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Overexpression of CD99 is associated with tumor adaptiveness and indicates the tumor recurrence and therapeutic responses in gliomas

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ABSTRACT

Glioma undergoes adaptive changes, leading to poor prognosis and resistance to treatment. CD99 influences the migration and invasion of glioma cells and plays an oncogene role. However, whether CD99 can affect the adaptiveness of gliomas is still lacking in research, making its clinical value underestimated. Here, we enrolled our in-house and public multiomics datasets for bioinformatic analysis and conducted immunohistochemistry staining to investigate the role of CD99 in glioma adaptive response and its clinical implications.

CD99 is expressed in more adaptative glioma subtypes and cell states. Under hypoxic conditions, CD99 is upregulated in glioma cells and is associated with angiogenesis and metabolic adaptations. Gliomas with overexpressed CD99 also increased the immunosuppressive tumor-associated macrophages. The relevance with tumor adaptiveness of CD99 presented clinical significance. We discovered that CD99 overexpression is associated with short-time recurrence and validated its prognostic value. Additionally, Glioma patients with high expression of CD99 were resistant to chemotherapy and radiotherapy. The CD99 expression was also related to anti-angiogenic and immune checkpoint inhibitor therapy response. Inhibitors of the PI3K-AKT pathway have therapeutic potential against CD99-overexpressing gliomas.

Our study identified CD99 as a biomarker characterizing the adaptive response in glioma. Gliomas with high CD99 expression are highly tolerant to stress conditions such as hypoxia and antitumor immunity, making treatment responses dimmer and tumor progression. Therefore, for patients with CD99-overexpressing gliomas, tumor adaptiveness should be fully considered during treatment to avoid drug resistance, and closer clinical monitoring should be carried out to improve the prognosis.

Introduction

Gliomas are the most prevailing central nervous system (CNS) tumors, accounting for approximately 80% of brain malignancies. Only 30% of patients with gliomas survive at five years after diagnosis, and this number drops to 6.8% for glioblastoma (GBM) [1]. Gliomas gradually develop tolerance to treatment, making recurrence almost inevitable [2].

Multiple adaptive responses exist in tumors to cope with various stressors, thus contributing to cancer malignancy and proposing challenges to therapy [3]. Proneural-mesenchymal transition (PMT) arises in gliomas under therapeutic pressure [4]. PMT enhances the tumor initiation and recurrence potential of cancer cells and produces resistance to treatments [5,6]. Hypoxia is the most common microenvironmental stress in glioma [7]. Through metabolic reprogramming and angiogenesis, gliomas adapt to low oxygen content [8,9]. Anti-tumor immunity,

Abbreviations: CGGA, the Chinese Glioma Genome Atlas; CPTAC, Clinical Proteomic Tumor Analysis Consortium; CNS, central nervous system; CTLs, cytotoxic T cells; FFPE, formalin-fixed paraffin-embedded; GBM, glioblastoma; GEO, Gene Expression Omnibus; GLASS, The Glioma Longitudinal AnalySiS; GSCs, glioma stem cells; GSEA, Gene Set Enrichment Analysis; GSVA, Gene Set Variation Analysis; IC50, half-maximal inhibitory concentration; LGG, low-grade glioma; PMT, Proneural-mesenchymal transition; TAMs, tumor-associated macrophages; TCGA, The Cancer Genome Atlas; TME, tumor microenvironment.

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such as activation of cytotoxic T cells (CTLs) in the tumor microenvironment (TME), also poses stress to gliomas [10]. Gliomas recruit immunosuppressive cells including tumor-associated macrophages (TAMs), facilitating their immune escape [11]. These adaptive mechanisms synergistically promote malignant progression and cause therapeutic resistance in glioma patients. Therefore, seeking biomarkers that reflect the adaptive responses is of biological and clinical significance.

The CD99 gene encodes a highly O-glycosylated transmembrane protein, which influence cell adhesion, differentiation, and transendothelial migration [12]. CD99 serves as a biomarker and potential therapeutic target for many tumors [13]. Previous studies have shown that CD99 is overexpressed in gliomas and plays an oncogene role [14]. CD99 affects the morphology and migration of glioma cells through the regulation of actin-related genes [15,16]. In GBM, CD99 is over-expressed mesenchymal and classical subtypes than that in proneural subtype [15], suggesting that it may be related to the PMT. In normal tissues, CD99 is an important functional molecule in the process of leukocyte transendothelial migration and is related to various immune response processes [17,18]. These implied CD99 may have potential immune functions in tumors. Taken together, we suspected that CD99 may be related to the adaptive response of glioma.

Herein, we aimed to use multiomics data to demonstrate the role of CD99 in glioma adaptative responses and expand its clinical significance. We discovered that CD99 is upregulated in more adaptive gliomas cell states, and relates to hypoxia response and immunosuppressive phenotype. CD99 is a biomarker associated with glioma adaptiveness, thus patients with overexpressed gliomas tend to undergo tumor recurrence in a shorter period and have a poor prognosis. CD99 expression is also associated with a variety of treatment responses in gliomas.

Material and methods

The Fudan PGx dataset

Thirty-five (35) glioma patients were enrolled in the Fudan PGx glioma cohort. All patients were newly diagnosed as WHO grade II and had at least 5 years of follow-up. During the five-year clinical monitoring, 12 patients had no recurrence were classified as non-recurrence patients, and 23 patients had recurrence were defined as relapse patients. The samples of these patients were divided into three groups: the primary operation samples of non-recurrence patients (NP samples), the first operation samples (RP samples) and the second operation samples (RS group) of relapse patients. Brain tissues of eight clinical donors (non-tumor patients) were included in this study as controls. The clinical data of the Fudan PGx cohort related to analyses were listed in **Supplementary Data 1**.

Multiomics data were generated using these tumor tissue samples. This work mainly used transcriptomic, proteomic, and metabolomic data. These data can be requested from the National Omics Data Encyclopedia (NODE, https://www.biosino.org/node/) and their accession numbers can be found in **Supplementary Table 1**. The clinical characteristics of the samples from this cohort can be found in **Supplementary Data 3**.

