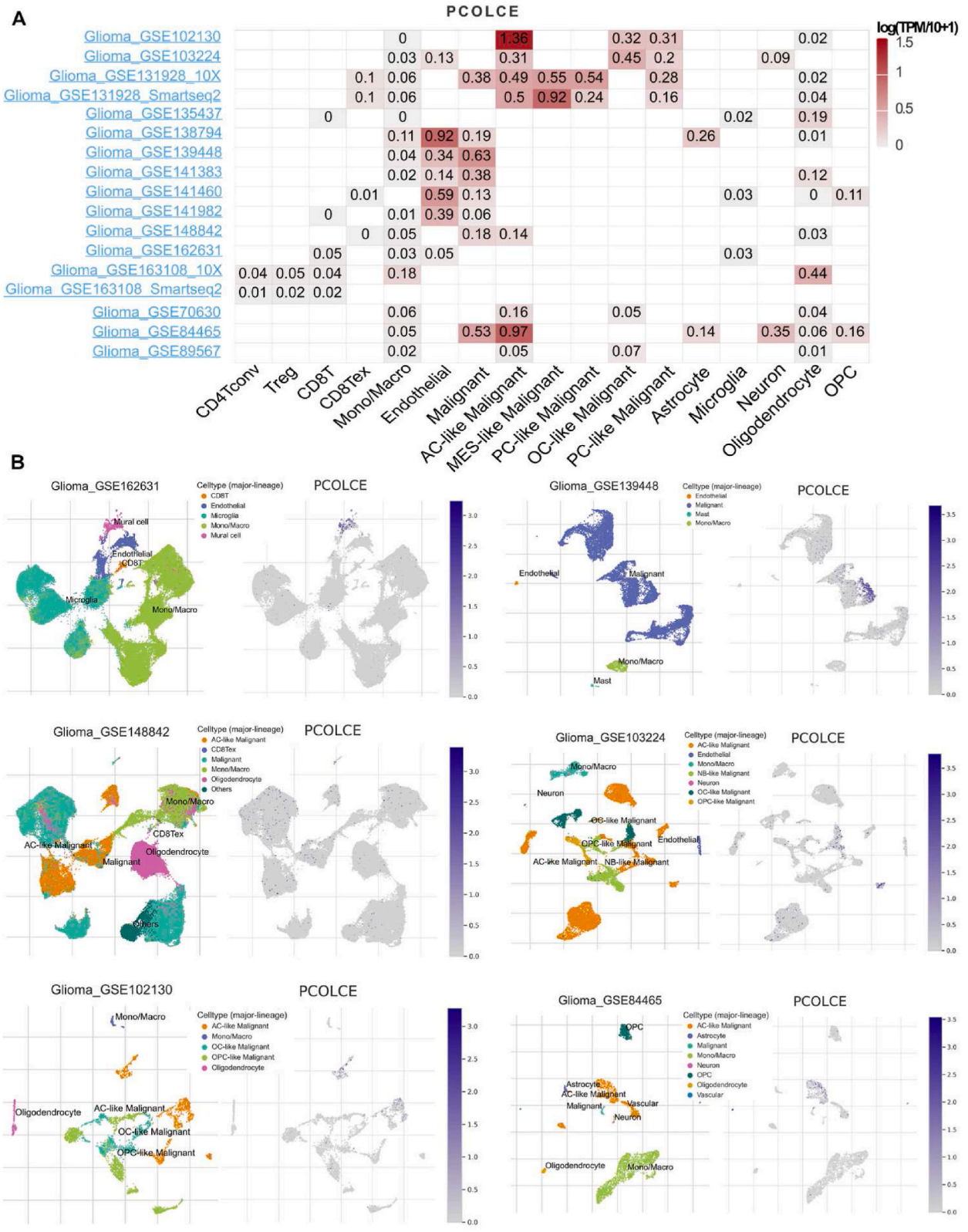

Fig. 2. ScRNA-seq analysis of PCOLCE in gliomas. (**A**) Heatmap showing the expression levels of PCOLCE in different cell types in 17 scRNA-seq datasets. (**B**) UMAP diagram for subclusters and the PCOLCE expression.

shown in Fig. 1F. Anaplastic oligodendrogliomas and anaplastic astrocytomas had higher PCOLCE expression than astrocytomas and oligodendrogliomas. Additionally, we discovered that PCOLCE expression was lower in IDH mutation status compared to wildtype (Fig. 1G), as well as in 1p/19q co-deletion status compared to the non-codeletion group (Fig. 1H). Results from WHO 2, 3, and 4 revealed similar findings (Fig. 1I–K). From the HPA dataset, the protein levels of PCOLCE in GBM, LGG, and normal tissues were shown in Fig. 1L and Fig. S1, the results demonstrated the high protein expression of PCOLCE in GBM.

Additionally, we used scRNA-seq analysis to investigate the cell types in which PCOLCE is expressed. The results indicated that PCOLCE was mainly expressed in endothelial, malignant, astrocyte-like malignant, and mesenchymal-like malignant cells (Fig. 2A and B). To further explore the spatial localization of PCOLCE expression in glioma, spatial transcriptomics analysis was applied. We revealed that PCOLCE was mainly expressed in the tumor-enriched section (Fig. 3A and B). By using the clustering analysis in STOmics databse, 15 segment clusters were identified (Fig. 3C). We further discovered that PCOCLE expression was mainly correlated with cluster7, which is characterized by collagen chain (Fig. 3D–I). These findings suggested a correlation between PCOLCE expression levels and glioma grade.

