

Research Paper

Low expression of CDHR1 is an independent unfavorable prognostic factor in glioma

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Received: 2021.03.01; Accepted: 2021.06.15; Published: 2021.06.26

Abstract

Background: Analysis of the differentially expressed genes between lower grade glioma (LGG) and glioblastoma (GBM) will identify genes involved in a more aggressive phenotype of glioma.

Methods: Differentially expressed genes between GBM and LGG were identified using published datasets. Kaplan-Meier estimator was used to determine the overall survival of different groups of glioma patients. The biological functions of CDHR1 in glioma were tested using CCK-8 and trans-well assays.

Results: CCDC109B, CD58, CLIC1, EFEMP2, EMP3, LAMC1, LGALS1, PDLIM1 and TNFRSF1A were over-expressed, while, CDHR1 was down-regulated in GBM in The Cancer Genome Atlas (TCGA), Chinese Glioma Genome Atlas (CGGA), GSE4412 and GSE43378 datasets. Compared with normal brain tissues, CDHR1 was down-regulated in glioma tissues. And low expression of CDHR1 was an unfavorable prognostic factor in glioma. Moreover, CDHR1 was lowly expressed in mesenchymal GBM subtype and lower expression of CDHR1 was associated with the worse clinical prognosis of GBM. Furthermore, CDHR1 was down-regulated in astrocytoma LGG subtype and low expression of CDHR1 was a bad prognosis of LGG. CDHR1 expression levels were also associated with IDH mutation. IDH mutant LGG or GBM patients were with higher CDHR1 expression. High expression of CDHR1 was a favorable prognosis in IDH mutant or IDH wild type LGG patients. CDHR1 expression was associated with MGMT methylation and CDHR1 was down-regulated in chemotherapy un-responsive LGG patients. CDHR1 was an independent prognostic factor and negatively associated with EMP3 expression. Glioma patients with low CDHR1 and high EMP3 expression had worse clinical outcomes. At last, we showed that over-expression of CDHR1 could inhibit glioma cell growth and invasion.

Conclusion: Low expression of CDHR1 was an independent unfavorable prognostic factor in glioma.

Key words: CDHR1, Lower grade glioma, Glioblastoma, TCGA, GEO datasets

Introduction

Glioma is originated from the central nervous system, representing the most common and aggressive type of brain tumor [1, 2]. Although with the deep understanding of the molecular alterations of glioma, therapeutic options for glioma patients are still limited to surgery, chemotherapy and radiation therapy [3, 4]. Moreover, most of glioma patients

become therapeutic resistant and recurrent during the treatment [5, 6]. So, it is important to search novel molecular targets and prognostic makers to predict the therapeutic responses and clinical outcomes of glioma.

Glioma could be divided into lower grade glioma (LGG) and glioblastoma (GBM) based on the

histological grades [7]. LGG is grade II-III glioma and GBM is grade IV glioma. The overall survival of patients with LGG is about 5-10 years [8]. On the contrary, the median survival of patients with GBM is only 12 to 15 months [9, 10]. Although, most primary GBMs are developed *de novo*, without effective treatments, some LGG patients would eventually progress to more malignant GBM [11, 12]. The molecular characteristics of LGG and GBM are extensively studied by The Cancer Genome Atlas (TCGA) [13, 14] and Chinese Glioma Genome Atlas (CGGA) [15, 16] groups, respectively. However, the differentially expressed genes between GBM and LGG are still not very clear. We thought that identification of the transcriptional signatures of different grades of glioma may provide new prognostic makers.

Cadherin-related family member 1 (CDHR1) is a photoreceptor-specific cadherin, belonging to the cadherin super-family [17]. Mutations of CDHR1 are detected in retinal dystrophies disease [18]. However, the functions of CDHR1 in tumor development are barely studied. Moreover, the expression and prognostic significance of CDHR1 in glioma are never reported. Here, using TCGA, CGGA and Gene Expression Omnibus (GEO) datasets, we studied the differentially expressed genes between GBM and LGG, and our data suggested that low expression of CDHR1 was an independent unfavorable prognostic factor in glioma.

Materials and Methods

Data collection

The gene expression, DNA methylation along with the clinical datasets of TCGA GBMLGG was downloaded from <https://tcga.xenahubs.net> website. The CGGA datasets were downloaded from <http://www.cgga.org.cn/index.jsp> website. The glioma GEO datasets were downloaded from www.ncbi.nlm.nih.gov/geo website, including GSE4412 [19], GSE43378 [20], GSE13041 [21], GSE44971 [22], GSE68848 [23], GSE74187 [24], GSE83300 [25] and GSE16011 [26] datasets. In TCGA, CGGA, GSE4412 and GSE43378 datasets, glioma patients with transcriptional data and clinical overall survival data were selected for further studies.

Identification of the differentially expressed genes between GBM and LGG

The differentially expressed genes between LGG and GBM patients were determined using two tails paired Student's t test in TCGA, CGGA, GSE4412 and GSE43378 datasets. Genes with fold change >2 and P value <0.001 were selected for further studies.

Heatmap presentation

R software 'pheatmap' package (version 1.0.12, <https://cran.r-project.org/web/packages/pheatmap/>) was used to create the figures of heatmaps. The clustering scale and clustering distance were determined by 'average' method and 'correlation' method, respectively.

ROC curves

R software 'pROC' package (version 1.16.2, <https://cran.r-project.org/web/packages/pROC/>) was used to determine the area under the ROC curve (AUC).

Survival analysis

The survival analysis was carried out using 'survival' package (version 3.1-8, <https://cran.r-project.org/web/packages/survival/index.html>) in R statistics software. Kaplan-Meier estimator was used to determine the overall survival of different groups of glioma patients. P values were determined using Log-rank test. P value less than 0.05 was chosen to be significantly different. R software 'survival' package was also used for univariate cox and multivariate cox regression analysis.

Correlation analysis

Correlation analysis was performed using "lm" method in R software. P values were determined by spearman correlation.

Cell culture and cell proliferation

Glioma cancer cell lines A172 and U87 were cultured in MEM medium (invitrogen) supplemented with 10% FBS at 37 °C in a humidified atmosphere with 5% CO₂. The cell proliferation was detected by CCK-8 assay. Briefly, A172 and U87 cells were seeded in 96-well plate with 10 μL CCK-8 per well. The cell growth rate (OD₄₅₀) was measured.

CDHR1 over-expression

CDHR1 cDNA was cloned into pCDNA 3.0 vector, and then transfected into A172 and U87 cells through lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol. The over-expression of CDHR1 was validated using western blot. Anti-human β-actin and CDHR1 antibodies were purchased from Santa Cruz Biotechnology.

Trans-well invasion assay

Trans-well invasion assays were performed in 24-well trans-well chamber with diluted matrigel (BD Biosciences, San Jose, CA, USA). The migrated cells from three random fields were photographed and counted. P values were determined using two tails

paired Student's t test. P value less than 0.05 was chosen to be significantly different.