Public datasets

Processed RNA-seq and microarray data of bulk tissues from the TCGA and four other public datasets were obtained from the GlioVis [19] website (http://gliovis.bioinfo.cnio.es/). The expression matrices from the CGGA project [20] were downloaded from the CGGA data portal (http://www.cgga.org.cn/download.jsp; dataset names: mRNA-seq_693, mRNAseq_325, and mRNA-array_301) and expression data were log2 transformed before analysis. CGGA referred to the mRNA-seq_693 dataset unless specified otherwise. The data from the GLASS project [21] were extracted from the synapse repositories (https://www.synapse.org/). The proteomics data of the GBM patients from the CPTAC cohort was downloaded from the PDC data portal (https://proteomic.datacommons.cancer.gov/pdc/) under the study ID of PDC000204 [22]. The fluorescence-activated cell sorting and deep sequencing (FACS-seq) profiles of glioma derived cell population were obtained from the Brain Tumor Immune Micro Environment (BrainTIME) data portal (https://joycelab.shinyapps.io/braintime/) [23]. The scRNA-seq profiles of pan-glioma samples and the patient-derived cell lines were extracted from the synapse repositories [24]. The scRNA-seq profiles of GBM samples conducted by Neftel et al. was obtained from the Single Cell Portal (https://singlecell.broadinstitute.org/single_cell) [25]. Three RNA expression datasets of hypoxic treatment glioma cells were obtained from Gene Expression Omnibus (GEO) under the accession of GSE45301 [26], GSE138535 [27], GSE118683 [28], and GSE78025 [29]. The expression profile of samples from bevacizumab-treated patients was obtained under the accession of GSE79671 [30], and the expression data of mouse tumor samples bearing glioma xenografts treated with different drugs was obtained under the accession of GSE39413 [31]. Data from the phase II clinical trial of nivolumab were collected from the supplementary materials of the literature [32]. All public datasets and their accessions were listed in **Supplementary Table 1**. The clinical characteristics of the patient samples mentioned above can be found in **Supplementary Data 3**.

Transcriptional subtype classification

We processed the RNA-seq profile using the "ssgsea.GBM.classification" R package [4]. This method separately scores the representation of each glioma subtype and simultaneously evaluated the *P* value indicating the significance of each subtype. We exhibit the *P* value of each subtype in the landscape. For analyses requiring assignment to a single subtype for each sample, we considered the subtype with the lowest *P* value. In cases where *P* values were equal, we assigned the sample to the subtype with the highest enrichment score.

Cell stemness index analysis

We used the method developed by Malta et al. to extract features from the transcriptome data in the Progenitor Cell Biology Consortium (PCBC) database [33]. The stemness signatures were applied to the expression profile data of patient samples from the TCGA dataset, and the mRNA-based cell stemness index (mRNAsi) was calculated in **Supplementary Figure 1**.

Enrichment analysis

Gene set enrichment analysis (GSEA) was performed using the "clusterProfiler" R package [34]. The gmt files were downloaded in MSigDB v7.5.1. Gene ranks were tested between the CD99 high expression group and the CD99 low expression group in the TCGA dataset. The intersection of highly correlated genes in the TCGA and CGGA datasets was used for over-representation analysis which was demonstrated in **Supplementary Figure 3**. The Pearson correlation coefficient *R* of other genes and the expression level of CD99 was calculated, *R* greater than 0.5 was considered to be highly correlated.

Deconvolution analyses of glioma microenvironment

We conducted the CIBERSORT algorithm [35] using the "IOBR" R package [36] to deconvolute the bulk expression matrices to infer the cell proportions of each sample which were demonstrated in Fig. 3. Reference signatures were created from the Brain TIME dataset. The raw count data from glioma samples were normalized by TPM quantification. Both the count and the TPM matrices served as input to generate the reference using the "GenerateRef" function under the mode of "DESeq2". Since the TCGA RNA-seq data obtained from the GlioVis were

log2 transformed, we first removed the log2 transformation and then deconvoluted the data using the CIBERSORT function under the relative mode with 500 permutations for significance analysis.

Calculation of immune and metabolism related signatures

The hypoxia score of single-cell dataset demonstrated in Fig. 2 was measured by gene set variation analysis (GSVA) performed by "GSVA" R