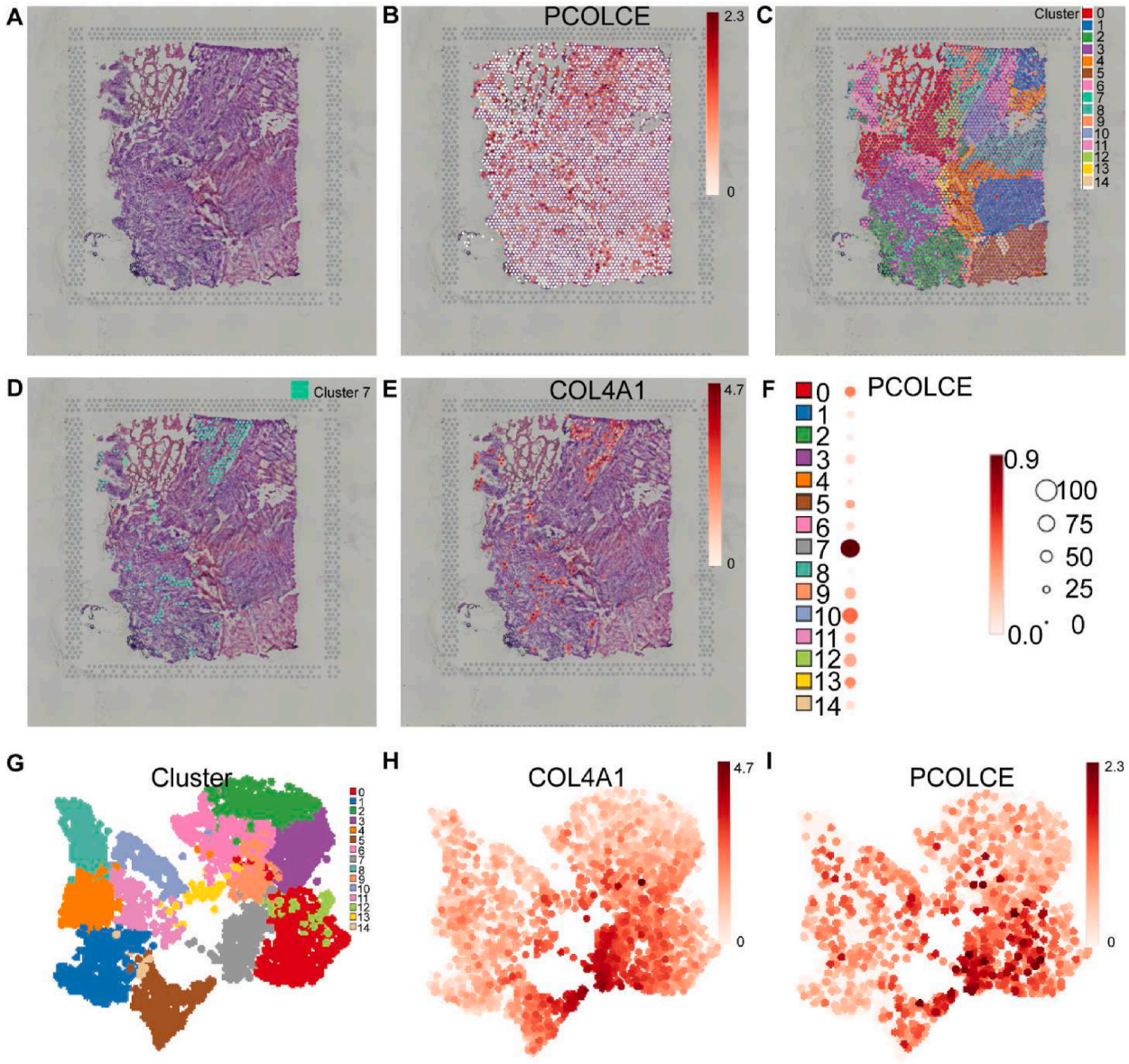

Fig. 3. Spatial transcriptomics analysis of PCOLCE in glioma. (**A**) H&E image of public glioblastoma dataset from the 10x Visium platform. (**B**) Spatial expression of PCOLCE in glioma. (C) Clusters analysis. The location of cluster 7 (**D**) and COL4A1 (**E**) in glioma. (**F**) Correlations in space between PCOLCE and clusters. (**G**) UMAP diagram for subclusters. UMAP diagram for COL4A1 (**H**) and PCOLCE (**I**).

3.2. Up-regulated PCOLCE expression predicted poor prognosis of glioma patients

First, we assessed the prognostic value of PCOLCE in patients with LGG and GBM in GEPIA. We found that poor OS and DFS in LGG were associated with increased PCOLCE expression (p = 6.8e-10 and 2.9e-06, respectively) (Fig. 4A and B). Similarly, elevated PCOLCE expression in GBM was associated with poor OS ($p = 0.008$) but not DFS ($p = 0.54$) (Fig. 4C and D). To further evaluate the predictive value of PCOLCE in various grades of glioma, the CGGA dataset was subsequently employed. High PCOLCE expression was linked to poor survival in all WHO grade survival of primary ($p < 0.0001$) or recurrent glioma ($p = 0.00032$) (Fig. 4E and F). Moreover, high PCOLCE expression was related to a poor survival probability in WHO grade 2 ($p = 0.0054$), 3 ($p = 0.022$), and 4 ($p = 0.018$) of primary glioma (Fig. 4G–I).