Results

Identification of the differentially expressed genes between GBM and LGG subtypes

Glioma GBM subtype have worse prognosis than LGG subtype. Identification of the differentially expressed genes between GBM and LGG may provide new prognostic makers for glioma. To do that, GBM and LGG expression profiles from same dataset were studied. Totally, we collected 834 LGG and 384 GBM

expression samples from TCGA [13, 14], CGGA [15, 16], GSE4412 [19] and GSE43378 [20] datasets. The detailed clinic parameters of glioma patients in TCGA, CGGA, GSE4412 and GSE43378 datasets were described in supplementary data. Univariate cox regression showed that GBM patients indeed had significantly shorter overall survival than LGG patients in all those four datasets (Fig. 1A).

Next, the differentially expressed genes between GBM and LGG subtypes in TCGA, CGGA, GSE4412 and GSE43378 datasets were determined. Based on the criteria of fold change>2 and P value<0.001, 2300

A

Univariate Cox regression analysis

Dataset	LGG	GBM	Coefficient	HR	P-value	95% CI
TCGA	516	158	1.94	6.97	<2e-16	5.69-8.54
CGGA	274	135	1.65	0.19	<2e-16	0.14-0.26
GSE4412	26	59	1.34	0.26	8.9e-05	0.13-0.51
GSE43378	18	32	1.31	0.27	0.0003	0.13-0.55

B

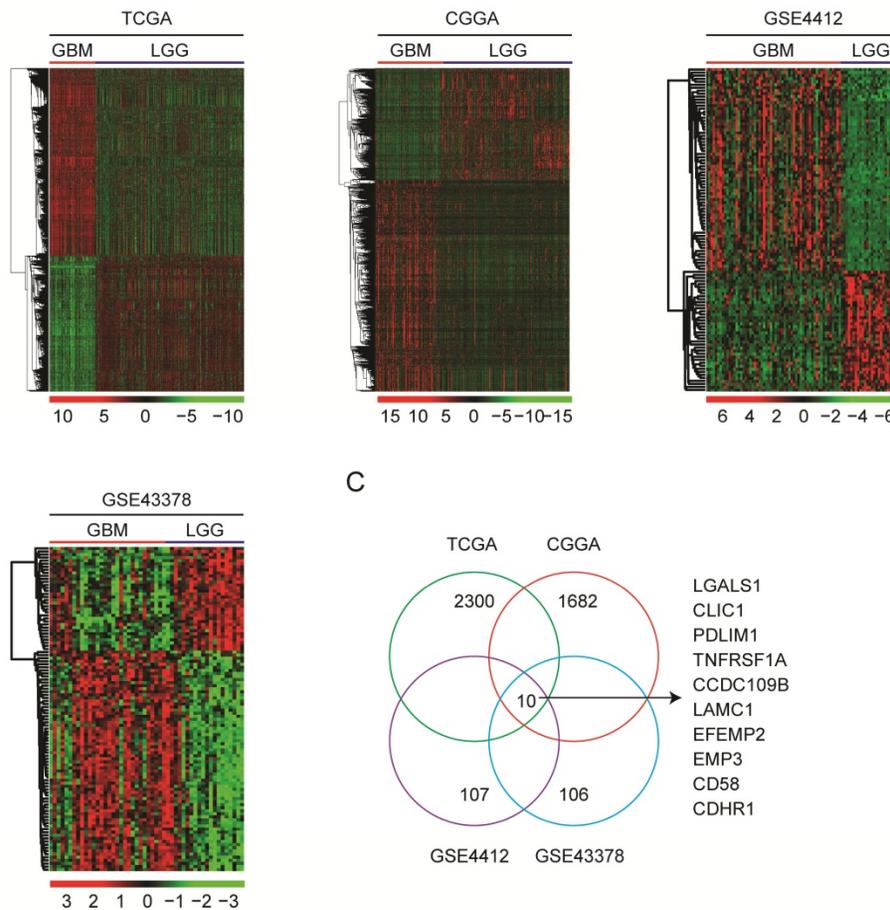


Figure 1. Identification of the differentially expressed genes between GBM and LGG subtypes. (A) The number of GBM and LGG patients in TCGA, CGGA, GSE4412 and GSE43378 datasets was demonstrated. Univariate cox regression was used to show the different clinical outcomes of GBM and LGG patients. The overall survival P-value was determined by log-rank test. HR represented hazard ratio. **(B)** Un-supervised clustering heatmaps demonstrated the differentially expressed genes (fold change>2 and P value<0.001) between GBM and LGG subtypes in TCGA, CGGA, GSE4412 and GSE43378 datasets. Up-regulated (red) and down-regulated (green) genes in GBM were delineated. **(C)** Venn diagram showed the number of differentially expressed genes between GBM and LGG in TCGA, CGGA, GSE4412 and GSE43378 datasets.

genes were differentially expressed in GBM patients in TCGA dataset, 1682 genes in CGGA dataset, 107 genes in GSE4412 dataset, and 106 genes in GSE43378 dataset, respectively (Fig. 1B). Venn diagram showed that ten genes *CCDC109B*, *CD58*, *CDHR1*, *CLIC1*, *EFEMP2*, *EMP3*, *LAMC1*, *LGALS1*, *PDLIM1* and *TNFRSF1A* were commonly differentially expressed between GBM and LGG subtypes in TCGA, CGGA, GSE4412 and GSE43378 datasets (Fig. 1C).

Compared with LGG, *CDHR1* is down-regulated in GBM patients

The fold changes of *CCDC109B*, *CD58*, *CDHR1*,

CLIC1, *EFEMP2*, *EMP3*, *LAMC1*, *LGALS1*, *PDLIM1* and *TNFRSF1A* in GBM were further illustrated in Fig. 2A. Compared with LGG, *CCDC109B*, *CD58*, *CLIC1*, *EFEMP2*, *EMP3*, *LAMC1*, *LGALS1*, *PDLIM1* and *TNFRSF1A* were all highly expressed in GBM. Particularly, *EMP3* was most significantly up-regulated in GBM patients. Compared with LGG, there were 18.1, 5.15, 3.35 and 5.31 fold changes of *EMP3* in GBM patients in TCGA, CGGA, GSE4412 and GSE43378, respectively (Fig. 2A). On the contrary, *CDHR1* was the only gene which was down-regulated in GBM patients. Compared with LGG, there were 0.15, 0.31, 0.37 and 0.32 fold changes of