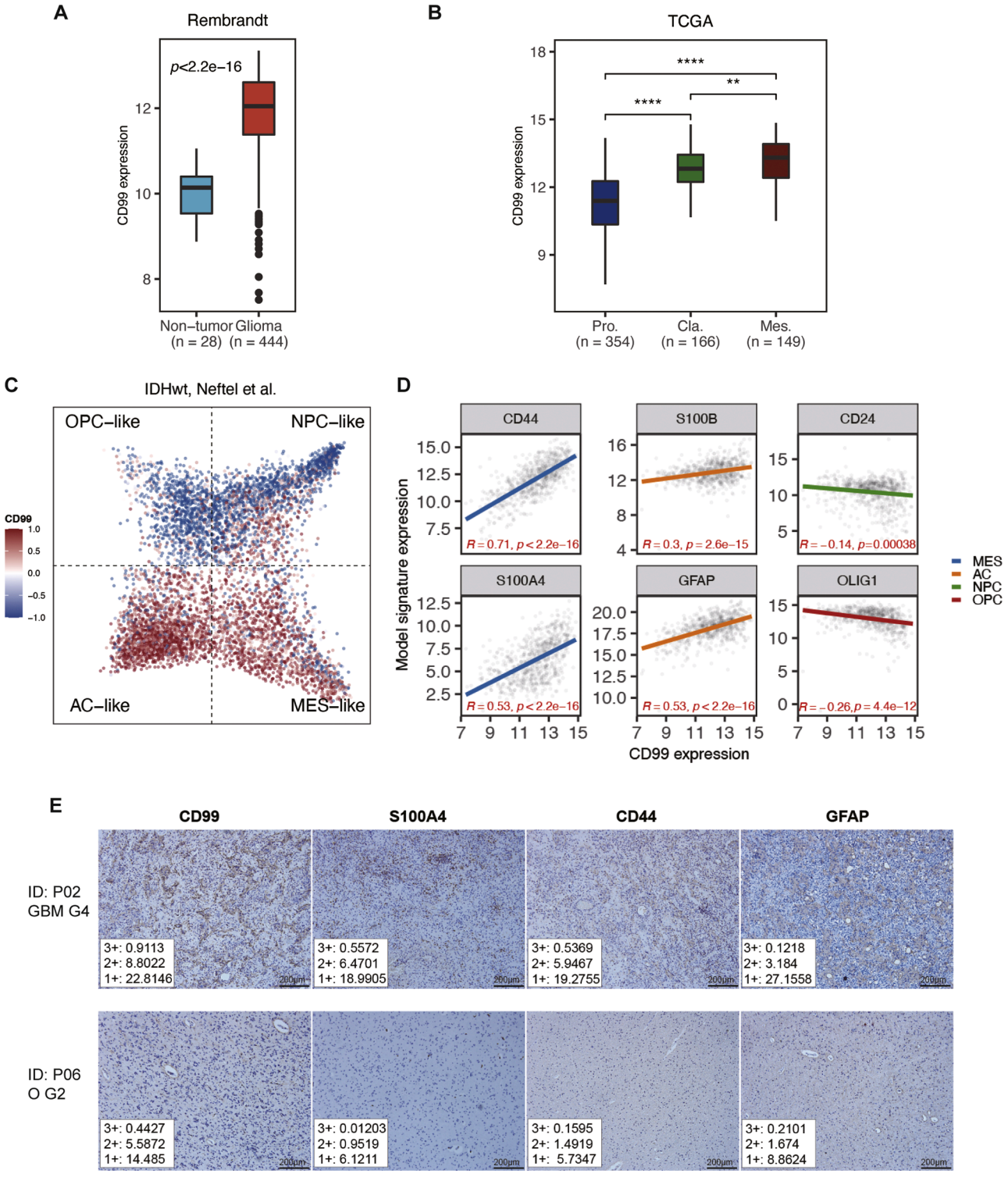

Fig. 1. CD99 is highly expressed in more adaptive glioma subtypes and cell states. (A) CD99 expression in gliomas and non-tumor tissues, results from the Rembrandt dataset. The *P* value was calculated by T-test. (B) CD99 expression of the different transcriptional subtypes in the TCGA dataset. Pro., proneural; Cla., classical; Mes., mesenchymal. ****, *P* value <= 0.0001, ***, *P* value <= 0.001. The *P* value was calculated by T-test. (C) CD99 expression was demonstrated in the Neftel single-cell dataset. The axes are relative meta-module scores. Glioma cells are divided into four cell states: OPC-like, NPC-like, MES-like, and AC-like. (D) Correlation between CD99 and cell state markers. R represents the Pearson correlation coefficient. (E) Representative IHC staining images of CD99 and cell-state markers. 3+, percentage of high positive area; 2+, percentage of positive area; 1+, percentage of low positive area.

package [37]. The immune and metabolism signatures in Fig. 3 and Fig. 4 were evaluated by the "IOBR" R package [36]. We inferred the tumor purity and stromal and immune cell admixture using the ESTIMATE algorithm [38]. The TAMs fraction in **Supplementary Figure 3** was estimated by three algorithms (xCell [39], EPIC [40], and quanTIseq [41]) and scored by three gene sets [42–44]. The metabolic signatures in Fig. 3 and T cell dysfunction gene set **Supplementary Figure 5** were scored using the "calculate_sig_score" function under the PCA mode.

Prediction of immunotherapy responses

The immune checkpoint blockade (ICB) resistance level demonstrated in **Supplementary Figure 5** was predicted using the TIDE method [45] on the webserver (http://tide.dfci.harvard.edu/). The Rembrandt dataset was normalized by non-tumor samples and then served as the input matrices.

Prediction of drug sensitivity

The half-maximal inhibitory concentration (IC50) demonstrated in Fig. 5 was predicted using the "oncoPredict" R package. The gene expression profiles and IC50 data of glioma cell lines in Sanger's Genomics of Drug Sensitivity in Cancer (GDSC) database [46] were used as training data to build ridge regression models. These were then applied to gene expression data of patient samples to predict their drug sensitivity.

Immunohistochemistry (IHC) staining

Ten formalin-fixed paraffin-embedded (FFPE) glioma tissues were obtained from Shandong Provincial Hospital. After deparaffinization, rehydration, antigen retrieval, quenching of endogenous peroxidase, and serum blocking, the sections were incubated with the primary antibodies for 1 to 2 h at room temperature (RT) or overnight at 4 °C. Then, the sections were incubated with horseradish peroxidase (HRP)-conjugated polymer for 30 min at RT. The color development was performed using diaminobenzidine (DAB) solution after thorough rinsing in PBS. Detailed information on the IHC kit and antibodies can be found in **Supplementary Table 2**. The IHC staining images were analyzed using the IHC profiler under ImageJ software.

Quantification and statistical analyses

The statistical analyses were conducted in R v4.2.1. and the "ggplot2" was used for data visualization. Survival analysis and Cox regression were conducted by the "survival" and "survminer" R packages. Time-dependent ROC curves were constructed using the "timeROC" R package [47].

Results

CD99 is highly expressed in more adaptive glioma subtypes and cell states

We validated that CD99 is overexpressed in gliomas compared with non-tumor brain tissues (Fig. 1**A** and **Supplementary Figure 1A**), implying its oncogene role. Next, we confirm that CD99 showed the highest expression in the mesenchymal subtype rather than in classical and proneural subtypes (Fig. 1**B** and **Supplementary Figure 1B**). In addition, we observed that the epithelial-to-mesenchymal transition process was also upregulated in gliomas with highly expressed CD99 (**Supplementary Figure 1C**). These indicate that CD99 expression is associated with PMT, which is an important adaptative response in glioma.

Gliomas adapt to dynamically changing microenvironmental conditions by switching to more adaptative cell states, thus eventually developing resistance to therapy [48]. Neftel et al. classified glioma neoplastic cells into four states according to the expression meta-module [25]. CD99 showed a strong expression inclination to Astrocyte-like (AC-like) and Mesenchymal-like (MES-like) states (Fig. 1**C**), which contribute to the major cell population of mesenchymal-subtype gliomas and are associated with hypoxia responses and immune microenvironment. CD99 was positively correlated with the MES-like and AC-like markers at both transcriptomic and proteomic levels. (Fig. 1**D** and **Supplementary Figure 1D**). This inclination was also observed in the IHC staining results (Fig. 1**E**), wherein glioma sections with high CD99 expression tend to express higher S100A4, CD44, and GFAP. Johnson et al. classified glioma cells into three pan-glioma states, and discovered that differentiated-like and proliferating stem-like states are associated with stress responses [24]. We observed that CD99 was also overexpressed in these cell states and negatively correlated with cell stemness (**Supplementary Figure 1E** and **1F**). Collectively, CD99 is expressed in subtypes and cell states related to adaptative responses.

CD99 is overexpressed in hypoxia adaptation process of gliomas

Hypoxia is the most common stress factor in gliomas. Mesenchymal gliomas are adaptive to hypoxia and thus gain more aggressive phenotypes [49]. Therefore, we suspected CD99 is associated with adaptive responses to hypoxia in glioma. To test this hypothesis, we first examined the hypoxia-related pathway in TCGA glioma samples and discovered that the hypoxia process was upregulated in gliomas with highly expressed CD99 (Fig. 2**A**). Angiogenesis is a predominant downstream response of hypoxia adaptation [7], we found that the angiogenesis gene set in the CD99 high expression group was also significantly upregulated (Fig. 2**B**). The expression of hypoxic markers was also associated with CD99. HIF1AN, the inhibitor of HIF1A, had a negative correlation. VEGFA, the vital regulator of angiogenesis, had a positive correlation (Fig. 2**C**). VEGFB expression also positively correlated with CD99, whereas no significant correlation was discovered with HIF1A, HIF3A, and KDR (**Supplementary Figure 2A**).

