CDC42—a promising target of glioma treatment related to Treg cell proliferation

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Abstract

Glioma is the worst prognostic neoplasm in the central nervous system. A polarity-regulating GTPase in cells, which is called CdC42, has been proven that its overactivation is tightly connected to the high malignancy of tumor. The RNA-seq and protein expression of CDC42 in tumor and comparison tissues were analyzed based on the online tools, CDC42 is remarkably boosted in tumor tissue compared to controls. 600 patients in the analysis set from the TCGA database and 654 patients in the validation set from the Chinese Glioma Genome Atlas (CGGA) database were adopted. The expression of CDC42 in various clinicopathological features was analyzed, including differential expression, survival analysis, GO and KEGG analysis, immune infiltration, correlated signaling pathway. It was found that CDC42 could be a potential biomarker of glioma transcriptional subtyping. The enrichment of CDC42 was shown to be an independent indicator of poor prognosis for glioma by Cox analysis and KM curves. Additionally, the concentration extent of CDC42 was closely related to immune infiltration, immune checkpoint inhibitors, and Treg cell markers (CD4, CD25, CD127). Further GSEA analysis demonstrated that CDC42 was significantly connected with the differentiation, migration and proliferation of T regulatory (Treg) cell through the PI3K/AKT signaling pathway.

Introduction

The most common primary intracranial tumors is gliomas\(^1\). Despite the widespread use of surgery, chemotherapy, radiotherapy\(^2\), glioma have a high probability of recurrence due to the residue of tumor and cancer stem cells, the average integral survival time of glioma patients is no more than 15 months\(^3\). In recent years, tumor-promoting microenvironment regulation and targeted immunotherapy have provided novel approaches for the treatment of glioma\(^4\). The development of immune targets including PD1, PDL-1 and KLRB1\(^5,6\) lays the groundwork for further tumor-free treatment of glioma. Treg immune cells are essential for the prevention of autoimmune diseases, and its infiltration in the tumor microstructure is conducive to microenvironment alleviation, tumor swelling, invasion and metastasis, angiogenesis and the suppression of antitumor immunity. Additionally, cancer tissue with a large number of Treg cells infiltrated participate in the adverse prognosis of patients\(^7\). CDC42 is a small G protein with GTPase activity belonging to the Rho protein family that regulates multiple signaling pathways and participates in multiple biological processes. These processes involve the formation of filamentous pseudopodia and the induction of finger-like protrusions, which play an indispensable role in cell migration, invasion, and metabolism\(^8\). Targeted inhibition of CDC42 expression has been shown to release the antineoplastic ability of effector T cells\(^9\), so further exploration the mechanism and efficacy of CDC42 in tumor evolution and extension is essential for targeted therapy of glioma. The TCGA and GTEx databases were adopted for differential analysis of CDC42 expression in pan-cancer, and further glioma RNAseq data and clinical information were downloaded from TCGA and CGGA databases to explore the relationship between CDC42 and glioma development through a collection of bioinformatics and survival analysis. Then the differential genes were screened to execute GO and KEGG enrichment analysis. The interrelation between CDC42 and immune checkpoint inhibitors or Treg
cell markers were revealed through analysis. GSEA analysis further confirmed the correlation between CDC42 and PI3K/AKT signaling pathway which was related to Treg cell migration, proliferation and metabolism in TCGA and CGGA databases gliomas\textsuperscript{10}.

**Materials And Methods**

**Data sources.** The RNAseq and matching clinical data of 598 patients (patients with incomplete data were deleted) with glioma in TCGA were obtained from the official website of TCGA, and 657 patients were used as validation set which gained from the public CGGA database. TIMER (http://TIMER.cistrome.org/) database was used to analyze the interrelation of CDC42 expression and immune infiltration between different tumors and controls. The GEPIA (http://geopia.cancer-pku.cn/) database and UALCAN (http://ualcan.path.uab.edu) database were adopted to supplement and discover the level of CDC42 total protein expression in tumor and controls. The c5.go.v2022.1.Hs.symbols and c2.cp.kegg.v2022.1.Hs.symbols.gmt datasets were obtained from the Molecular Signatures Database (MsigDB)

