CDH2 expression is of prognostic significance in glioma and predicts the efficacy of temozolomide therapy in patients with glioblastoma

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Abstract. Glioma is the most common and malignant primary brain cancer in adults. Radical surgical excision accompanied by radiotherapy and chemotherapy is the prevailing standard therapy for patients with glioblastoma (GBM). Cadherin 2 (CDH2) encodes the N-cadherin protein, a classical cadherin and a member of the cadherin superfamily, which sustains the integrity of the cell and is involved in several cell signal transduction pathways. In the present study, the association between CDH2 expression and clinical features was investigated based on the Chinese Glioma Genome Atlas (CGGA), the Rembrandt datasets and The Cancer Genome Atlas datasets (TCGA). Medical statistical methods, including Kaplan-Meier analysis and Cox regression model were used. The expression of CDH2 was identified to be strongly associated with glioma World Health Organization grade in the CGGA and Rembrandt datasets. Patients with low CDH2 expression had an improved prognosis and benefited from temozolomide therapy. In conclusion, these findings revealed that CDH2 may serve as a prognostic and predictive molecular biomarker for the grading and treatment of glioma.

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Abbreviations: CGGA, Chinese Glioma Genome Atlas; TCGA, The Cancer Genome Atlas; TMZ, temozolomide

Key words: Cadherin 2, glioma, temozolomide, survival, chemotherapy

Introduction

Glioma is the most common malignant brain cancer in adults. In patients with grade IV glioma according to the World Health Organization (WHO) guidelines (1) their condition is similar to glioblastoma (GBM). At present, the standard therapy is surgical excision accompanied by chemotherapy and radiotherapy (1). Even in patients who actively cooperate with treatment, the median overall survival (OS) time of patients who suffer from GBM is <15 months (2), and drug resistance is partially accountable for the poor prognostic outcome of GBM.

The epithelial-mesenchymal transition (EMT) process serves an important function in tumor invasion (3), metastasis and drug resistance in a number of types of cancer, including lung cancer (4) and pancreatic carcinoma (5). Conversely, the role of EMT in gliomagenesis remains vague; however, several EMT-associated factors, including Twist (6), zinc finger E-box-binding homeobox (ZEB)1 (7), ZEB2, and the SNAI family, have been confirmed to accelerate the invasion, progression and drug resistance of glioma (8,9). Cadherin 2 (CDH2), which encodes the N-cadherin protein, is also a marker of EMT. An increasing amount of evidence has suggested that CDH2 has a close association with the WHO grade of glioma (10). By contrast, a previous study demonstrated that GBMs express lower CDH2 levels than low-grade gliomas (11). Therefore, the association between CDH2 and glioma malignancy requires further study.

In the present study, a detailed and systematic analysis was performed using the The Cancer Genome Atlas (TCGA), Chinese Glioma Genome Atlas (CGGA) and Rembrandt databases, and identified that CDH2 expression was associated with glioma grade and may serve as a prognostic indicator for OS in patients with glioma. In addition, in patients with GBM expressing low levels of CDH2, temozolomide (TMZ) therapy had an improved curative effect, among other independent prognostic factors. The results of the present study demonstrated the prognostic and predictive value of CDH2 for glioma patients and suggests that CDH2 levels could be used to identify which patients are likely to benefit from TMZ therapy in the clinical setting.