To further confirm whether CD99 is upregulated under hypoxic conditions, we tested its expression in glioma cells under hypoxic and normoxic treatment. In three datasets containing the U78-MG cell line, the A-172 cell line, and patient-derived glioma stem cells (GSCs), we found that the expression of CD99 was consistently upregulated after hypoxia treatment (Figs. 2**D**, 2**E**, and 2**F**). We also discovered that compared with the normal human astroglia HEB cell line, CD99 expression had more steep changes in glioma cells U87-MG under hypoxia treatment (**Supplementary Figure 2B**). Due to the tumor heterogeneity, we tested the hypoxia response at the single-cell level. Johnson et al. performed scRNA-seq analyses of patient-derived glioma spheroid-forming cells exposed to continuous hypoxic stress. We evaluated the hypoxia level score of individual cells in this dataset with the GSVA method. CD99 showed a trend consistent with the level of hypoxia response (Figs. 2**G** and 2**H, Supplementary Figures 2C** and **2D**). These discoveries implied that CD99 is a responder of hypoxia adaptation in gliomas.

Metabolic reprogramming is an important mechanism during the hypoxic response. We found that the glycolytic pathway was positively correlated with CD99 expression, whereas the citric acid cycle was negatively correlated with CD99 expression (Fig. 2**I**). These results suggest that gliomas with highly expressed CD99 tend to behave stronger Warburg effect under hypoxic conditions. Next, we analyzed the metabolome data in the Fudan PGx dataset (Fig. 2**J**). The concentration of D-glucose, 6-phosphofructose, and glyceric acid was upregulated in glioma with highly expressed CD99, indicating that their glucose uptake was enhanced and more intermediates of glucose metabolism were accumulated. Fatty acids were negatively correlated with CD99 expression, such as adrenic acid, a common polyunsaturated fatty acid in the CNS. This suggested that fatty acids may serve as an alternative energy source for gliomas under hypoxia. Collectively, we confirm that gliomas with highly expressed CD99 had stronger hypoxia

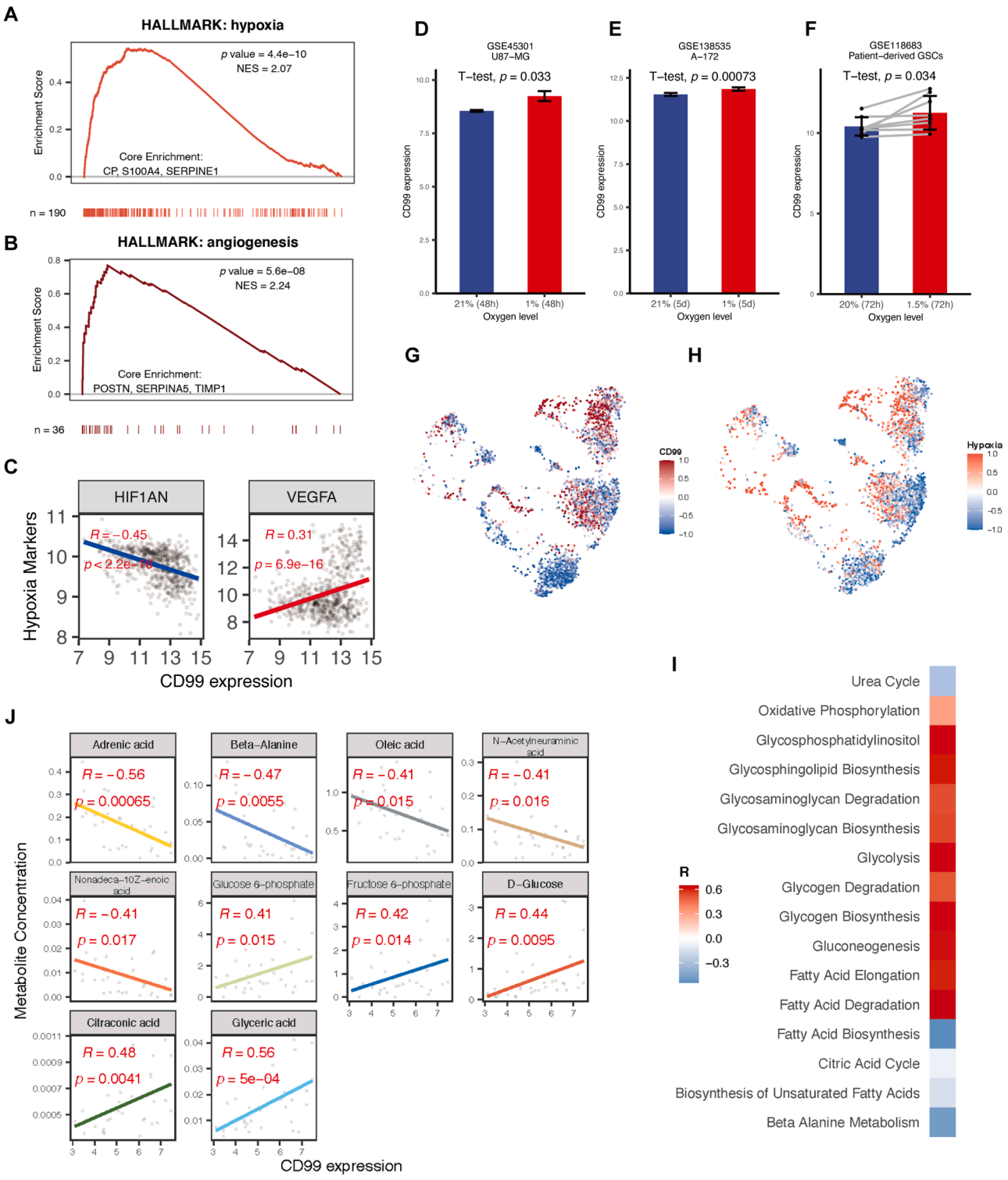

Fig. 2. CD99 is overexpressed in hypoxia adaptation process of gliomas. (A) The GSEA plot in the hypoxia genes set of the TCGA dataset. (B) The GSEA plot in angiogenesis genes set of the TCGA dataset. (C) Correlation between CD99 and hypoxia response markers. R represents the Pearson correlation coefficient. (D) CD99 expression in the U78-MG cell line of the GSE45301 dataset. (E) CD99 expression in the A-172 cell line of the GSE138535 dataset. (F) CD99 expression in eight patient-derived GSCs in the GSE118683 dataset. Paired *t*-test, gray lines represent GSCs from the same patient. (G) The UMAP plot of CD99 expression level in HF3016 GSCs from the Johnson et al. dataset. (H) The UMAP plot of hypoxia score in HF3016 GSCs from the Johnson et al. dataset. (I) The correlation between CD99 expression and hypoxia-related metabolic pathways in the TCGA datasets. (J) The correlation between CD99 expression and metabolites in the PGx dataset. The panel showed metabolites with strong correlations (Pearson correlation coefficient $R > 0.4$).

adaptation.