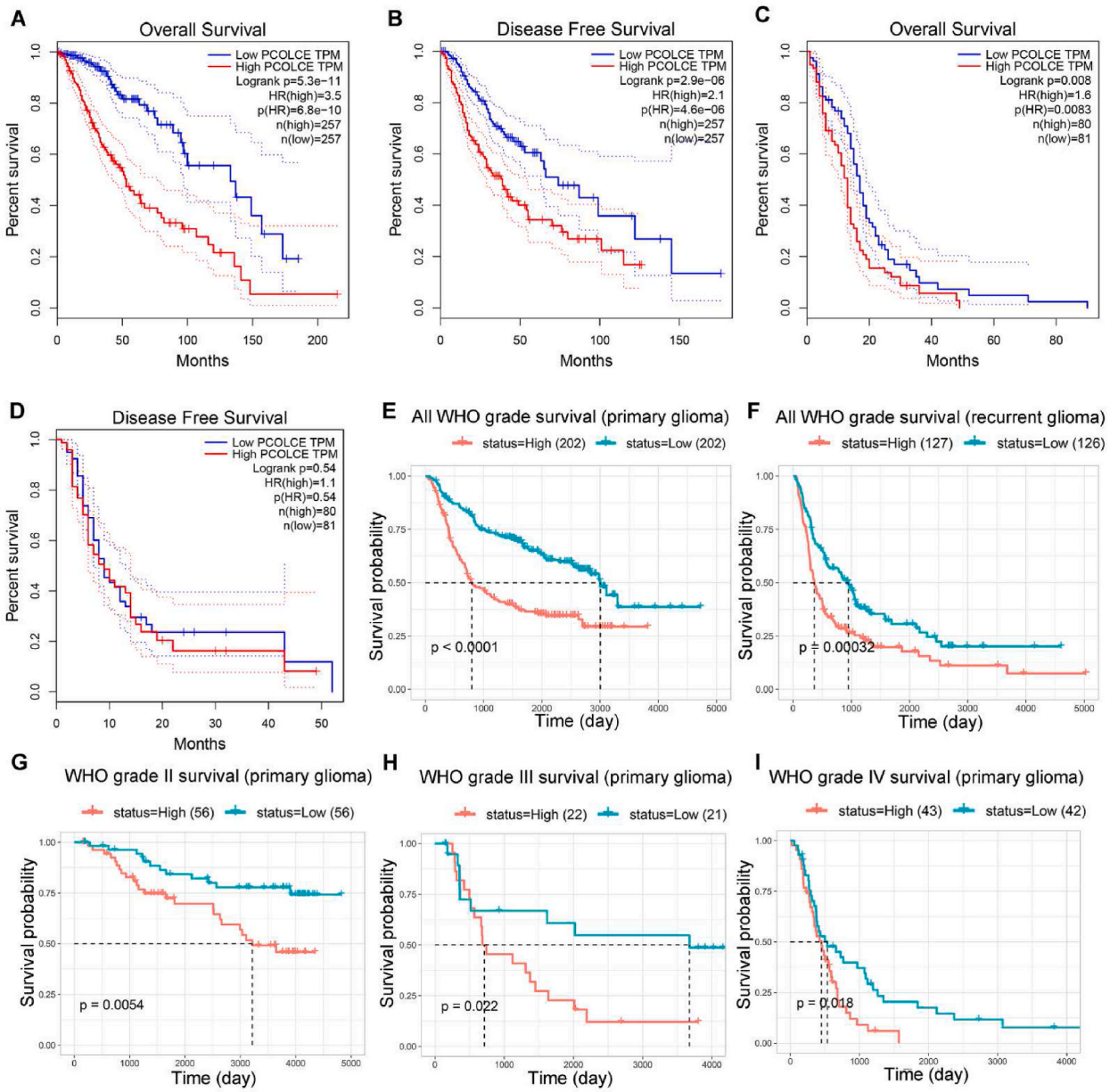

Fig. 4. Predictive value of PCOLCE in the OS and DFS of glioma. OS (**A**) and DFS (**B**) of LGG in high and low PCOLCE-expression group. OS (**C**) and DFS (**D**) of GBM in high and low PCOLCE-expression groups. Association between PCOLCE and survival probability of all WHO grade primary (**E**) and recurrent (**F**) glioma in mRNA_array_693 dataset in CGGA. (**G**) Association between PCOLCE and survival probability of primary glioma in WHO II grade in mRNAseq325 dataset in CGGA. (**H**) Association between PCOLCE and survival probability of primary glioma in WHO III grade in mRNAseq_325 dataset in CGGA. (**I**) Association between PCOLCE and survival probability of primary glioma in WHO IV grade in mRNAseq_325 dataset in CGGA.

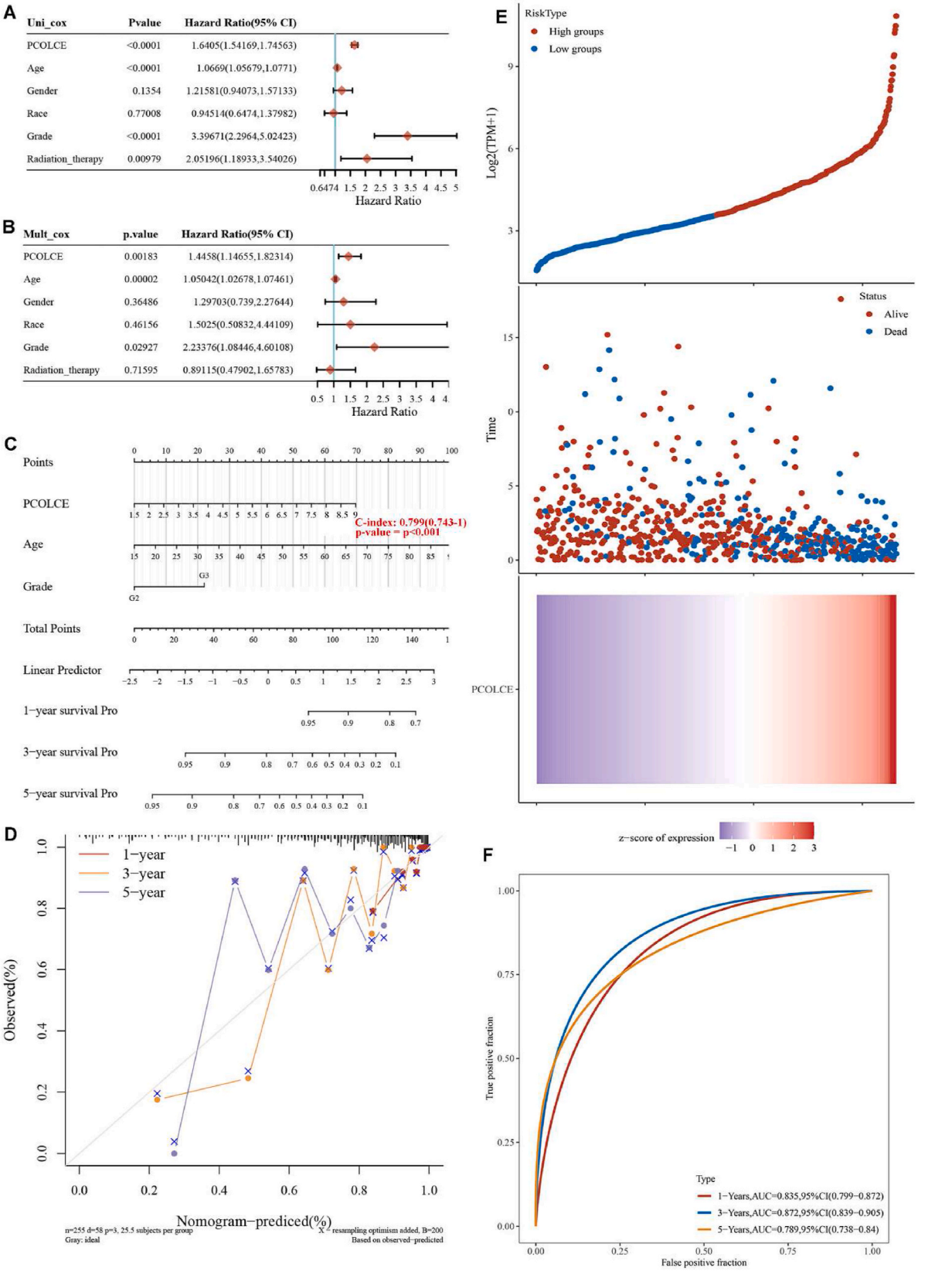

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Fig. 5. Prognostic value of PCOCLE in glioma. Forrest plot of univariate Cox regression (**A**) and multivariate Cox regression (**B**) analysis in glioma. (**C**) Nomogram integrated PCOLCE, age, and grade. (**D**) Calibration curve for the overall survival nomogram model. (**E**) Survival status of the patients. More dead patients corresponding to the higher PCOLCE expression. (**F**) Time-dependent ROC analysis of PCOLCE.

To examine the independent prognostic force of PCOLCE in gliomas, we also used the univariable and multivariable Cox regression models. PCOLCE (HR = 1.6405, $p < 0.0001$), age (HR = 1.0669, $p < 0.0001$), grade (HR = 3.39671, $p < 0.0001$), and radiation treatment (HR = 2.05196, $p = 0.00979$) showed predictive significance for the OS of glioma, according to univariable assessment results (Fig. 5A). Similarly, PCOLCE (HR = 1.00183, $p = 0.00183$), age (HR = 1.05042, $p = 0.00002$), and grade (HR = 2.23376, $p = 0.02927$) had predictive values in the following multivariable cox regression analysis (Fig. 5B). These findings proved that PCOLCE expression may be a reliable prognostic indicator in gliomas. PCOLCE, grade, and age were subsequently presented in the nomogram. Nomograms for the 1-, 3-, and 5-year OS have been verified in Fig. 5C. We used the statistics from the TCGA dataset to create a ROC curve to analyze the prediction effectiveness of the PCOLCE gene in the 1-, 3-, and 5-year survival rates. The calibration plot for predicting the OS is shown in Fig. 5D. Additionally, our results showed that the mortality rate in the group with high PCOLCE expression was significantly higher than that in the PCOLCE-low group (Fig. 5E). The area under the ROC curve (AUC) was 0.835 at 1-year, 0.872 at 3-years, and 0.789 at 5-years, respectively, indicating a high predictive value (Fig. 5F).