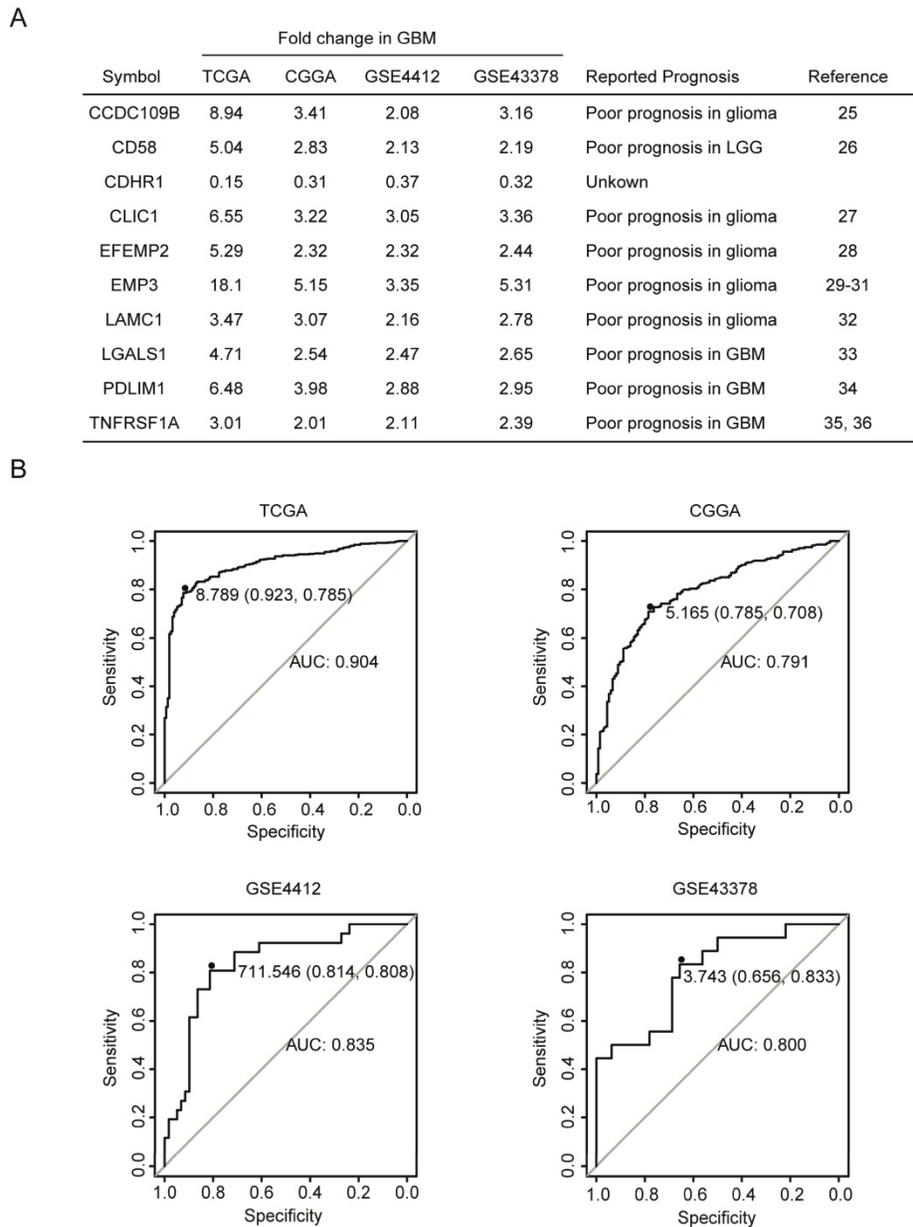


Figure 2. Compared with LGG, *CDHR1* is down-regulated in GBM patients. (A) Compared with LGG, the fold changes of the ten commonly differentially expressed genes in GBM were demonstrated in TCGA, CGGA, GSE4412 and GSE43378 datasets. The previously reported prognostic effects of *CCDC109B*, *CD58*, *CLIC1*, *EFEMP2*, *EMP3*, *LAMC1*, *LGALS1*, *PDLIM1* and *TNFRSF1A* in glioma were also demonstrated by literature research. **(B)** ROC curves showed the specificity and sensitivity of using expression levels of *CDHR1* to distinguish GBM from LGG in TCGA, CGGA, GSE4412 and GSE43378 datasets.

CDHR1 in GBM patients in TCGA, CGGA, GSE4412 and GSE43378, respectively (Fig. 2A). Moreover, ROC analysis in TCGA, CGGA, GSE4412 and GSE43378 datasets showed that expression levels of CDHR1 could distinguish GBM from LGG patients with high specificity and sensitivity (Fig. 2B).

Consistent with the high expression in GBM subtype, previous reports suggested that CCDC109B [27], CD58 [28], CLIC1 [29], EFEMP2 [30], EMP3 [31-33], LAMC1 [34], LGALS1 [35], PDLIM1 [36] and TNFRSF1A [37, 38] were all bad prognostic factors in glioma, as were summarized in Fig. 2A. However, the prognostic effects of CDHR1 in glioma were never reported and searching with the keywords of "CDHR1 in glioma" had failed to retrieve any related publication in PubMed. We speculated that, like other nine genes, CDHR1 was a potential prognostic marker in glioma. So, the expression and prognostic effects of CDHR1 in glioma were further studied.

CDHR1 is lowly expressed in glioma tissues and low expression of CDHR1 is a bad prognostic factor of glioma

First, the expression levels of CDHR1 in normal brain tissues and glioma tissues were tested. Totally, 42 normal brain tissues and 446 glioma tissues were collected from TCGA [13, 14], GSE44971 [22] and GSE68848 [23] datasets. Compared with normal brain tissues, the expression levels of CDHR1 were significantly down-regulated in glioma tissues in all TCGA, GSE44971 and GSE68848 datasets (Fig. 3A).

Second, the associations between expression levels of CDHR1 and glioma overall survival were tested using TCGA, CGGA, GSE4412 and GSE43378 datasets. We found that, consistent with the down-regulation of CDHR1 in GBM, the low expression of CDHR1 was a bad prognosis in glioma. Glioma patients with higher expression of CDHR1 had longer overall survival than glioma patients with lower expression of CDHR1 in all TCGA, CGGA, GSE4412 and GSE43378 datasets (Fig. 3B).

CDHR1 is lowly expressed in mesenchymal GBM subtype and low expression of CDHR1 is a bad prognostic factor of GBM

As a subtype of glioma, GBM could be further divided into classical, mesenchymal, neural and proneural four subtypes based on the molecular alterations and mesenchymal subtype of GBM had unfavorable prognosis [39]. We found that, compared with classical, neural and proneural GBM subtypes, CDHR1 was down-regulated in mesenchymal subtype of GBM in TCGA dataset (Fig. 4A). The low expression levels of CDHR1 in mesenchymal subtype of GBM were further validated in GSE13041 dataset

[21] (Fig. 4A).