Increased CD99 expression is associated with the immune adaptation dominated by TAMs in gliomas

Anti-tumor immunity is detrimental to cancer cells, but gliomas reshape the tumor immune microenvironment adapting to this stress and promoting cancer progression [50]. Genes highly correlated with CD99 are enriched in immune-related pathways (**Supplementary 3A** and **3B**). We found that leukocyte transendothelial migration and inflammatory response pathways were significantly upregulated in gliomas with highly expressed CD99 (Fig. 3**A** and **Supplementary Figure 3C**). Next, we found that CD99 was positively correlated with immune scores, and negatively correlated with tumor purity, indicating that gliomas with high CD99 expression had more immune infiltration (Fig. 3**B** and **Supplementary Figure 3D**). The CD99 expression is associated with CD45, a ubiquitous surface protein marker of immune cells (**Supplementary Figure 3E**). In glioma sections with higher expression of CD99, we also observed more positive areas for CD45 (Fig. 3**C**).

To further explore the association between CD99 and the glioma TME, we analyzed different cell fractions to confirm the major cell type influenced by CD99. The expression level of CD99 is related to the composition of immune cells, and the association with TAMs is the

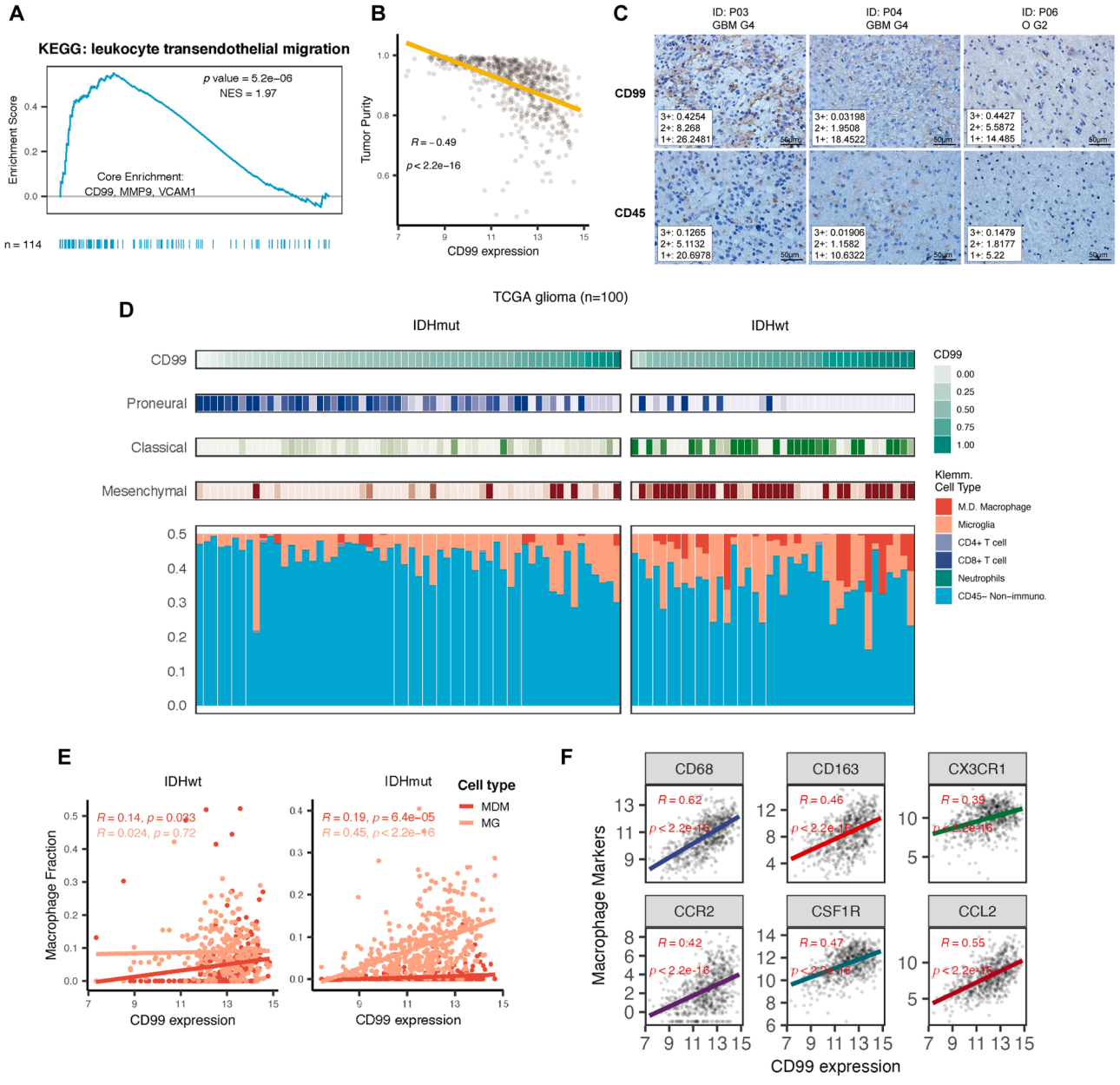

Fig. 3. Increased CD99 expression is associated with the immune adaptation dominated by TAMs in gliomas. (A) The GSEA plot in the leukocyte transendothelial migration and inflammatory response of the TCGA dataset. (B) The correlation between tumor purity and CD99 expression in the TCGA dataset. (C) Representative IHC staining images of CD99 and immune cell marker CD45. 3+, percentage of high positive area; 2+, percentage of positive area; 1+, percentage of low positive area. (D) The landscape of TME heterogeneity in gliomas. Each column represents a sample in the TCGA dataset. Samples are divided by IDH mutation status and then arranged by CD99 expression level. The first track shows the normalized CD99 expression. The next three tracks indicate the *P* value of three transcriptional subtypes. The stacked bar plot in the middle demonstrates the cell fractions of each TME component. (E) The correlation between macrophage fraction and CD99 expression. Left, results from IDHwt gliomas. Right, results from IDHwt gliomas. MDM, marrow-derived macrophages; MG, microglia. (F) The correlation between CD99 expression and immune-related markers in the TCGA dataset.

greatest (Fig. 3**D**). This association is consistent between multiple methods (**Supplementary Figure 3F**). CD99 expression was positively correlated with TAMs in different types of gliomas, and microglia were the main cause of this trend in the IDHmut subtype, while the elevated TAMs components in IDHwt were mainly due to marrow-derived macrophages (MDMs) (Fig. 3**E**). The CD99 expression is correlated with a variety of macrophage expression markers, such as CD68 universally expressed by macrophages, M2 polarization macrophage marker CD163, TAMs surface receptor CSF1R, microglia cell marker CX3CR1, MDMs marker CCR2, and CCL2 secreted by TAMs (Fig. 3**F**). These analyses together suggested that the increased TAMs are the main variation under CD99 expression changes. Since TAMs produce low levels of pro-inflammatory cytokines and lack T-cell co-stimulation factors, causing T-cell exclusion which facilitates tumor escape from immunosurveillance [51]. Thus, the function of CD99 in glioma TME is related to the immune adaptation dominated by TAMs.