3.3. PCOLCE was correlated with TIICs in gliomas

In the TIMER database, we evaluated the correlation between PCOCLE expression and TIICs. The findings demonstrated a positive correlation between PCOCLE expression level and the infiltration of B cells (Cor = 0.12, $p = 8.79e\text{-}03$), $CD4^+$ cells (Cor = 0.248, $p = 4.13e\text{-}08$), macrophages (Cor = 0.24, $p = 1.30e\text{-}07$), neutrophils (Cor = 0.224, $p = 8.15e\text{-}07$), and dendritic cells (DCs) (Cor = 0.274, $p = 1.15e\text{-}09$) in LGG (Fig. 6A). A low cumulative survival was seen in LGG patients with high infiltration levels of B cells ($p < 0.001$), $CD8^+$ cells ($p < 0.001$), $CD4^+$ cells ($p = 0.01$), macrophages ($p < 0.001$), or DCs ($p = 0.001$) (Fig. 6B). PCOLCE expression in GBM was negatively related to neutrophils and $CD8^+$ cells (Cor = −0.106, $p = 2.97e\text{-}02$ and Cor = −0.145, $p = 2.97e\text{-}03$, respectively), but positively correlated with DC (Cor = 0.415, $p = 7.86e\text{-}19$) (Fig. 6C). A low cumulative survival was seen in GBM patients with significant DC infiltration ($p = 0.002$) (Fig. 6D). It is important to study the relationship between PCOLCE expression and TME since it has been reported that TME plays a crucial role in carcinogenesis and progression. The ESTIMATE method was used to analyze the correlation between PCOLCE. In both LGG and GBM, the expression of PCOLCE was positively correlated with the stomal score (R = 0.391, $p < 0.001$ in LGG; R = 0.446, $p = 2.27e\text{-}09$ in GBM) (Fig. 6E), immunological score (R = 0.359, $p = 1.35e\text{-}17$ in LGG; R = 0.276, $p = 0.000328$ in GBM) (Fig. 6F), and ESTIMATE score (R = 0.382, $p < 0.0001$ in LGG, R = 0.362, p = 1.77e-06 in GBM) (Fig. 6G). We also assessed the amounts of 22 different immune cell types invading gliomas using the CIBERSORT method. High levels of macrophage M0, M1, and M2 infiltrates were related to high levels of PCOLCE expression, but the levels of plasma B cells, monocytes, and resting mast cells were negatively correlated with low levels of PCOLCE expression (Fig. 7A and B). By using the XCELL algorithm, we further confirmed that PCOLCE was positively related to immune, stroma, and microenvironment scores (Fig. 7A). We also measured the degree of immune cell infiltrates in LGG and GBM. As seen in Fig. 7C, PCOLCE expression was negatively correlated with the infiltration of eosinophils (R = −0.28, $p = 0.00025$), monocytes (R = −0.22, $p = 0.0061$), and follicular helper T cells (R = −0.21, $p = 0.0072$) in GBM, but positively correlated with the infiltration of macrophage M0 (R = 0.24, $p = 0.002$). PCOLCE expression in LGG was positively linked with the infiltration of M0 macrophages (R = 0.33, $p = 1.9e\text{-}10$), M1 macrophages (R = 0.38, $p = 3.6e\text{-}13$), neutrophils (R = 0.13, $p = 0.013$), resting mast cells (R = 0.13, $p = 0.014$), resting CD4 memory T cells (R = 0.24, $p = 4.6e\text{-}06$) (Fig. 7C). These findings showed that PCOLCE expression may be associated with the TIICs and affected glioma prognosis. For instance, a poor prognosis for LGG ($p = 0.001$, Fig. 6B) and GBM ($p = 0.002$, Fig. 6D) was associated with strong DC infiltration and high PCOLCE expression.

3.4. Glioma patients with low PCOLCE expression had a stronger response to ICB

Glioma patients were split into two groups (PCOLCE-high and PCOLCE-low) based on the PCOLCE expression levels. In glioma patients with high PCOLCE expression, we discovered that the expression of these immune checkpoints, except TIGIT, was up-regulated (Fig. 8A and B). Additionally, LGG and GBM responses to ICB have been studied. Patients with LGG and GBM who had low PCOLCE expression responded better to ICB (Fig. 8C and D). It's interesting to note that PCOLCE expression was negatively correlated with temozolomide's IC50 in gliomas (Fig. 8E).

3.5. Co-expression of PCOLCE with immune-related genes

We examined the co-expression of PCOLCE and genes involved in immunity. PCOLCE was significantly co-expressed with every MHC gene in LGG but was only co-expressed with a small number of MHC genes in GBM (Fig. 9A). Several immune activation genes, including CD276, CD28, CD40, CXCR4, IL2RA, IL6, STING1, TNFRSF14, TNFRSF18, TNFRSF25, TNFRSF9, TNFRSF4, and TNFRSF15, were positively co-expressed with PCOLCE in LGG and GBM (Fig. 9B). The majority of immunosuppressive genes, including CD274, CD96, CTLA4, IL-10, IL-10RB, KDR, KIR2DL1, NECTIN2, PDCD1, PDCD1LG2, TGFB1, and TGFBR1, were co-expressed with PCOLCE (Fig. 9C). Additionally, PCOLCE was co-expressed CCR3, CCR4, CCR5, CCR7, CCR8, CXCR3, CXCR4, CXCR6, XCR1 and multiple chemokines (Fig. 9D and E).