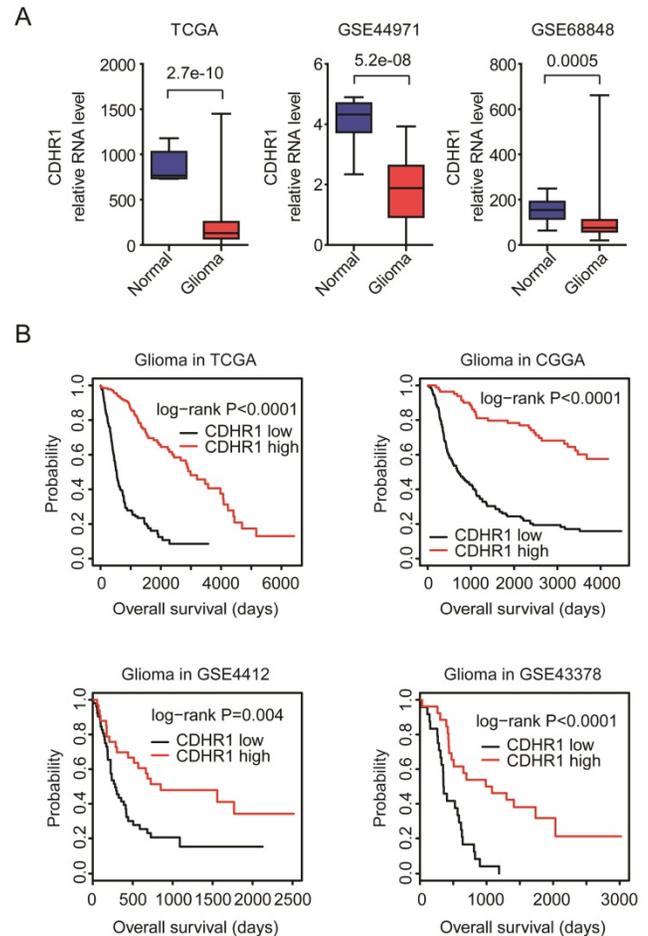


Figure 3. CDHR1 is lowly expressed in glioma and low expression of CDHR1 is a bad prognostic factor of glioma. (A) Box plots demonstrated the different expression levels of CDHR1 in normal brain tissues (blue) and glioma tissues (red). P values were generated by using the two tails paired Student's t test. **(B)** The Kaplan-Meier Plotters demonstrated the associations between expression levels of CDHR1 and glioma overall survival using TCGA, CGGA, GSE4412 and GSE43378 datasets. The P-values showed the different overall survival of CDHR1 highly expressed glioma (red) and CDHR1 lowly expressed glioma patients (black).

The prognostic effects of CDHR1 were further tested in glioma GBM subtype. As showed in the Kaplan-Meier Plotters, there was no significantly different overall survival in CDHR1 highly expressed GBM or CDHR1 lowly expressed GBM patients in TCGA and GSE43378 datasets (Fig. 4B). However, in CGGA dataset, CDHR1 highly expressed GBM patients had significantly longer overall survival than CDHR1 lowly expressed GBM patients (Fig. 4B). The prognostic effects of CDHR1 in GBM were further tested using GSE74187 [24] and GSE83300 [25] datasets. Similar to the results derived from CGGA dataset, low expression of CDHR1 was a significantly bad prognosis of GBM in GSE83300 dataset, but not significant in GSE74184 dataset (Fig. 4C).

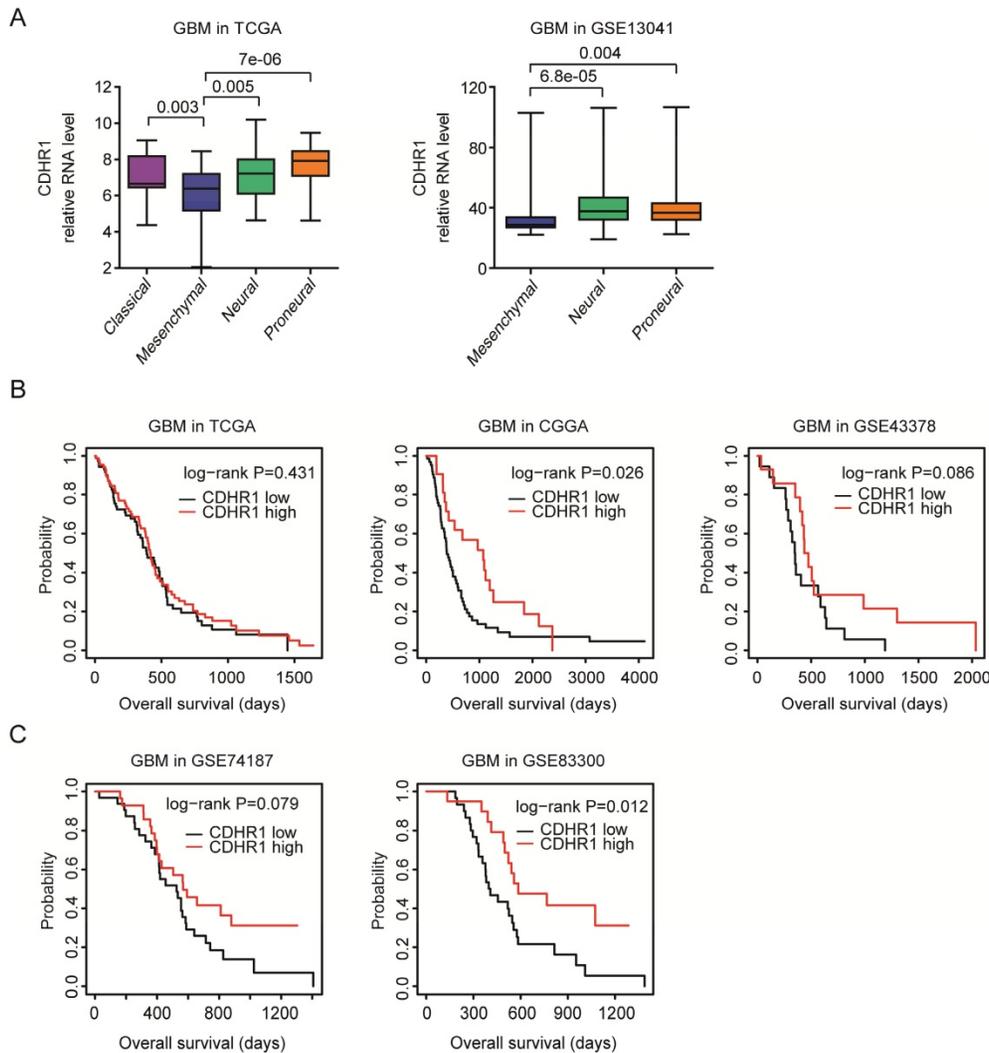


Figure 4. CDHR1 is lowly expressed in mesenchymal GBM subtype and low expression of CDHR1 is a bad prognostic factor of GBM. (A) Box plots demonstrated the different expression levels of CDHR1 in classical, mesenchymal, neural and proneural GBM subtypes in TCGA and GSE13041 datasets. **(B)** The Kaplan-Meier Plotters demonstrated the different overall survival of CDHR1 highly expressed GBM (red) with CDHR1 lowly expressed GBM patients (black) in TCGA, CGGA and GSE43378 datasets. **(C)** The Kaplan-Meier Plotters demonstrated the prognostic effects of CDHR1 in GBM patients derived from GSE74187 and GSE83300 datasets.