**Gene and protein expression analysis of pan-cancer.** The TIMER 2.0 database is a comprehensive resource \textsuperscript{11}, CDC42 were submitted in the "Gene_DE" column affiliated exploration module of the TIMER 2.0 website. Then CDC42 expression differences between tumors and adjacent tumor-free tissues for 32 cancer types from the TCGA database were analyzed. Using matched TCGA normal and GTEx data as controls, we supplemented the expression of CDC42 in TCGA tumors using the web-based tool GEPIA\textsuperscript{12}. UALCAN is an interactive portal\textsuperscript{13} to analyze CDC42 protein expression in tumor and normal samples using data from the Clinical Proteome Tumor Analysis Consortium (CPTAC, http://ualcan.path.uab.edu/Analysis prot.html).

**Analysis of CDC42 expression in gliomas.** The profiles of relevant clinicopathological and molecular biological features of CDC42 expression in gliomas were analyzed from TCGA and CGGA databases, including age, sex, pathology, isocitrate dehydrogenase IDH mutation status, 1p/19q codeletion status, MGMT promoter methylation status, WHO grade and transcriptional subtyping. The significance of the dissimilarity was examined by t-test and one-way ANOVA, and the effectiveness of the predictive model with high expression of CDC42 in mesenchymal classification was verified using ROC curves.

**Survival analysis.** Kaplan-Meier curve was portrayed to estimate the prognostic value of CDC42 in glioma patients through TCGA and CGGA database. With median survival as the cut-off value, patients were separated into two groups of low and high levels, and results were visualized utilizing the "survival" R package. The prognostic-related independent risk factors were evaluated through univariate Cox and multivariate Cox proportional hazards regression model. P<0.05 was regarded as statistically significant.

**Gene function enrichment analysis.** The CDC42-combination protein were obtained from the STRING online tool (https://string-db.org)\textsuperscript{14} and the functional annotations of each binding protein were downloaded. The CDC42 were entered in the “Proteins by name” query and “Homo sapiens” were selected
for organisms. Furthermore, following main parameters were confirmed: network type ("full STRING network"), required score ["medium confidence (0.400)"], and size cutoff ("no more than 20 interactors"). R statistical software “limma” package\textsuperscript{15} screened differential genes (Log Fc>0.5, p<0.05), the genes most related to CDC42 expression were analyzed by GO and KEGG with R package, the top 15 pathways most relevant to expression were screened, and the column and bubble charts were drawn.

**Immune infiltration and correlation analysis.** We entered the CDC42 gene into the TIMER database in the “Genes” module, which is connected to the “Immunity” board and defined the type of immune infiltration to acquire information about the level of immune invasion in LGG and GBM. Several major immune infiltrating cells were covered, including B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, dendritic cells. The correlation between CDC42 and relevant immune checkpoint inhibitors was studied and circos plot (R pack) was plotted. Finally, the correlation between CDC42 and Treg cell markers expression levels were analyzed and the relevant scatter plots were plotted using SPSS v.25.0 software.

**GSEA analysis.** To excavate the relevance between CDC42 expression and PI3K/AKT signaling pathway which play a critical status for migration, proliferation and metabolism of Treg cells, GSEA\textsuperscript{4.3.2} software \textsuperscript{16} was used. Depending on the median expression values for CDC42, samples were divided into high and low levels. NOM P values < 0.05, |NES|>1 and FDR q<0.25 were supposed statistically significant.

**Statistical analysis.** All statistical analysis were performed using RStudio and SPSS v.25.0 software (IBM Corp.). The significance of the distinction between two groups was tested by unpaired t test. One-way ANOVO was performed on multiple groups of samples to assess significant differences among three groups and above. Pearson correlation analysis was utilized to assess the degree of the correlation between two objects.