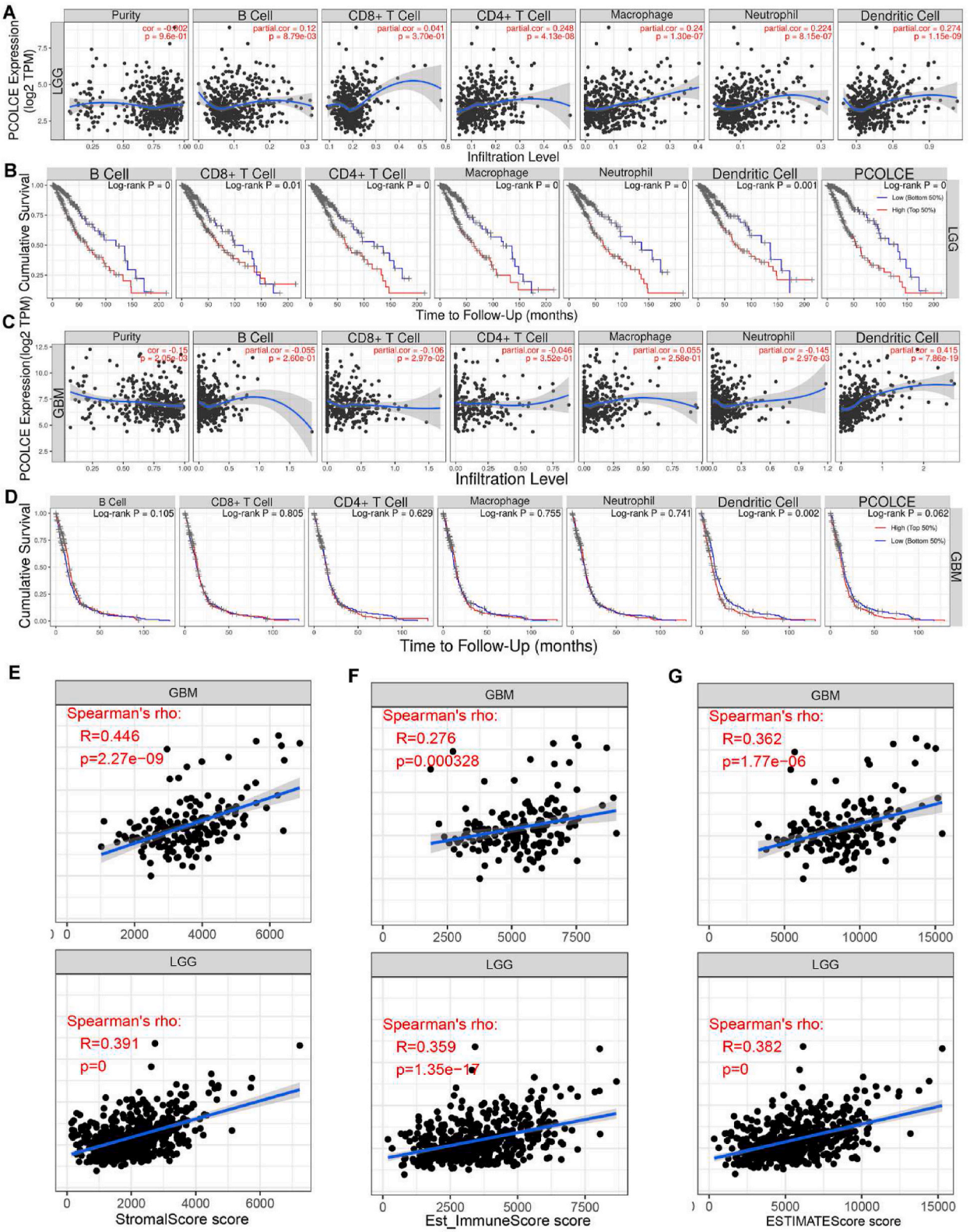

Fig. 6. TIIC infiltration and TIIC-related prognosis analysis in LGG and GBM. (**A**) Association between PCOLCE and TIIC infiltration of LGG in TIMER. (**B**) TIIC-related prognosis in LGG. (**C**) Association between PCOLCE and TIIC infiltration of GBM in TIMER. (**D**) TIIC-related prognosis in GBM. Correlation between PCOLCE and stromal score (**E**), immune score (**F**), and ESTIMATE score (**G**) in LGG and GBM.

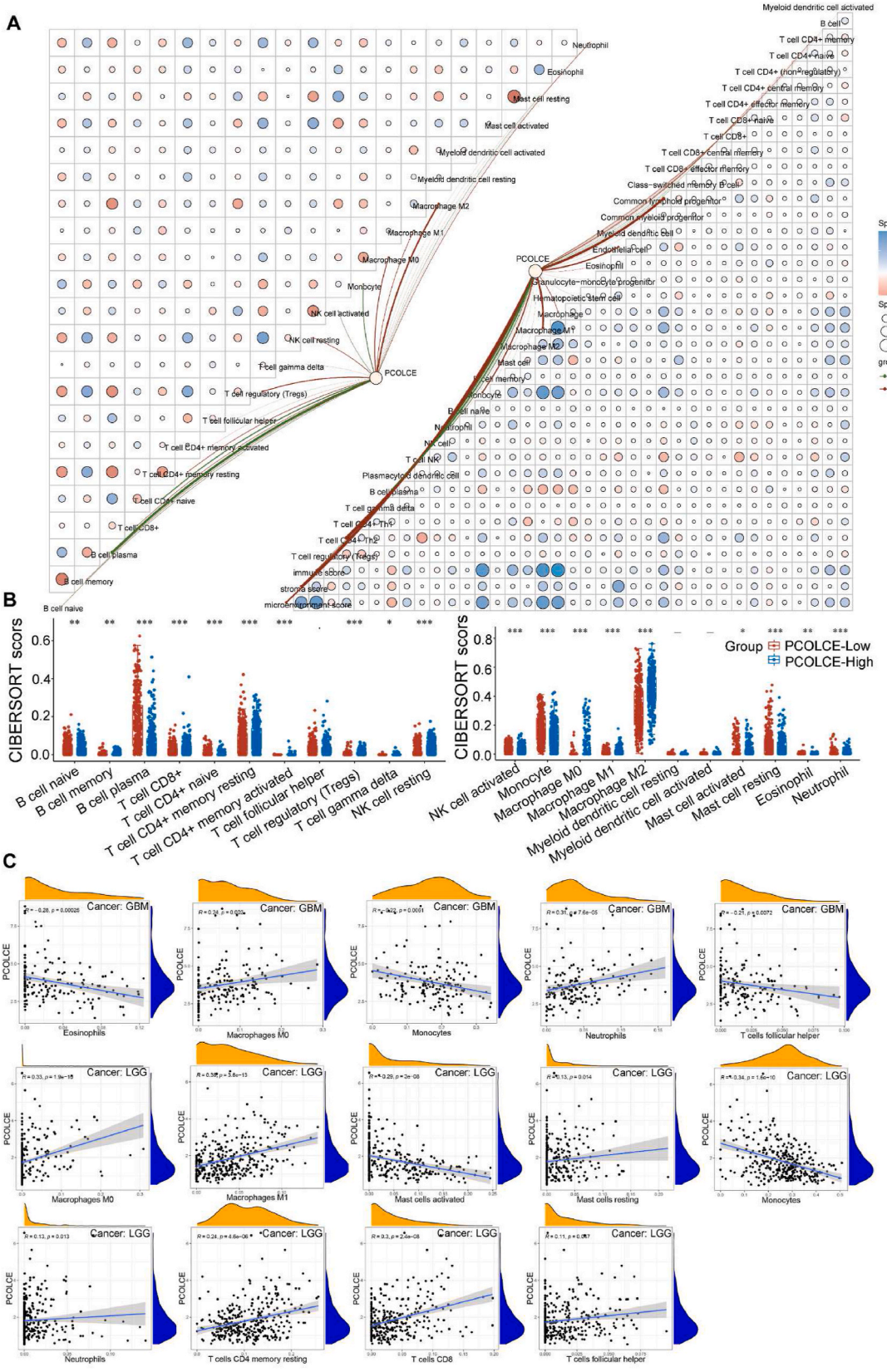

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Fig. 7. Correlation of PCOLCE with TIIC infiltration based on CIBERSORT method. (**A**) Correlation of PCOLCE and immune cells analyzed by CIBERSORT algorithm (left) and XCELL algorithm (right). (**B**) TIIC infiltration in high and low PCOLCE expression group of gliomas. (**C**) Relationship between PCOLCE and TIIC infiltration in LGG and GBM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

We investigated the co-expression genes and protein-coding genes of PCOLCE to learn more about the potential mechanism by which it regulates gliomas. The following top 10 genes showed a strong positive correlation with PCOLCE in LGG: COL1A2, TIMP1, COL6A2, CD248, COL1A1, GPX8, SERPINH1, COL3A1, COL5A1, and ZYX (Fig. 9F). FUT9, AKAP6, ZMYND11, TMEM170B, ALDH5A1, NAP1L3, NALCN, GLUD2, GLASP2, ZFYVE20, and ZRANB1 were the top 10 significant negatively related genes in LGG (Fig. 9G). The following 10 genes in GBM showed a strong positive correlation with PCOLCE: COL1A2, LEPRE1, SERPINH1, COL3A1, LUM, RCN3, ANPEP, LAMB1, COL1A1, and COL6A3 (Fig. 9H). PHYHIPL, KCNJ10, RFTN2, FAM169A, CRB1, ABAT, GKAP1, CTNND2, ASCL1, and RAB39B were the top 10 significant negatively linked genes in GBM (Fig. 9I). Furthermore, the PCOLCE protein interacted with other partners was established by the STRING and Gene MANIA databases (Fig. 9J and K).