CDHR1 is lowly expressed in astrocytoma LGG subtype and low expression of CDHR1 is a bad prognostic factor of LGG

LGG is grade II-III glioma. We further showed that, compared with grade II LGG, CDHR1 was down-regulated in grade III LGG patients in TCGA dataset (Fig. 5A). However, in CGGA and GSE16011 [26] datasets, there was no significant difference of CDHR1 expression between grade II or III LGG patients (Fig. 5A). LGG could be further divided into astrocytoma, oligoastrocytoma and oligodendroglioma subtypes [7]. Next, we evaluated the expression levels of CDHR1 in different LGG subtypes. In TCGA and CGGA datasets, we showed that, compared with oligoastrocytoma and oligodendroglioma, CDHR1 was down-regulated in astrocytoma subtype of LGG (Fig. 5B). Moreover, in GSE16011 dataset, the expression of CDHR1 was also

lower in astrocytoma LGG subtype, compared with oligodendroglioma LGG subtype (Fig. 5B).

The prognostic effects of CDHR1 were also tested in glioma LGG subtype. CDHR1 highly expressed LGG patients had significantly longer overall survival than CDHR1 lowly expressed LGG patients in TCGA and CGGA datasets (Fig. 5C). Those results suggested that CDHR1 was also a prognostic factor in glioma GBM or LGG subtypes, respectively.

CDHR1 is highly expressed in IDH mutant glioma and high expression of CDHR1 is a favorable prognosis in IDH mutant or IDH wild type LGG patients

IDH mutation is a common mutation in glioma. IDH mutant glioma patients have better clinical outcomes [40, 41]. Indeed, in both TCGA and CGGA datasets, IDH wild type LGG patients had significantly shorter overall survival than LGG

patients with IDH mutations (Fig. 6A). Similarly, in GBM patients, IDH mutation was associated with better clinical outcomes in TCGA and CGGA datasets (Fig. 6B).

We then studied the different expression levels of CDHR1 in glioma patients with or without IDH mutations. First, CDHR1 expression was lower in IDH wild type LGG patients, compared with LGG patients with IDH mutation 1p19q deletion or IDH mutation 1p19q non-deletion in both TCGA and CGGA datasets (Fig. 6C). IDH was also highly expressed in GBM patients with IDH mutation, compared with IDH wild type GBM patients in TCGA and CGGA datasets (Fig. 6D). Moreover, CDHR1 low expression was associated with the unfavorable prognosis of IDH mutant LGG patients in CGGA datasets (Fig. 6E).

However, in TCGA datasets, there was no significantly different overall survival between CDHR1 highly or lowly expressed IDH mutant LGG patients (Fig. 6E). Furthermore, in IDH wild type LGG patients, CDHR1 low expression was associated with the unfavorable prognosis in TCGA and CGGA datasets (Fig. 6F).

CDHR1 expression is correlated with MGMT methylation intensity

MGMT methylation was another important prognostic factor in glioma. Epigenetic hyper-methylation of MGMT could increase the sensitivity of temozolomide therapy, and represented a favorable prognostic maker [42-44]. We showed that in TCGA dataset, LGG patients with

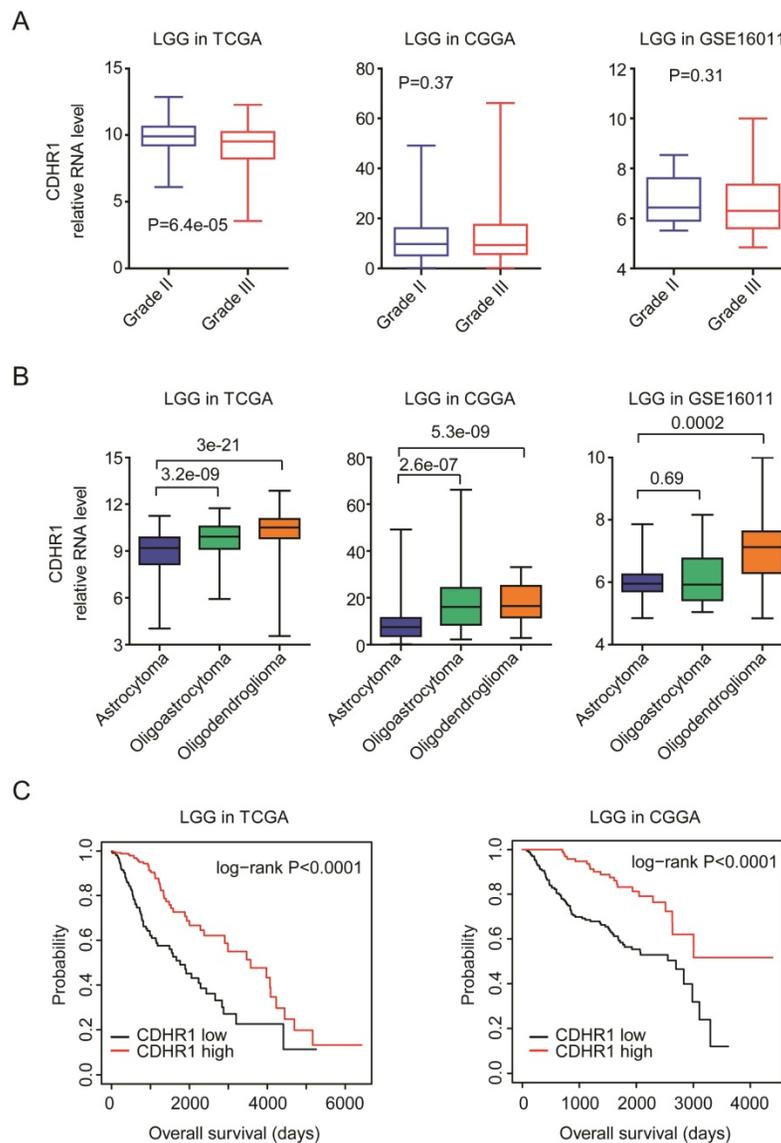


Figure 5. CDHR1 is lowly expressed in astrocytoma LGG subtype and low expression of CDHR1 is a bad prognostic factor of LGG. (A) Box plots demonstrated the different expression levels of CDHR1 in grade II and grade III LGG subtypes in TCGA, CGGA and GSE16011 datasets. **(B)** Box plots demonstrated the different expression levels of CDHR1 in astrocytoma, oligoastrocytoma and oligodendroglioma LGG subtypes in TCGA, CGGA and GSE16011 datasets. **(C)** The Kaplan-Meier Plotters demonstrated the different overall survival of CDHR1 highly expressed LGG (red) with CDHR1 lowly expressed LGG patients (black) in TCGA and CGGA datasets.

hyper-methylated MGMT had longer overall survival than LGG patients with hypo-methylated MGMT (Fig. 7A). Moreover, the methylation levels of MGMT

were higher in chemotherapy responsive LGG patients than chemotherapy un-responsive LGG patients (Fig. 7B).