**Results**

**CDC42 is enriched in human pan-cancer.** Pan-cancer analysis was accomplished through the TCGA and GTEx databases, including mRNA and protein expression distinction between tumor and adjacent normal tissues in different organs. And the CDC42 expression levels in various clinical and molecular biological characteristics in gliomas was explored. The functional enrichment of differential genes was analyzed, subsequently the correlation between CDC42 and relevant immune checkpoint inhibitors and cell markers were investigated. GSEA was applied to the correlation study of CDC42 and related signaling pathways. The specific flow chart is as follows (Figure 1)

TIMER2.0 was applied to study the mRNA expression levels of CDC42 for the entire 32 tumors in the TCGA database in order to explore the difference in CDC42 expression between tumors and adjacent tumor-free tissues. The outcome demonstrated that the expression rates is relatively high in BRCA (breast invasive carcinoma), CHOL (cholangiocarcinoma), ESCA (esophageal carcinoma), HNSC (head and neck
squamous cell carcinoma), LIHC (liver hepatocellular carcinoma), STAD (stomach adenocarcinoma) compared with the corresponding tumor-free tissues (Figure 2A).

In addition we considered that TIMER 2.0 lacked certain normal tissues as controls, we supplemented GTEx database normal tissues as controls, and found that CDC42 was highly expressed in CHOL (cholangiocarcinoma), GBM (glioblastoma multiforme), PAAD (pancreatic adenocarcinoma), STAD (stomach adenocarcinoma) and TGCT (testicular germ cell tumors) than normal tissues (Figure 2B).

The normal metabolism of tissues and various life activities are closely related to protein functions. The CTPAC analysis implied that CDC42 protein was enriched in UCEC (uterine corpus endometrial carcinoma), KIRC (kidney renal clear cell carcinoma), HNSC (head and neck squamous cell carcinoma) and HCC (hepatocellular carcinoma) than normal tissues (Figure2C). All evidence suggested that CDC42 was enriched in multiple tumors, it manifested that this molecule may be a promising pan-cancer marker.

**CDC42 is associated with glioma clinicopathological characteristics.** CDC42 with different expression levels of showed diverse clinicopathological characteristics, showing an asymmetric distribution in IDH mutation status, MGMT promoter methylation status, 1p/19q codeletion status and WHO grade (Figure 3), which were analyzed in the TCGA database and validated in the CGGA database. We found that CDC42 was highly enriched in high-grade gliomas, IDH wild-type patients (Figure3 C, G), non-methylated patients (Figure3 D, I) and non-codel patients (Figure3 E, J). Glioma transcriptional subtyping is a widely used molecular diagnostic technique\(^{17}\). CDC42 expression was higher in the mesenchymal categorization (Figure 4 A, C), which may predict that CDC42 was the molecule with prediction in transcriptional subtyping. The ROC prediction curve (Figure4 B, D) was established and the effectiveness of this model was confirmed by

**CDC42 highly expressed tumors have a significantly poor prognosis.** According to an analysis of the KM curve and the cox proportional hazard model based on the TCGA and CGGA databases (Figure 5A), patients with high CDC42 expression (median survival 639 days) in the TCGA database had considerably shorter overall survival compared to patients with low CDC42 expression (median survival 3519 days), this conclusion was verified in the CGGA database (Figure 5B). CDC42 expression was a predictive predictor in univariate and multivariate cox regression studies, independent of other known prognostic markers, such as WHO grade, age at diagnosis, IDH mutation, deletion of 1p/19q coding, and MGMT promoter methylation. These results imply that CDC42 in the TCGA and CGGA datasets is an independent predictive factor (Tables 1 and 2).
Table 1. Prognostic factors in the TCGA database: a multivariate and univariate analysis of overall survival (OS). Abbreviations: CI, confidence interval; HR, hazard ratio; IDH, isocitrate dehydrogenase; WHO, world health organization.

Table 2. Prognostic factors in the TCGA database: a multivariate and univariate analysis of overall survival (OS). Abbreviations: CI, confidence interval; HR, hazard ratio; IDH, isocitrate dehydrogenase; WHO, world health organization.