Increased CD99 expression indicates short-term recurrence and unfavorable survival for glioma

The adaptive responses, especially the PMT, keep the cell viability under therapeutic stress and detrimental survival conditions, enhancing the recurrence potential of glioma [4,49]. Thus, CD99 may be related to glioma recurrence. To test this hypothesis, we used the PGx cohort to examine CD99 expression in the first-onset samples. At both transcriptomic and proteomic levels, compared to the NP group there was significant overexpression of CD99 in the RP group with short-term recurrence (Figs. 4**A** and 4**B**). In the GLASS cohort containing more recurrence patient, the first-onset samples overexpressing CD99 presents a shorter interval between the first and the second surgery (Fig. 4**C**). These results suggested that overexpression of CD99 is an indicator of glioma recurrence.

Moreover, adaptive tumors often become more aggressive and cause less favorable outcomes in patients, thus making CD99 overexpression to be a risk factor for glioma prognosis. Patients with CD99-overexpressed

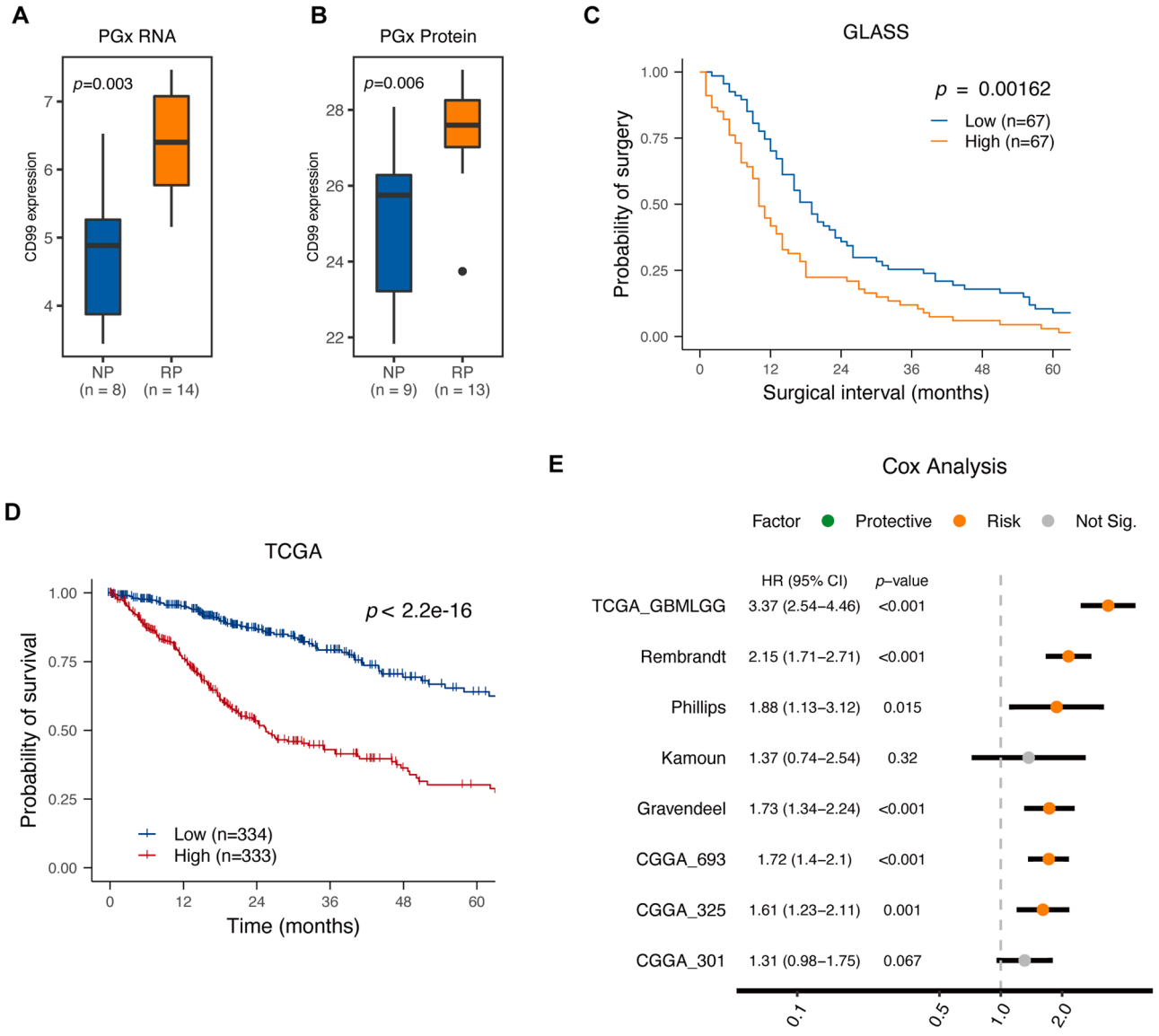

Fig. 4. Increased CD99 expression indicates short-term recurrence and unfavorable survival for glioma. (A) CD99 RNA expression level in the PGx cohort. (B) CD99 protein expression level in the PGx cohort. RP represents the first surgical sample taken from patients with short-term (under 5 years) recurrence, whereas NP represents patients without recurrence in 5 years. (C) Kaplan-Meier curves of the GLASS dataset. The patients were divided into two groups based on the CD99 expression level of the first-onset sample. The x-axis represents the time interval between the first and the second surgery. (D) Kaplan-Meier curves of all glioma patients in the TCGA dataset. (E) Cox-analysis of eight different datasets.

gliomas bear many malignant phenotypes, including higher WHO grade, IDHwt, and malignant histological diagnosis (**Supplementary Figures 4A-C**). The IHC staining showed that GBMs expressed higher CD99 compared to low-grade gliomas (LGG) (**Supplementary Figures 4E** and **4D**). We validate the prognosis value of CD99 in glioma in multiple datasets. The Kaplan-Meier curves consistently showed that the high-expression group presents less favorable survival (Fig. 4**D**). Univariate Cox regression analysis of eight cohorts confirmed that CD99 upregulation is a risk factor for patient survival, with six of the eight datasets showing a significant *P*-value and all hazard ratios over 1 (Fig. 4**E**). After considering other variables, multivariate Cox regression analysis revealed that the CD99 high expression is an independent risk factor associated with the overall survival of glioma patients (**Supplementary Figure 4F** and 4 G, Hazard Ratio [HR] = 1.5, $p = 0.022$). The time-dependent ROC analysis in the TCGA dataset showed that the CD99 expression was able to predict patient survival (**Supplementary Figure 4H**). Taken together, the ability of CD99 for stratifying glioma prognosis was widely demonstrated.