3.6. Pathway enrichment analyses of PCOLCE in gliomas

We then used the GESA web tool Linkedomics to annotate GO functions and analyze KEGG pathways. The biological process GO analysis results for LGG illustrated that PCOLCE was positively correlated with a wide range of immune-related biological processes, including the response to interferon-gamma, response to type I interferon, leukocyte migration, adaptive immune response, T cell activation, lymphocyte mediated immunity, leukocyte cell-cell adhesion, humoral immune response, angiogenesis, granulocyte activation, leukocyte proliferation, neutrophil mediated immunity, and interleukin-8 (Fig. 10A). PCOLCE also regulated the collagen metabolic process and extracellular structure, as shown in Fig. 10A. According to the findings of KEGG analysis, PCOLCE was positively correlated with autoimmune thyroid disease, ECM-receptor interaction, allograft rejection, viral myocarditis, the intestinal immune network for IgA production, complement and coagulation cascades, hematopoietic cell lineage, antigen processing and presentation, and cytokine-cytokine receptor interaction in LGG (Fig. 10B). Furthermore, biological process GO analysis illustrated that PCOCLE was positively correlated with immune-related biological processes, including adaptive immune response, granulocyte activation, neutrophil mediated immunity, and lymphocyte activation involved in immune response in GBM, in addition to collagen metabolic process (Fig. 10C). PCOLCE was found to be positively correlated with ECM-receptor interaction, complement and coagulation cascades, cytokine-cytokine receptor interaction, hematopoietic cell lineage, NF-κB, and TNF pathway, according to the KEGG pathway enrichment study in GBM (Fig. 10D). These findings demonstrated that PCOLCE may be involved in a variety of immune-related biological processes in gliomas.

4. Discussion

In this study, we discovered that PCOLCE expression was elevated in GBM than in normal tissues. Furthermore, a correlation between PCOLCE expression and WHO glioma grade was found. PCOLCE was most strongly expressed in WHO grade IV gliomas and least expressed in WHO grade II gliomas, as shown in Fig. 1C and D. PCOLCE expression was higher in glioma patients over 42 years of age than in glioma patients under 42 years of age. Additionally, 1p/19q co-deletion and IDH mutation both had a negative connection with PCOLCE expression. IDH mutation and 1p/19q codeletion were regarded as key markers for glioma classification in the 2021 WHO classification system. Therefore, PCOCLE expression may also aid in accurately determining the grade of gliomas. Additionally, our findings showed that high levels of PCOLCE expression may be a predictor of poor outcomes for LGG and GBM patients. The CGGA data also showed that all WHO-grade primary gliomas had a poor prognosis when PCOLCE was over-expressed, consistent with a most recent study [35]. It has illustrated that PCOLCE actively increases the production of BMP1. The removal of C-propeptides from procollagen I, II, and III in the extracellular matrix by BMP1, a zinc metalloproteinase, causes collagen to be deposited [13]. The increase of collagen deposition is widely acknowledged to be the most prominent ECM modification during cancer development [36], suggesting that PCOLCE may also play a role in the acceleration of cancer metastasis. A link between high PCOLCE expression and a poor prognosis for recurrent gliomas may also be explained in part by this. In patients with gastric cancer [19], ovarian cancer [19], and osteosarcoma [17] the higher PCOLCE expression level was associated with a worse prognosis. Interestingly, the increased expression of PCOLCE may affect the prognosis of patients with lymph node metastases from gastric cancer [19], indicating that the expression of PCOLCE may be employed as an index to predict metastases from gastric tumors.

There was a strong correlation between the expression of PCOLCE and the stromal, immune, and ESIMATE scores in LGG and GBM. Furthermore, our results demonstrated a correlation between PCOLCE expression and the level of immune infiltration in LGG and GBM. According to the data from TIMER, B, $CD4^+$ T, macrophage, neutrophil, and DCs cell infiltration levels in LGG were positively correlated with PCOLCE expression. Patients with LGG who had high levels of infiltration of these cells had a poor prognosis (Fig. 6B). These findings illustrated that PCOLCE may partially help the progress of LGG tumors by up-regulating the levels of B, $CD4^+$ T, macrophage, neutrophil, and DC cell infiltration. Additionally, PCOLCE was positively related to DC cells infiltration, the presence of which was associated with a poor prognosis for GBM patients, suggesting that PCOLCE may also facilitate the process of the tumor by up-regulating DC infiltration. The expression of PCOLCE was also positively related to infiltration of M2 macrophage, this also may be a reason why PCOLCE was positively related to the microenvironment and immune score. Additionally, we employed the CIBERSORT method to identify the invasion of various immune cells. Notably, PCOLCE expression in LGG was positively correlated with M1 macrophage, resting memory $CD4^+$ T cells, and negatively correlated with monocyte and active mast cell invasion. These results