CDC42 is significantly associated with cellular immune activities. 20 CDC42 binding proteins were screened using for PPI network analysis (Figure 6A) and the binding protein-related annotations (Table s1) were downloaded. In order to investigate the biological functions and molecular mechanism connection between CDC42 and tumors, differential analysis of genes associated with CDC42 expression was performed using the “limma” package, the genes most relevant to CDC42 were screened in the TCGA database and the CGGA database (p<0.05, log Fc>0.5), a differential gene expression heatmap was
drawn (Figure S1 S2), and R packages were used to screen the genes for GO and KEGG analysis. Bioinformatics was applied to observe the enrichment results using the Gene Ontology bar and bubble plots. GO analysis of TCGA and CGGA databases manifested that CDC42 high-expression genes were intimately related to leukocyte migration and leukocyte migration regulation (Figure 6B). KEGG enrichment analysis showed that CDC42 was associated with signaling pathways such as Neuroactive ligand receptor interaction, MAPK signaling pathway, and CAMP signaling pathway and was involved in tumorigenesis and development (Figure 6C). These results indicate that CDC42 on glioma cells may be crucial for immune response and disease regulation.

**CDC42 is highly correlated with tumor immune infiltration.** The association between CDC42 and immune cells in LGG and GBM was examined using the online program TIMMER2.0 to investigate the interaction between CDC42 and the tumor microenvironment, and the results (Figure 7) showed that B cells (0.682, p=1.26e-66), CD8+ T cells (0.52, p=1.91e-34), CD4+ T cells (r=0.422, p=4.96e-22), Macrophage (r=0.473, p=1.06e-27), Neutrophil (r=0.62, p=8.42e-52) and Dendritic cell (r=0.604, p=1.32e-48) cells were positively correlated with CDC42 expression in LGG, and B cells (r=0.03, p=5.35e-01), CD8+ T cells (r=0.165, p=6.82e-04), Macrophage (r=0.054, p=2.75e-01) and Neutrophil (r=0.242, p=5.77e-07) were positively correlated in CDC42 expression in GBM. Immune cells express a class of immunosuppressive molecules known as immune checkpoint inhibitors, which can control the level of immunological activation. Immune evasion of tumors can be accomplished by upregulating immune checkpoint inhibitors. The relationship between the expression of CDC42 and the pertinent immune checkpoint inhibitors was investigated (Figure 8). The TCGA database showed that CDC42 expression was positively correlated with TIM-3, HAVCR2, HVEM, TNFRSF14, IDO, IDO1, CD27L, CD70, CD200R1, PD-L1, PD274, PD-1, PDCD1, CTLA4, BTLA, LAG3, and CD47 immune checkpoint inhibitors in gliomas, and this result was verified in the CGGA database.

**CDC42 is associated with the development of Treg cells in the tumor microenvironment.** According to the literature, Treg cells play an indispensable role in tumor immune evasion. The correlation between CDC42 expression in glioma and three markers of Treg cells (CD4, IL2RA (CD25), IL7R (CD127)) (Figure 9) was explored by person correlation analysis using Stata software, the results showed that CDC42 expression correlated with CD4 (r=0.552, p<0.001), IL2RA (r=0.560, p<0.001) in the TCGA database. The expression results of CD4 (r=0.326, p<0.001), IL2RA (r=0.244, p<0.001), IL7R (r=0.447, p<0.001) were demonstrated in the CGGA database, and the level of CDC42 expression may be related to the effectiveness of immunotherapy.

**CDC42 is positively correlated with Treg cell development-related pathways.** PI3K/AKT signaling pathway occupies a critical role in Treg cell development, functional implementation and cell stability, it has been reported that PI3K-AKT signaling pathway is involved in the migration, proliferation, metabolism and other processes of Treg cells. In order to further explore the role of CDC42 expression in PI3K/AKT pathway in the tumor microenvironment Treg cell migration and proliferation, GSEA analysis was performed. The results of GSEA provide a clearer explanation of the critical role played by CDC42 in the
migration and proliferation of Treg cells in gliomas by demonstrating a positive correlation between CDC42 expression and the PI3K/AKT signaling pathway (Figure 10).