CD99 expression indicates therapeutic responses for glioma

Tumor cells activate adaptive responses under therapy and eventually became resistant, thus we suspected CD99 may be associated with treatment response in glioma. The conventional treatment for glioma is

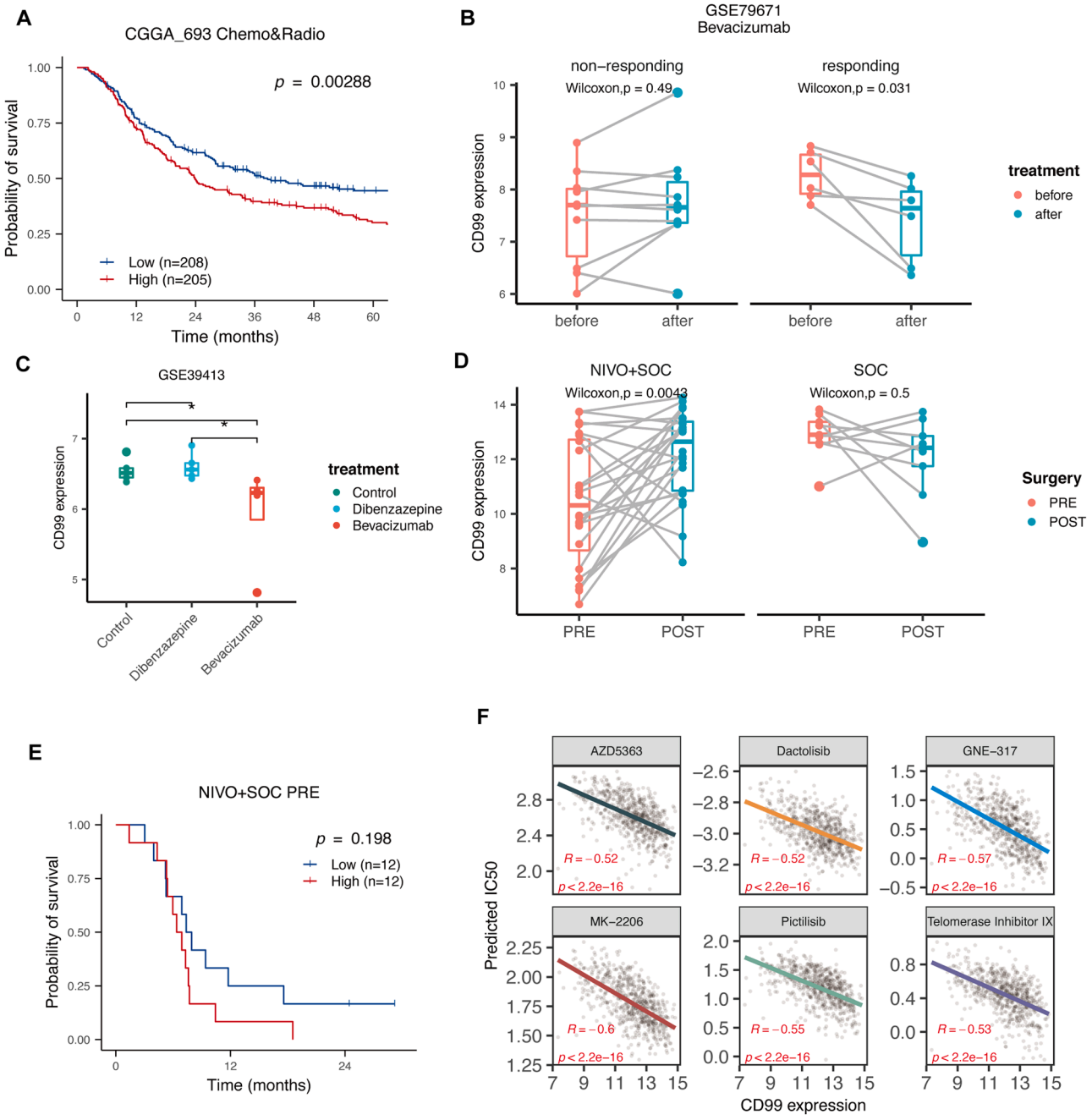

Fig. 5. CD99 expression indicates therapeutic responses for glioma. (A) Kaplan-Meier curves of glioma patients who underwent chemotherapy and radiotherapy in the CGGA 693 dataset. (B) The CD99 expression changes of bevacizumab responding and non-responding patients. (C) The CD99 expression of bevacizumab-treated mice versus dibenzazepine-treated mice and control group. (D) The CD99 expression changes under different treatments. NIVO, Nivolumab adjuvant treatments. gray lines connect the samples before and after the treatment of the same patient. (E) Kaplan-Meier curves of the Schalper dataset; patient groups were divided by pre-treatment CD99 expression level. (F) The correlation between CD99 expression and the predicted IC50 of PI3K-AKT pathway inhibitors of the TCGA glioma samples.

surgical resection adjuvanted with radiotherapy and/or chemotherapy. Survival outcomes were analyzed in the CGGA data set by screening patient samples who received concurrent chemoradiotherapy, chemotherapy alone, and radiotherapy alone. We found that patients with CD99-overexpressed gliomas had poorer survival after treatment regardless of whether they chose monotherapy or combination therapy, indicating that the treatment effect is poor (Fig. 5**A** and **Supplementary Figures 5A-D**).

Since gliomas which highly expressed CD99 are adaptable to hypoxia, we speculated CD99 expression is associated with the response to anti-angiogenic therapy. To test this hypothesis, we analyzed samples from a cohort of patients with recurrent glioma treated with bevacizumab who underwent bevacizumab therapy followed by resection of the recurrent tumor [30]. In the responding patients, the expression of CD99 in the tumor after treatment decreased significantly compared with the tumor from the first onset. However, in the non-responding group, there was no significant difference in the expression level of CD99 before and after administration (Fig. 5**B**). In mice bearing glioma xenografts treated with bevacizumab, the expression of CD99 after treatment was significantly down-regulated compared with two control groups (placebo group and dibenzazepine-treated group) (Fig. 5**C**). In summary, we believe that whether CD99 is down-regulated after bevacizumab treatment is related to the treatment response.