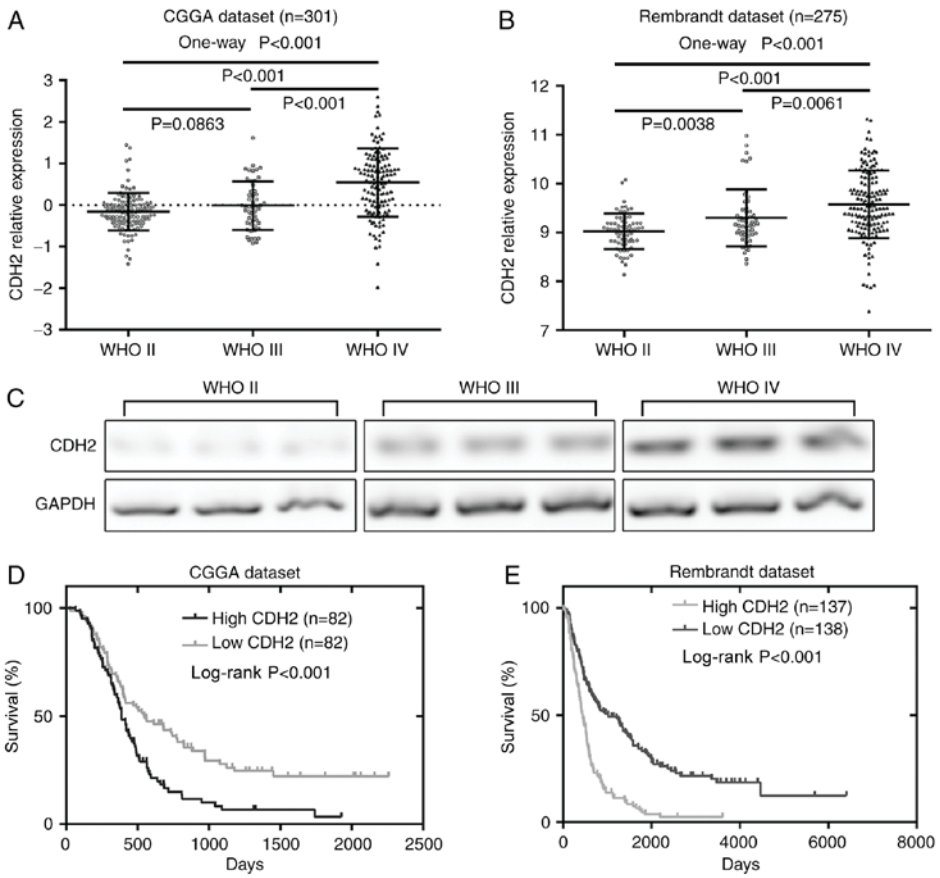

Figure 1. CDH2 expression level is associated with WHO grade and patient prognosis in glioma. (A and B) The expression of CDH2 was analyzed in glioma tissues of different grades from (A) the CGGA dataset and (B) the Rembrandt dataset. (C) CDH2 mRNA levels in 9 frozen glioma samples of different grades were examined using 1% agarose electrophoresis following reverse transcription-polymerase chain reaction, using GAPDH as a control. (D and E) Association of CDH2 expression with overall survival in glioma cases of (D) the CGGA dataset and (E) the Rembrandt dataset. CDH2, cadherin 2; WHO, World Health Organization; CGGA, Chinese Glioma Genome Atlas.

Materials and methods

Clinical samples. Clinical characteristics and CDH2 mRNA expression data of 301 glioma specimens were obtained from the microarray data stored in the Chinese Glioma Genome Atlas (CGGA; http://www.cgga.org.cn). The histological diagnoses were determined according to the WHO criteria (12). Publicly available Rembrandt microarray data were obtained online (https://wiki.nci.nih.gov/display/ICR/Rembrandt+Data+Portal) on May 8, 2014. Any patients lost to follow-up were not included in the survival analysis. TCGA dataset, which consists of RNA-seq data, was downloaded from the website (https://cancergenome.nih.gov/). Nine clinical glioma samples (fresh-frozen) were selected according to WHO grade classification (1) and age (≤45) were obtained from the Department of Neurosurgery of the Second Affiliated Hospital of Harbin Medical University (Harbin, China, Table I). All patients provided written informed consent, and all human experiments were approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University.

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from patient samples using TRIzol® reagent (Life Technologies; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Then cDNAs were synthesized using the PrimeScript RT Reagent kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. The following primers (Beijing *Tianyi Huiyuan* Bioscience & Technology Inc., Beijing, China) were used: CDH2 forward, 5'-ACCTTTGCCAGGAGCTGTTT-3'; CDH2 reverse, 5'-TGT GCTCCCTATGACCCAGA-3'; GAPDH forward, 5'-AGA AGGCTGGGGCTCATTTG-3'; and GAPDH reverse, 5'-AGG GGCCATCCACAGTCTTC-3' were used for PCR. Following amplification (denaturation 95˚C for 10 secs, annealing 53˚C for 10 secs and elongation 72˚C for 60 secs, 40 cycles) of the PCR product, 1% agarose gel electrophoresis (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), DNA ladder (Beijing Solarbio Science & Technology Co., Ltd.) and ethidium bromide (Beijing Solarbio Science & Technology Co., Ltd.) were used to assess the amount of CDH2. All PCR experiments were conducted in triplicate.