Discussion

Glioma is the most common and deadliest tumor of the nervous system, and even with the implementation of various treatments such as surgery, radiotherapy, and chemotherapy, the treatment of glioma has not achieved satisfactory results. With the advancement of immunotherapy (immune checkpoint inhibitors, tumor-associated macrophages, dendritic cell vaccines, CAR-T), tumor microenvironment and other related therapies, actively exploring new immunotherapy targets in the treatment of glioma is the current mainstream research direction. Because new targets have achieved positive results on various tumors, exploring and finding immunotherapy targets for glioma provides an active and important role for the treatment of glioma.

CDC42 is a signaling molecule with GTPase activity in the Rho family, which is closely associated with regulating the dynamic tissue and membrane transport of the cytoskeleton to promote physiological processes such as cell proliferation, motility, polarity, cell division and cell growth. The upregulation of the expression of CDC42 is closely related to the development and metastasis of gastric cancer, breast cancer, lung cancer and other tumors. It was found that CDC42 was highly expressed in a variety of tumor tissues and there are significant differences in the early stages of tumors. CDC42 was found to be closely related to prognosis-related pathological features of glioma, KM curves indicate that patients with high CDC42 expression have a poor prognosis and shorter total OS, and UCR and MCR indicate that CDC42 expression can be used as an independent risk factor for glioma. Functional enrichment analysis showed that CDC42-related genes were related to regulatory functions such as leukocyte migration, and KEGG pathway analysis showed that cAMP, MPKA and other signaling pathways were involved in tumorigenesis. In addition, CDC42 expression was positively correlated with several immune cell and Treg cell markers in LGG and GBM. Finally, GSEA showed that CDC42 expression may affect the PI3K/AKT signaling pathway and promote the proliferation and migration of Treg cells.

By releasing inhibitory brakes on T cells, immune checkpoint inhibitors effectively activate the immune system and trigger effective antitumor immune responses. Immune checkpoint inhibitors that currently target three different molecules have been approved for use in humans by the US Food and Drug Administration (FDA). CDC42 was found to be positively correlated with numerous immune checkpoint inhibitors (TIM-3, HVEM, IDO, CD27L, CD200R1, PD-L1, PD-1, CTLA-4, BTLA, LAG-3, CD47) in the TCGA and CGGA databases. This showed that CDC42 articulation levels might be related with cancer resistance and immunotherapy.

Our findings show that CDC42 is favorably connected with the crucial PI3K-AKT signaling system, which regulates cell division and proliferation. It has also been proposed that CDC42 may promote the growth and metastasis of cancer cells by altering the PI3K-AKT pathway. Additionally, the PI3K/AKT pathway is crucial for the differentiation of CD4 T cells into Treg cells and for preserving the homeostasis of the
peripheral T cell environment. However, the relationship between CDC42 and PI3K/AKT signaling pathways in the pathogenesis and development of glioma and Treg cells proliferation and differentiation needs to be further elaborated.

Although the relationship between CDC42 and a variety of cancers in multiple databases was integrated, and the expression characteristics of CDC42 in glioma was also analyzed, the study still has certain limitations. In vivo and in vitro experiments are needed to confirm the findings and to conduct additional mechanistic research. In conclusion, the prognoses of glioma patients are influenced by CDC42, which is associated to immune infiltration, and CDC42 could be an immuno-therapeutic target for glioma. The treatment of gliomas and other types of tumors may benefit from these findings.

Declarations

Data availability.

The online datasets for this article can be found on the relevant online repositories. Both the accession numbers and the names of the repository(s) are listed in the article. The datasets used for analysis provided by the Chinese Glioma Genome Atlas (CGGA) database (https://www.cgga.org.cn) and the Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). The enrichment gene set was obtained from the Gene Set Enrichment Analysis (GSEA) database (https://www.gsea-msigdb.org/gsea).

Author contribution statement

T.J.: Article design, Conception, Data acquisition, Data collation, Statistical analysis, Software, Picture drawing, investigation, Writing-review and editing. X.W. and J.H. investigation, Writing-review and editing. D.C. Final approval, Writing-review and editing.

Competing interests

The authors declare no competing interests.

Additional information

The additional information was shown below

Figure S1 The differential gene expression heatmap in TCGA database.