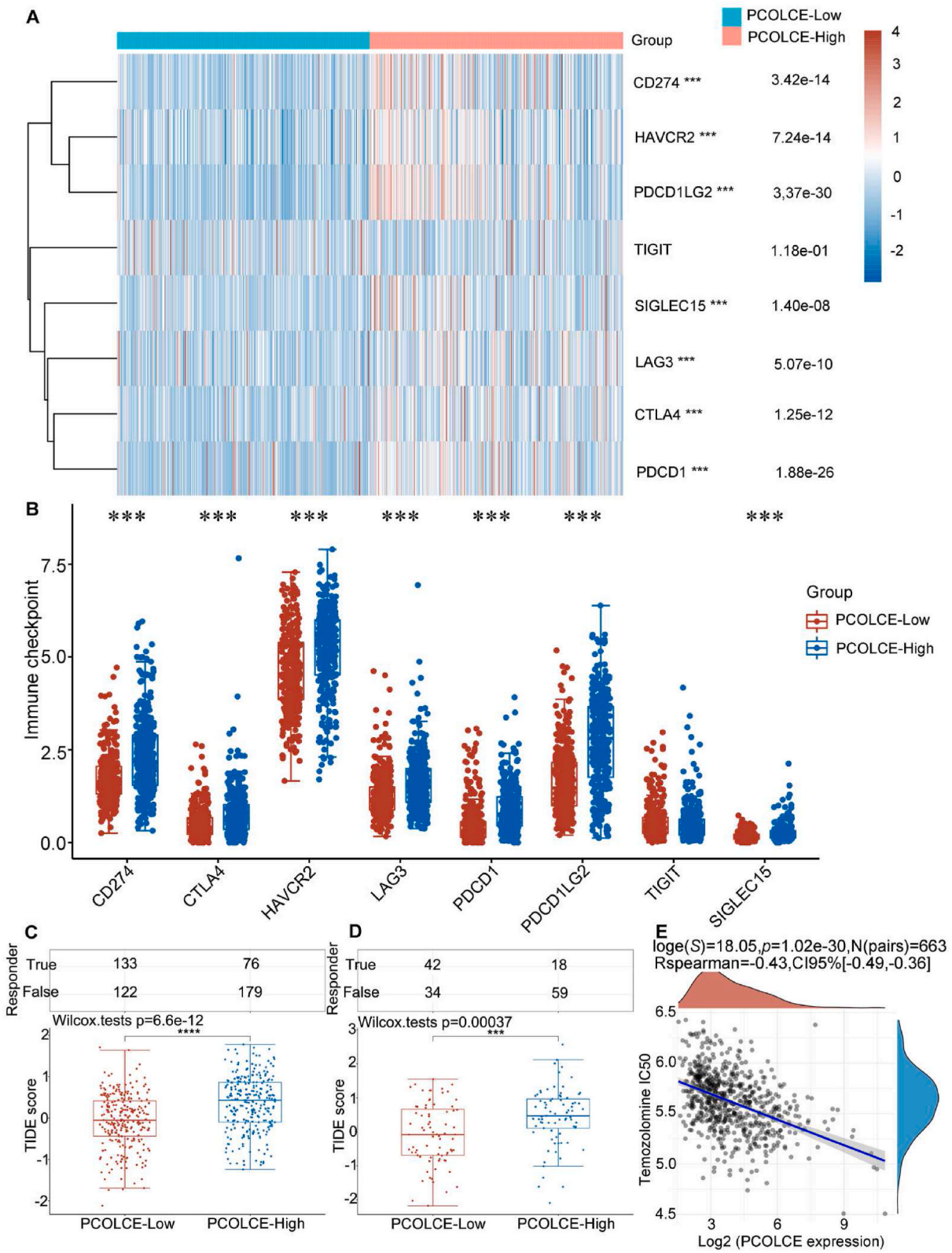

Fig. 8. Correlation of PCOLCE with immune checkpoints, response to ICB, and IC50 of temozolomide. (**A, B**) Differential expressions of immune checkpoints in PCOLCE-high and PCOLCE-low expression group of gliomas. Different TIDE scores in PCOLCE-high and PCOLCE-low groups in LGG (**C**) and GBM (**D**). (**E**) Association between PCOLCE and IC50 of temozolomide. ***$p < 0.001$.

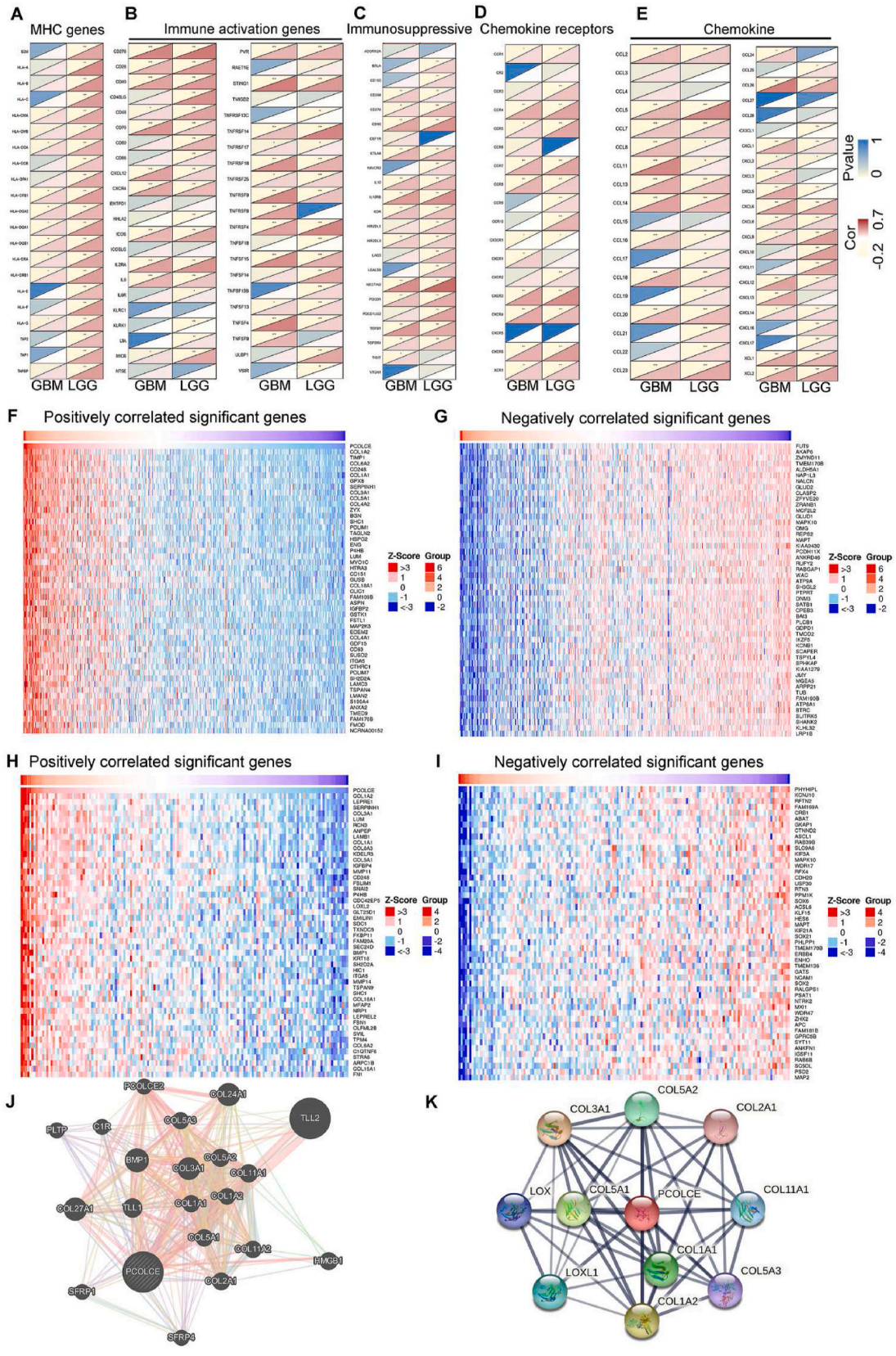

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Fig. 9. Co-expression between PCOLCE and MHC genes (**A**), immune activation genes (**B**), immunosuppressive genes (**C**), chemokine receptors (**D**), and chemokine (**E**) in LGG and GBM. Top 50 positively (**F**) and negatively (**G**) PCOLCE-correlated significant genes in LGG. Top 50 positively (**H**) and negatively (**I**) PCOLCE-correlated significant genes in GBM. PPI network of PCOLCE from GeneMANIA (**J**) and STRING (**K**). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