Statistical analysis. Differences in OS and progression-free survival (PFS) were evaluated using the Kaplan-Meier method and analyzed using the log-rank test in the univariate analysis. Student's t-test was used to examine the differences between two groups. Multigroup comparisons of the means were carried out using a one-way analysis of variance test with post hoc contrasts performed using the Student-Newman-Keuls

Table I. The corresponding clinical and pathological information of nine patients.

	Grade	Histology	Age (years)	Sex
Patient 1	II	Astrocytoma	40	Female
Patient 2	II	Oligodendroglioma	30	Female
Patient 3	II	Astrocytoma	42	Male
Patient 4	III	Anaplastic oligodendroglioma	38	Male
Patient 5	III	Anaplastic oligodendroglioma	41	Female
Patient 6	III	Anaplastic oligodendroglioma	44	Female
Patient 7	IV	Glioblastoma	45	Female
Patient 8	IV	Glioblastoma	45	Male
Patient 9	IV	Glioblastoma	39	Female

test. A χ^2 test was used to evaluate the distribution of patient characteristics between subgroups. Cox proportional hazards regression analysis was used to assess the prognostic value of CDH2 expression among other factors. All statistical calculations were performed with SPSS 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 6.01 (GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

CDH2 is associated with WHO grade and the prognosis of glioma patients. The expression of CDH2 was detected in 301 glioma samples in the CGGA dataset (grade II, n=122; grade III, n=51; grade IV, n=128). CDH2 expression was significantly higher in gliomas of grade IV than in those of grades II (P<0.001) or III (P<0.001) (Fig. 1A). However, glioma of grade III presented no significant difference compared with grade II (P=0.0863). Furthermore, the public dataset Rembrandt was used to further confirm these findings. The results demonstrated that CDH2 expression had an evident association with the WHO grade of glioma (P<0.001; Fig. 1B). In the Rembrandt dataset, CDH2 expression in grade III glioma was higher than that in grade II (P=0.0038). Furthermore, in the clinical glioma tissues obtained from our hospital (n=9), the mRNA level of CDH2 was demonstrated to be higher in grade IV than in grade II and III glioma samples (Fig. 1C).

High expression of CDH2 confers an unfavorable prognosis in glioma patients. The median CDH2 expression level in 164 patients with high-grade glioma (WHO III and IV) from the CGGA data set was used as the cut-off point to divide the patients into low CDH2 (n=82) and high CDH2 (n=82) expression groups. Kaplan-Meier survival curves and the log-rank test were employed to identify any associations between CDH2 expression and OS. Patients in the low CDH2 expression group lived longer compared with those in the high expression group (P<0.001) (Fig. 1D). The Rembrandt dataset was also analyzed for confirmation of these findings, and the results demonstrated that the group with a high expression of CDH2 had a significantly worse outcome (P<0.001) (Fig. 1E). This data demonstrated that high expression of CHD2 may be indicative of an unfavorable survival outcome.

Table II. Clinical and pathological characteristics of 164 patients with high-grade glioma in association with CDH2 expression.

	CDH2 expression		
Variable	Low (n=82)	High (n=82)	P-value
Age, years			0.0030
<45	52	33	
≥45	30	49	
IDH1 status			0.0136
Mutant	15	50	
Not mutant	67	32	
Sex			0.1596
Male	50	49	
Female	32	33	
Chemotherapy			0.2678
Yes	49	51	
No	27	31	
NA	6	0	
Radiotherapy			0.0661
Yes	64	65	
No	11	16	
NA	7	1	
TCGA subtype			0.0326
Neural	9	20	
Proneural	12	13	
Mesenchymal	50	40	
Classical	11	9	
WHO grade			0.1046
III	16	25	
IV	66	57	

CDH2, cadherin 2; IDH1, isocitrate dehydrogenase 1; TCGA, The Cancer Genome Atlas; WHO, World Health Organization.

CDH2 is an independent prognostic factor in patients with high-grade glioma. The clinicopathological information of 164 patients with high-grade glioma in the CGGA dataset

Table III. Univariate and multivariate Cox regression analyses for overall survival in 164 glioma samples of the Chinese Glioma Genome Atlas dataset.