Figure S2 The differential gene expression heatmap in CGGA database.

Table S1 Annotations on CDC42-binding proteins.

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References


Figure 1

The process of this study. Pan-cancer analysis was carried out using the TCGA and GTEx databases. For glioma-related analysis, the Cancer Genome Atlas (TCGA) databases and Chinese Glioma Genome Atlas (CGGA) were employed.
mRNA expression level of CDC42 in pan-cancer. (A) Human CDC42 expression levels in different cancer types from TCGA data in TIMER. *p <0.05, **p <0.01, ***p < 0.001; For the type of CHOL, GBM, PAAD, STAD, and TGCT in the TCGA project, the normal tissues of the GTEx database were included as controls. Data for the box plot were provided. *p <0.05, **p <0.01; (B) CDC42 proteomic expression profile in UCEC, KIRC, HNSC and HCC from CPTAC samples. Standard deviations from the median for samples for a given cancer type are shown by z-values. N indicates how many samples there are.
Figure 3

Association between CDC42 and clinicopathological characteristics of gliomas. (A) Overview of TCGA database glioma clinicopathological characteristics connected to CDC42; (B) Overview of CGGA database glioma clinicopathological characteristics connected to CDC42; (C) CDC42 was significantly increased in gliomas without isocitrate dehydrogenase (IDH) mutation in the TCGA and CGGA databases. An unpaired t test was performed to determine the difference’s significance; (D) and (H) CDC42 was
increased in the O6-methylguanine-DNA methyltransferase (MGMT) promoter–unmethylated gliomas in the TCGA and CGGA databases. An unpaired t test was performed to determine the difference's significance; (E) and (I) CDC42 was significantly increased in gliomas without 1p/19q codeletion in the TCGA and CGGA databases. An unpaired t test was performed to determine the difference's significance; (F) and (J) CDC42 was significantly increased in higher-grade gliomas in the TCGA and CGGA databases. One-way ANOVA was performed to determine the difference's significance.

Figure 4
CDC42 is specifically enriched in the mesenchymal subtype of gliomas. 

(A) and (C) CDC42 was enriched in the mesenchymal subtype of gliomas in the TCGA and CGGA databases. One-way ANOVA was performed to determine the difference's significance; (B) and (D) The TCGA and CGGA databases' receiver-operating characteristic (ROC) curve revealed that CDC42 had a high expression specificity for the mesenchymal subtype of gliomas. AUC, area under the curve.

![Graphs showing survival probability and survival days for TCGA and CGGA databases.](image)

**Figure 5**

Gliomas with high expression of CDC42 have a poor prognosis. (A) and (B) CDC42 expression in gliomas according to the Kaplan-Meier curve in the TCGA and CGGA datasets. The median expression of CDC42 serves as the group's cutoff point. A log-rank test was used to determine the significance of the prognostic value.
Figure 6

CDC42-related gene enrichment analysis  A  CDC42 interaction network analysis; B  GO analysis of CDC42 in the TCGA and CGGA databases; C  KEGG enrichment analysis of CDC42 in the TCGA and CGGA databases.
Figure 7

The correlation between CDC42 and immune cells in LGG and GBM.
Figure 8

The immune checkpoint inhibitors and CD161 Pearson association. The R-value was reflected by the band's width. The P-value was denoted by the band's color. Pearson correlation analysis was used to verify the correlation.
Figure 9

Correlation analysis between CDC42 expression and Treg cell markers. (A) and (B) The expression of CDC42 was significantly correlated with CD4 in the TCGA and CGGA databases; (C) and (D) The expression of CDC42 was significantly correlated with IL2RA(CD25) in the TCGA and CGGA databases; (E) and (F) The expression of CDC42 was significantly correlated with IL7R(CD127) in the TCGA and CGGA databases.
Figure 10

Enrichment plots from GSEA. (A) CDC42 expression positively correlated with the PI3K/AKT signaling pathway in the TCGA database; (B) CDC42 expression positively correlated with the PI3K/AKT signaling pathway in the CGGA database.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.csv
- SupplementaryFig.S1.png
- SupplementaryFig.S2.png