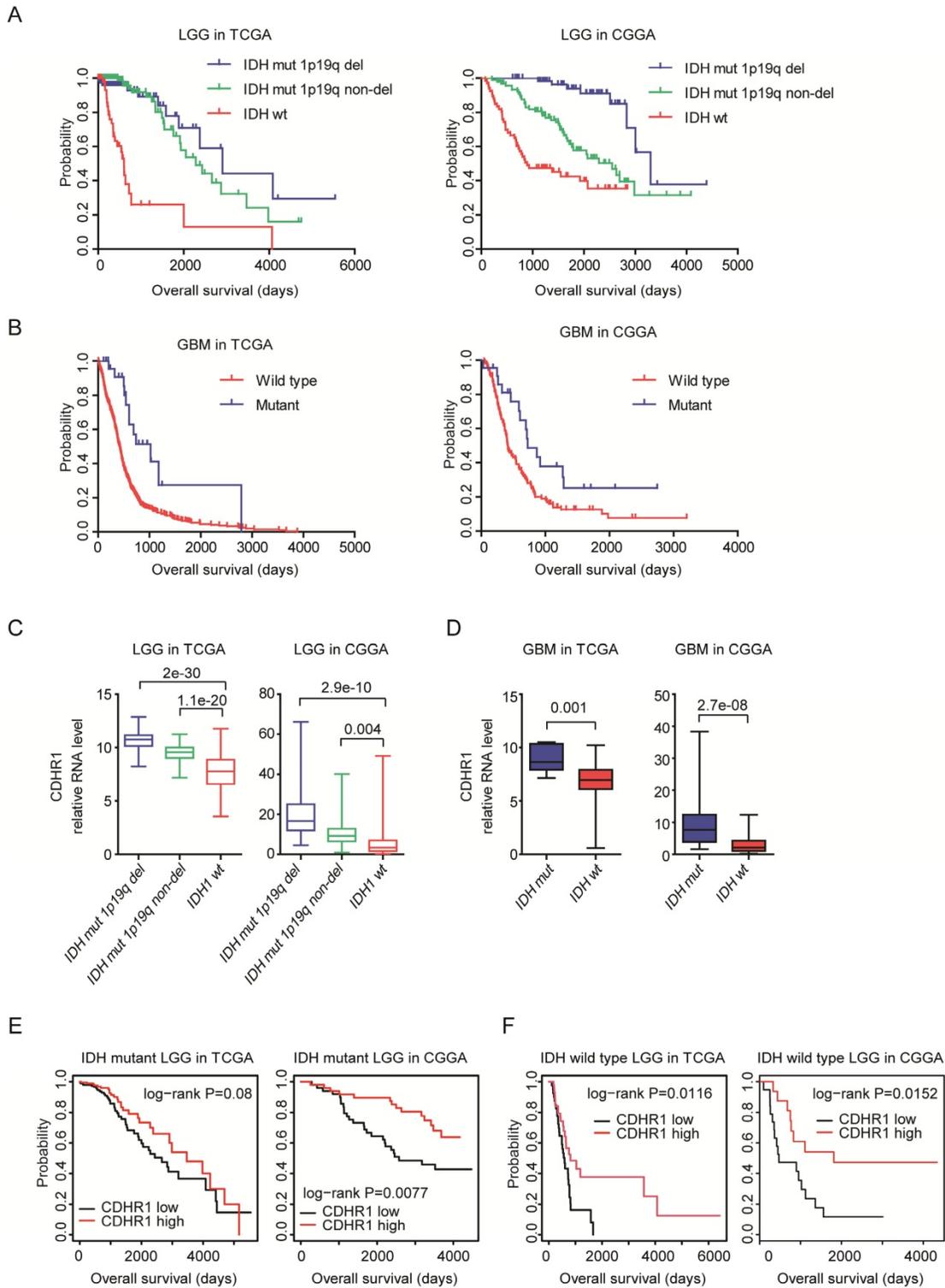


Figure 6. CDHR1 is highly expressed in IDH mutant glioma and high expression of CDHR1 is a favorable prognosis in IDH mutant or IDH wild type LGG patients. (A) The Kaplan-Meier Plotters demonstrated the different clinical outcomes of LGG patients with IDH mutation 1p19q deletion, IDH mutation 1p19q non-deletion or without IDH mutation. (B) The Kaplan-Meier Plotters demonstrated the different clinical outcomes of IDH mutant and IDH wild type GBM patients. (C) Box plots demonstrated the different expression levels of CDHR1 in IDH mutation 1p19q deletion, IDH mutation 1p19q non-deletion and IDH wild type subtypes of LGG in TCGA and CGGA datasets. (D) Box plots demonstrated the different expression levels of CDHR1 in IDH mutant and IDH wild type subtypes of GBM in TCGA and CGGA datasets. (E) The Kaplan-Meier Plotters demonstrated the prognostic effects of CDHR1 in IDH mutant LGG patients derived from TCGA and CGGA datasets. (F) The Kaplan-Meier Plotters demonstrated the prognostic effects of CDHR1 in IDH wild type LGG patients derived from TCGA and CGGA datasets.

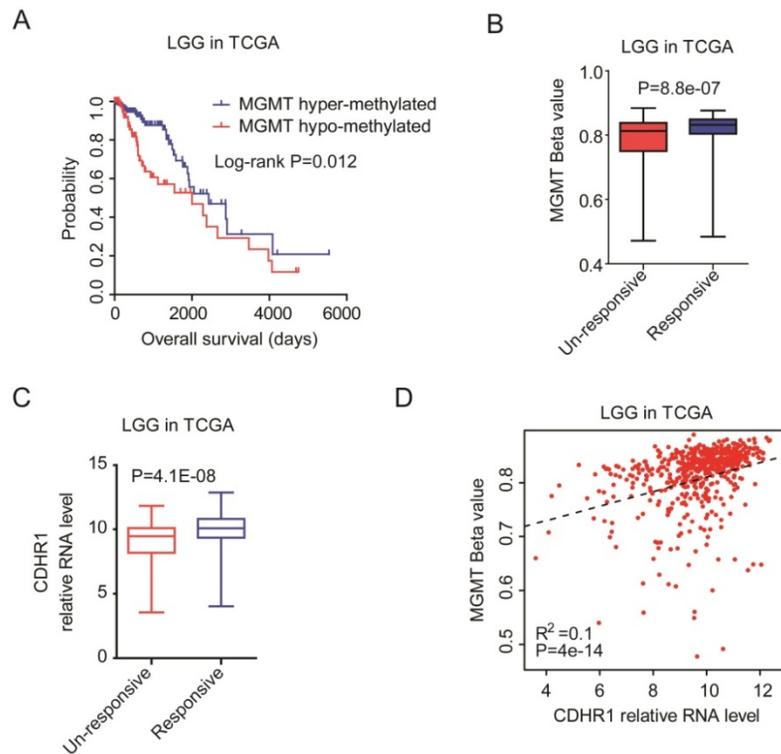


Figure 7. CDHR1 expression is correlated with MGMT methylation intensity. (A) The Kaplan-Meier Plotters demonstrated the different clinical outcomes of LGG patients with MGMT hypo-methylation or with MGMT hyper-methylation. (B) Box plots demonstrated the different methylation intensity of MGMT in chemotherapy responsive or chemotherapy un-responsive LGG patients. (C) Box plots demonstrated the different expression levels of CDHR1 in chemotherapy responsive or chemotherapy un-responsive LGG patients. (D) Spearman correlation between CDHR1 expression and MGMT methylation in LGG patients in TCGA dataset.