In previous analyses, we found that CD99 is highly correlated with TAMs, therefore, we speculate that it also affects the immunotherapy effect of glioma. To test this hypothesis, we first analyzed T cell dysfunction and immune checkpoint blockade (ICB)-related features in the TCGA dataset (**Supplementary Figure 5E**). We found that increased CD99 expression was negatively correlated with T cell accumulation but positively correlated with T cell exhaustion and other adverse factors, including TAMs, myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs). Next, we used the TIDE algorithm to predict the level of resistance to ICB therapy in the Rembrandt dataset, and CD99 was positively correlated with the TIDE score, suggesting that CD99 is an unfavorable factor for ICB therapy (**Supplementary Figure 5F**). To further explore the impact of CD99 expression on ICB therapy, we analyzed a cohort including primary and secondary surgery samples from 27 patients with recurrent glioma who received neoadjuvant nivolumab therapy [32]. These patients received nivolumab administration followed by tumor resection. Compared with patients who received standard of care (SOC) alone, patients treated with nivolumab showed a significant upregulation of CD99 after dosing, suggesting that CD99 is a responsive factor to ICB therapy (Fig. 5**D**). In addition, patients with higher expression levels of CD99 before dosing had poorer survival (Fig. 5**E**). But this inclination was not reflected by post-dosing CD99 expression (**Supplementary Figure** 5 G).

We further explored the association between CD99 and drug sensitivity of glioma to screen for potential therapeutic targets. We found that CD99 was significantly negatively correlated with the IC50 of PI3K-AKT pathway inhibitors, which indicated that gliomas with high CD99 expression may be more sensitive to these drugs (Fig. 5**F**). GSEA analysis showed that gliomas with high expression of CD99 were up-regulated in the PI3K-AKT pathway (**Supplementary Figure 5H**). Therefore, PI3K-AKT pathway inhibitors may provide new therapeutic strategies for patients with high CD99 expression.

Discussion

Glioma cells develop survival advantages by adapting to stressors. Under the pressure of treatments, glioma cells acquire resistance through PMT [4]. At the same time, the interaction between glioma cells and TME cells inhibits anti-tumor immunity, which brings challenges to immunotherapy [52]. Therefore, discoveries of biomarkers related to glioma adaptiveness enable understanding of glioma malignancy and thus assist clinical decision-making.

In this study, we proposed that CD99 is a biomarker related to glioma adaptive responses by integrating multi-dimensional omics data, and further proved its clinical value. CD99 is highly expressed in MES-like and AC-like cell states of glioma and is related to PMT. Overexpression of CD99 implies a higher level of hypoxic responses, including the initiation of transcriptional programs and metabolic reprogramming. Gliomas with high expression of CD99 also recruit more TAMs to help the immune escape of cancer cells.

Glioma patients with high expression of CD99 are prone to relapse in a short period of time and often have worse postoperative survival. In addition, they have relatively insufficient responses to ICB therapy. Therefore, for patients with gliomas with high expression of CD99, tumor adaptability should be fully considered clinically. Such patients are prone to resistance to monotherapy thus multi-drug combinations should be taken into consideration, such as combining with anti-angiogenic therapy and PI3K-AKT pathway inhibitors. Moreover, closer clinical detection and follow-up should be carried out.

However, the studies require further experimental verification for the mechanism of CD99 signaling. Previous studies have launched gene silencing experiments in U87MG cells and observed a significant morphological change and a decrease in cell migration, which is related to the down-regulation of actin dynamics genes [15]. Whether the change of CD99 expression can lead to the transition of cell states still needs knockout and overexpression experiments in multiple glioma cell lines with transcriptomic profiling to identify the changes in cell state markers. Although we observed the increase of CD99 expression in glioma cells under hypoxic conditions, the upstream and downstream molecules of CD99 signaling involved in the hypoxic response still require further studies. Additionally, we found that gliomas with high expression of CD99 are related to the increase of TAMs, but the cellular mechanism is still unknown. To address this problem, patient-derived xenograft (PDX) models need to be constructed to observe the recruits of TME cells in vivo. This work points out several future directions for studies of the biological function of CD99 in glioma.

Recent studies have shown that the adaptive responses in gliomas are interconnected. For instance, hypoxia induces mitochondrial dysfunction, which attenuates the cytotoxic and inflammatory functions of immune cells, allowing TAMs to exhibit an immunosuppressive M2-like phenotype [53]. The accumulation of lactic acid in the TME due to hypoxia can activate the function of Tregs, thereby further suppressing the anti-tumor immune response. TAMs can also induce PMT in glioma cells. The Oncostatin M (OSM) on TAMs can interact with the OSM receptor (OSMR) on GBM cells to induce the up-regulation of a series of major histocompatibility complex (MHC) genes, thus making the GBM cells transform into an MES-like state [54]. Since CD99 is related to many types of adaptive responses, it may be at the crossroad of these signaling pathways.

In terms of the clinical value of CD99 expression, the results are mainly based on public data sets from retrospective research, lacking validation and quantitative research of prospective cohorts. The studies on anti-angiogenic therapy and ICB therapy also require larger cohorts. The screening of drugs based on bioinformatic prediction lacks pharmacodynamic experiments.

Notably, the role of CD99 in glioma anti-angiogenic therapy has recently attracted interest. It was found that in glioma samples with a high cuproptosis signature, both VEGFA and CD99 were highly expressed [55]. In a study using proteomic analysis to predict resistance to antiangiogenic therapy in recurrent GBM, CD99 was screened as a potential biomarker positively correlated with drug response and used for modeling [56]. In a study of various solid tumors such as osteosarcoma, targeting CD99 with a tumor vaccine can inhibit angiogenesis and tumor growth [57]. Based on our observation, we suspected that CD99 involve in hypoxia-induced angiogenesis and became an indicator of response to anti-angiogenesis therapy. Therefore, further exploration of the biological mechanism of CD99 in glioma angiogenesis has promising clinical significance.

Conclusions

In conclusion, gliomas with high CD99 expression have significant tumor adaptiveness and malignancy. For glioma patients with overexpressed CD99, the combination of therapies should be considered to avoid drug resistance, and closer clinical monitoring and post-treatment follow-up should be carried out.

Declarations

Ethics approval and consent to participate

This study involving human participants was reviewed and approved by the Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University (Accession: SWYX.NO.2020–180).

Consent for publication

All the authors listed have contributed significantly and approved the manuscript for publication.

Data statement

The public datasets and the original contribution analyzed in this study can be found in online repositories. The accession can be found in Supplementary material. Further inquiries can be directed to the corresponding author.

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Authors' contributions

YTZ, YCL, SW and EFS conceived the study. EFS conducted most of the bioinformatics analysis and data interpretation. SYS conducted the patient sample collection and multiomics sample preparation for the Fudan PGx cohort and performed the IHC staining experiment. RLZ contributed to the bioinformatics analysis and interpretation and helped refine the data visualization. ZHC helped with the data interpretation and revised the manuscript. QWC contributed to the data processing of the Fudan PGx cohort and manuscript revision. EFS drafted the manuscript. YTZ, YCL, SW, JSW, and LMS contributed to data interpretation and revised the manuscript. All authors reviewed and approved the manuscript.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2023.101759.

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