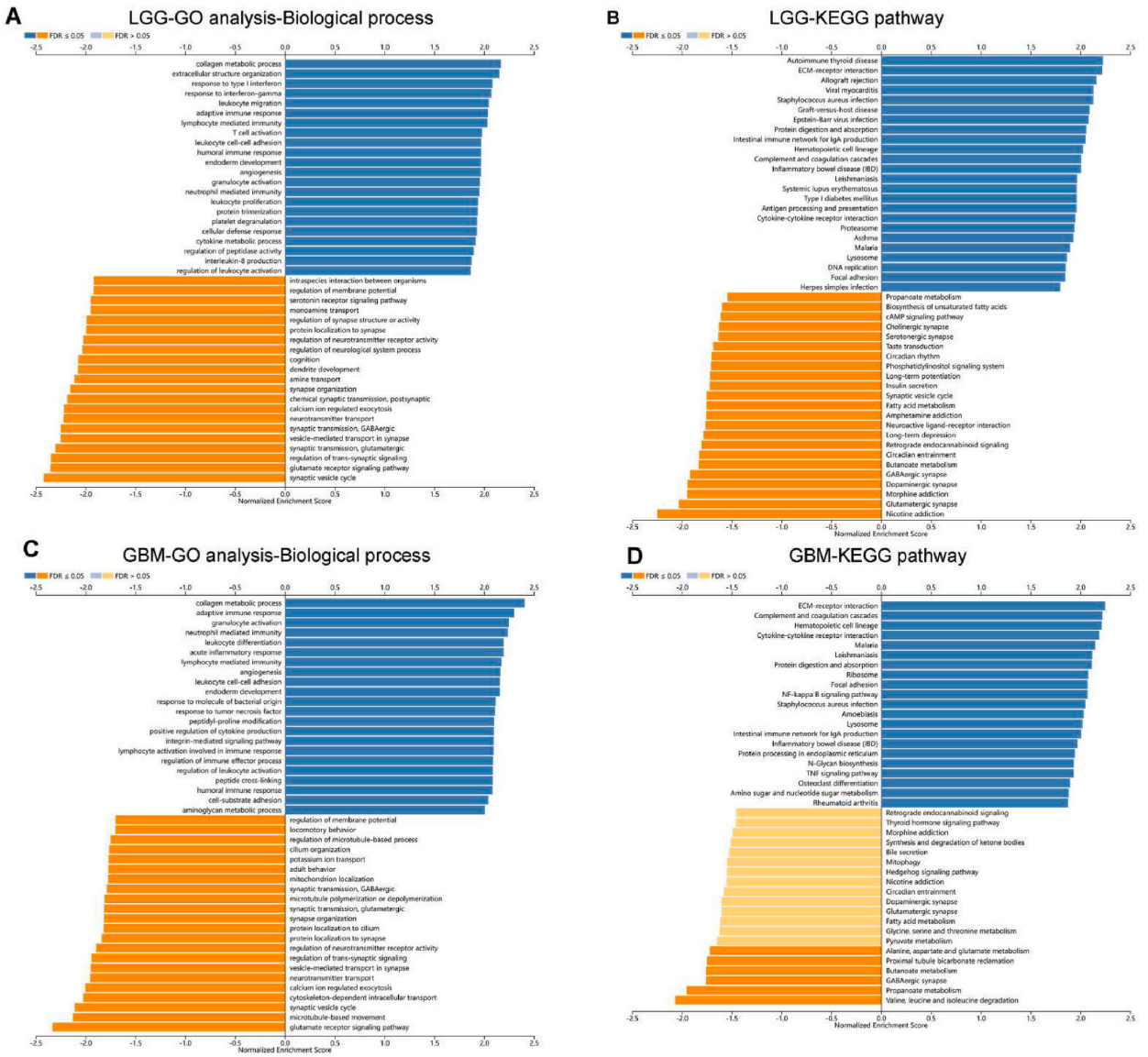

Fig. 10. Pathway enrichment analyses of PCOLCE. (**A, B**) GO biological process analysis and KEGG analysis in LGG. (**C, D**) GO biological process analysis and KEGG analysis in GBM.

proved that PCOLCE may have a potential function in tumor immunity.

Immunotherapies have been used to treat glioma patients, and this has altered the paradigm of the treatment for this disease. Immunotherapy with immune checkpoint inhibitors like LAG-3 [37], anti-PD-1 [38,39], or CTLA4 [40,41] demonstrated potential anticancer efficacy in glioblastoma. Here, we assessed the relationship between immune checkpoint expression and PCOLCE. Positive correlations between PCOLCE expression and PDCD1, CTLA4, LAG3, SIGLEC15, PDCD1LG2, HAVCR2, and CD274 expression were found. Furthermore, high PCOLCE expression was linked to a stronger response to ICB in both LGG and GBM, suggesting that patients with gliomas who have low PCOLCE expression may also respond more favorably to ICB therapy. It's interesting to note that PCOLCE expression was negatively correlated with temozolomide's IC50, which suggests that glioma patients with high PCOLCE expression may benefit more from temozolomide chemotherapy. These findings may have implications for the accurate treatment of glioma patients.

In LGG and GBM, PCOLCE was co-expressed with the majority of immune-related genes. For instance, in LGG, PCOLCE positively co-expressed with each MHC gene. PCOLCE also interacted with genes linked to collagen, including COL5A1, COL5A2, COL5A3, COL3A1, and COL2A1. This was confirmed by the results of spatial transcriptomics analysis. According to our previous research, COL5A1 promotes glioma growth and migration [42]. These findings suggested that PCOLCE may also partially promote tumor growth by interacting with COL5A1. Additionally, GO and KEGG studies confirmed that PCOLCE was correlated with collagen metabolism, extracellular structural organization, and the interaction of the ECM with receptors in gliomas. Increased collagen deposition is the most well documented ECM modification in the course of cancer development, according to prior research. These findings highlighted the potential tumor-promoting function of gliomas. Additionally, the enrichment analyses also revealed that PCOLCE may be a novel immune-related gene that partially influences the development of glioma through several immunological pathways, including leukocyte migration, adaptive immune response, lymphocyte mediated immunity, T cell activation, leukocyte cell-cell adhesion, humoral immune response, granulocyte activation, neutrophil mediated immunity, and leukocytic leukocytosis, the intestinal immune network that produces IgA, the NF-κB signaling pathway, and the TNF signaling pathway.

Some limitations of the present report should be noted. In vitro and in vivo experiments to validate PCOLCE expression are needed for subsequent studies. The biological function of PCOLCE and the correlation of PCOLCE with TME in gliomas needs to be validated by further in vitro and in vivo experiments. We will continue to explore the specific mechanism of PCOLCE in subsequent studies.

To sum up, PCOLCE was up-regulated in gliomas and related to clinical characteristics. The prognosis of primary and recurrent gliomas was negatively linked with high expression of PCOLCE. In glioma patients, PCOLCE was associated with immune cell infiltration, immune checkpoints, and ICB immunotherapy response. PCOLCE may also be a novel immune-related gene that influences the progress of glioma via a variety of biological or immunological mechanisms.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets presented in this study can be found in online repositories TCGA (https://portal.gdc.cancer.gov/), CGGA (http://www.cgga.org.cn/index.jsp), GEPIA(http://gepia.cancer-pku.cn/index.html), TISCH2 (http://tisch.comp-genomics.org/) 10x genomics (support.10xgenomics.com/" title="https://support.10xgenomics.com/">https://support.10xgenomics.com/) and HPA (http://www.proteinatlas.org/). The authors did not have special access privileges.

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CRediT authorship contribution statement

Luli Yu: Writing – review & editing, Writing – original draft. **Xinyao Hu:** Formal analysis, Data curation, Conceptualization. **Hua Zhu:** Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28089.

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