On the contrary, the expression levels of CDHR1 were higher in chemotherapy responsive LGG patients than chemotherapy un-responsive LGG patients (Fig. 7C). Also, we observed significantly positive correlations of CDHR1 expression and MGMT methylation (Fig. 7D). All those results suggested that CDHR1 was associated with IDH mutation and MGMT methylation, and CDHR1 was an important prognostic factor of glioma.

CDHR1 is an independent prognostic factor and combination of CDHR1 with EMP3 achieves better clinical predication

Next, we determined the relationships of CDHR1 with *CCDC109B*, *CD58*, *CLIC1*, *EFEMP2*, *EMP3*, *LAMC1*, *LGALS1*, *PDLIM1* and *TNFRSF1A*. Multivariate cox regression showed that *EMP3* and *LGALS1* were independent prognostic factors in TCGA dataset (Fig. 8A), and in CGGA dataset, CDHR1 was an independent prognostic factor (Fig. 8A). Previously, our results showed that *EMP3* was highly expressed in old LGG patients and was a critical prognostic factor in glioma [33]. We observed significantly negative correlations between CDHR1 expression and *EMP3* expression in both TCGA and GSE43378 datasets (Fig. 8B).

Furthermore, we determined the synergistic prognosis of CDHR1 with *EMP3*. We found that

glioma patients with CDHR1 low *EMP3* high expression had worse clinical outcomes in TCGA and CGGA datasets (Fig. 8C). While, glioma patients with CDHR1 high *EMP3* low expression had better clinical outcomes (Fig. 8C). Those results suggested that combination of CDHR1 and *EMP3* may achieve better prognostic significance than CDHR1 or *EMP3* alone.

Over-expression of CDHR1 inhibits glioma cell growth and invasion

At last, we determined the biological functions of CDHR1 in glioma. Two glioma cell lines A172 and U87 were transfected with CDHR1 over-expression plasmid. Western blot showed the high expression of CDHR1 in A172 and U87 cells after plasmid transfection (Fig. 9A). Then, we tested the cell growth using CCK8 assay. We found that, the cell growth was significantly inhibited by CDHR1 over-expression in both A172 and U87 cells (Fig. 9B). Furthermore, over-expression of CDHR1 inhibited glioma cell invasion as tested using trans-well assay (Fig. 9C). The number of invasive cells was significantly suppressed by CDHR1 over-expression in A172 and U87 cells (Fig. 9D). Those results were consistent with our previous results that CDHR1 was lowly expressed in glioma cells and low expression of CDHR1 was an unfavorable prognostic factor in glioma.

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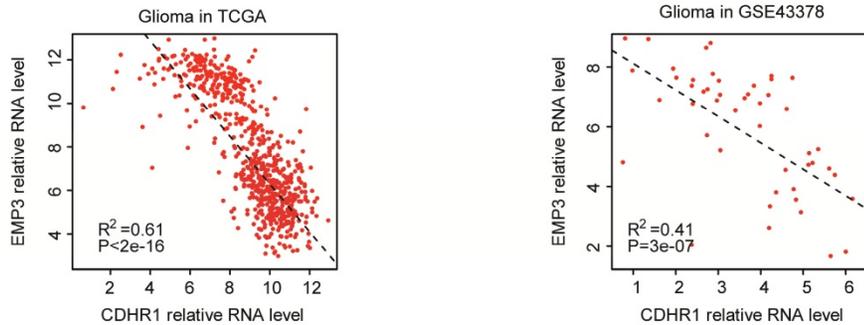
Multi-variable Cox regression analysis of glioma in TCGA

Variable	Coefficient	HR	p-value	95% CI
CCDC109B	0.04	1.04	0.68	0.85-1.27
CD58	0.21	1.23	0.057	0.99-1.52
CDHR1	-0.045	0.96	0.39	0.86-1.06
CLIC1	0.22	1.25	0.12	0.94-1.1.65
EFEMP2	0.04	1.05	0.65	0.86-1.27
EMP3	0.3	1.36	0.0006	1.14-1.61
LAMC1	0.17	1.18	0.05	1-1.41
LGALS1	-0.22	0.8	0.028	0.66-0.98
PDLIM1	-0.03	0.97	0.69	0.86-1.11
TNFRSF1A	-0.15	0.86	0.21	0.68-1.09

Multi-variable Cox regression analysis of glioma in CGGA

Variable	Coefficient	HR	p-value	95% CI
CCDC109B	0.018	1.02	0.016	1-1.03
CD58	0.035	1.04	0.022	1-1.07
CDHR1	-0.08	0.92	1.9e-09	0.89-0.95
CLIC1	-0.001	1	0.53	1-1.002
EFEMP2	0.001	1	0.41	1-1.005
EMP3	0.00001	1	0.96	1-1.002
LAMC1	0.007	1	0.4	1-1.02
LGALS1	-0.0001	1	0.6	0.999-1
PDLIM1	-0.001	1	0.83	0.999-1
TNFRSF1A	-0.003	1	0.57	0.98-1

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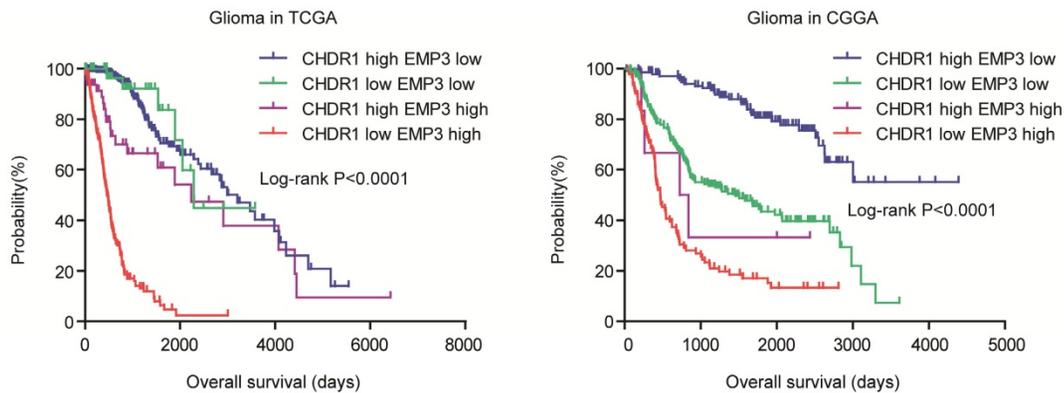


Figure 8. CDHR1 is an independent prognostic factor and combination of CDHR1 with EMP3 achieves better clinical predication. (A) Multivariate cox regression was used to test the relationships of CCDC109B, CD58, CDHR1, CLIC1, EFEMP2, EMP3, LAMC1, LGALS1, PDLIM1, TNFRSF1A expressions and overall survival in glioma patients in TCGA and CGGA datasets. (B) Spearman correlation between CDHR1 and EMP3 expression in glioma patients was tested in TCGA and GSE43378 datasets. (C) Kaplan-Meier plotters demonstrated the different overall survival of glioma patients with different expression levels of EMP3 and CDHR1 in TCGA and CGGA datasets. Log-rank test was used to determine the P values.

Discussion

Glioma is a heterogeneous disease. Histological grades, IDH mutation [40, 41], MGMT methylation [42-44] and EMP3 expression [31-33] were all validated prognostic factors of glioma. Interesting, those prognostic factors were coordinated with each other. For example, EMP3 was over-expressed in grade IV glioma (GBM). And grade II-III glioma (LGG) had high frequency of IDH mutation [11, 45]. In our studies, using TCGA, CGGA and GEO

datasets, we further confirmed the prognostic significance of histological grades, IDH mutation, MGMT methylation and EMP3 expression. Moreover, we believed that genes associated with histological grades, IDH mutation, MGMT methylation and EMP3 expression in glioma were also potential prognostic factors for glioma.

In this analysis, we started with the differentially expressed genes between GBM and LGG. We identified ten differentially expressed genes shared in TCGA, CGGA, GSE4412 and GSE43378 datasets.

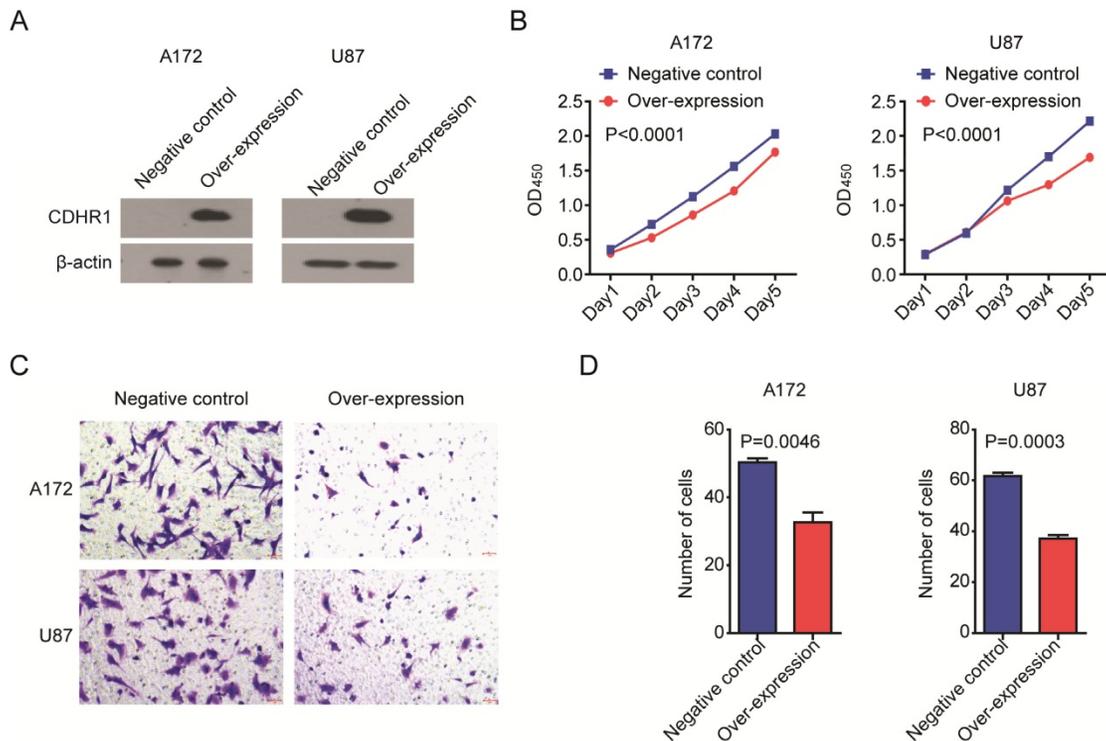


Figure 9. Over-expression of CDHR1 inhibits glioma cell growth and invasion. (A) Western blot showed the over-expression of CDHR1 in glioma A172 and U87 cell lines, using an empty vector as negative control. (B) Cell growth in A172 and U87 with or without CDHR1 over-expression was detected using CCK8 assay. The cell growth rate was determined by OD₄₅₀. (C) Representative images of invasive A172 and U87 cells with or without CDHR1 over-expression in trans-well assay. (D) Statistical quantification of migrated A172 and U87 cells with or without CDHR1 over-expression in trans-well assay. Results were representative of three independent experiments.

Among them, nine genes, including EMP3 were previously reported prognostic makers of glioma. However, the expressions and prognostic effects of CDHR1 were never known. We showed that, CDHR1 was down-regulated in GBM and CDHR1 was down-regulated in IDH mutant glioma patients. Moreover, CDHR1 expression was positively correlated with MGMT methylation and negatively correlated with EMP3 expression. All those features suggested that CDHR1 was a potential prognostic maker.

Indeed, CDHR1 was down-regulated in glioma tissues, compared with normal brain tissues. Moreover, the down-regulation of CDHR1 was associated with poor clinical outcomes of glioma in all TCGA, CGGA, GSE4412 and GSE43378 datasets. Furthermore, the bad prognostic effects of CDHR1 were detected in GBM or LGG patients in at least one dataset. GBM or LGG patients with CDHR1 high expression had longer overall survival. At last, we confirmed the function of CDHR1 by over-expression CDHR1 in glioma cell lines. The over-expression of CDHR1 could significantly inhibit glioma cell growth and cell invasion. All those results suggested that low expression of CDHR1 was an independent unfavorable prognostic factor in glioma.

Our analysis highlighted that genes differentially expressed in different glioma grades

were potential prognostic factors. Next, it is interesting to identify the differentially expressed genes between IDH mutant and IDH wild type glioma patients or identify the genes most associated with MGMT methylation and EMP3 expression. Those analyses may provide new understandings of the development of glioma and suggest novel therapeutic targets and prognostic makers for glioma.

Conclusion

Low expression of CDHR1 was an independent unfavorable prognostic factor in glioma.

Abbreviations

LGG: Lower grade glioma; GBM: glioblastoma; TCGA: The Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas; CDHR1: Cadherin-related family member 1; GEO: Gene Expression Omnibus; HR: hazard ratio.

Supplementary Material

Supplementary data.

<http://www.jcancer.org/v12p5193s1.xlsx>

Acknowledgements

We appreciate the generosity of the researches from TCGA, CGGA and GEO groups for sharing the

huge amount of data.

Funding

The present study was supported by grants from the Fujian Maternity and Child Health Hospital (grant nos. YCXB 18-10 and YCXM 19-04). This study was also supported by Fujian Natural Science Foundation (grant nos. 2020J01337).

Author Contributions

HW designed the study and wrote the manuscript. HW, XW and LX performed the data analysis. YY.L performed the CCK8 and trans-well assay. HC and JZ designed the study and supervised the work.

Competing Interests

The authors have declared that no competing interest exists.

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