	Univariate			Multivariate		
Variables	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.590	1.116-2.265	0.0100	0.934	0.619-1.411	0.7460
IDH1 status	0.471	0.307-0.722	0.0010	0.566	0.350-0.916	0.0210
Sex	0.835	0.589-1.183	0.3100	-	-	-
Chemotherapy	0.640	0.457-0.897	0.0100	0.641	0.463-0.887	0.0070
Radiotherapy	0.774	0.518-1.156	0.2110	-	-	-
TCGA subtype	1.121	0.910-1.382	0.2830	-	-	-
WHO grade	1.872	1.226-2.858	0.0040	1.418	0.884-2.274	0.1470
CDH2 expression	1.910	1.342-2.719	<0.0010	1.746	1.211-2.518	0.0030

IDH1, isocitrate dehydrogenase 1; TCGA, The Cancer Genome Atlas; WHO, World Health Organization; CDH2, cadherin 2; HR, hazard ratio; CI, confidence interval; age (<45 vs. ≥45 years); IDH1 status (mutant and not mutant); sex (female and male); chemotherapy (yes, no and NA); radiotherapy (yes, no and NA); TCGA subtype (neural, proneural, mesenchymal and classical); WHO grade (II, III and IV); CDH2 expression (low and high expression).

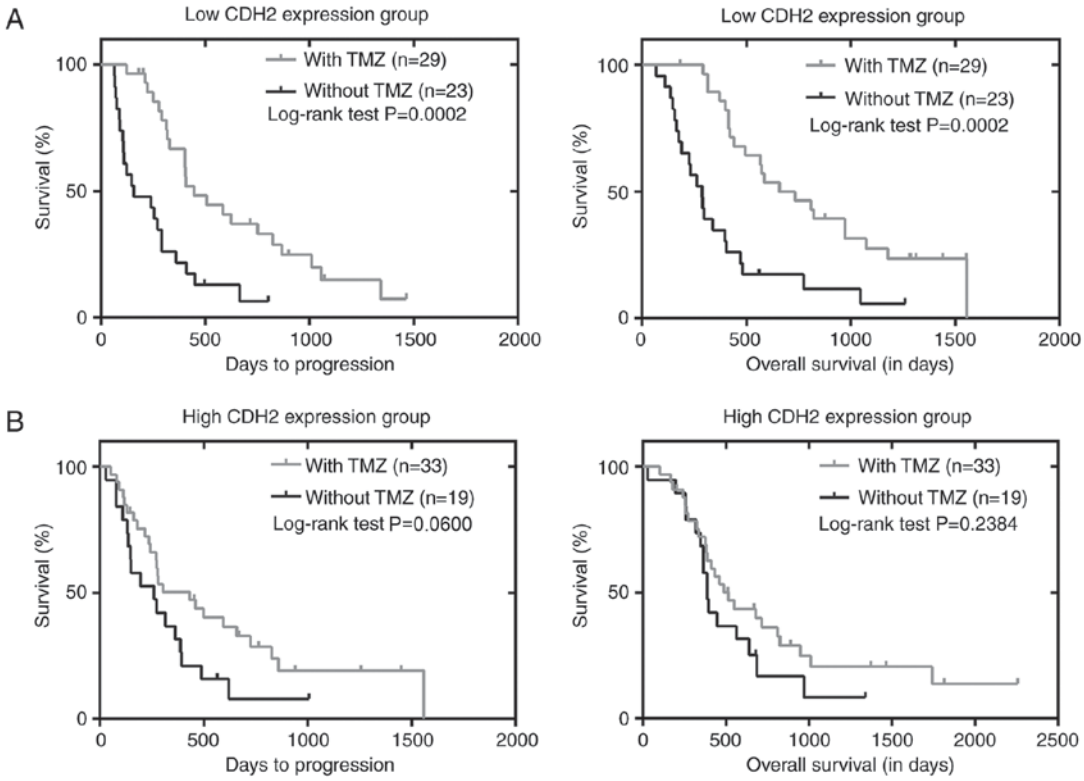

Figure 2. Survival analysis of patients with high-grade glioma treated with or without TMZ in the Chinese Glioma Genome Atlas dataset. (A) Kaplan-Meier analyses of PFS (left) and OS (right) in patients in the low CDH2 expression group treated with or without TMZ. (B) Kaplan-Meier analyses of PFS (left) and OS (right) patients in the high CDH2 expression group treated with or without TMZ. TMZ, temozolomide; PFS, progression-free survival; OS, overall survival; CDH2, cadherin 2.

was investigated, and revealed that CDH2 expression was associated with age at diagnosis (P=0.003), isocitrate dehydrogenase 1 (IDH1) mutation status (P=0.0136) and TCGA subtype (P=0.0326) (Table II). Univariate Cox regression analysis was conducted to analyze the genetic and clinical variables with respect to survival. OS was identified to be associated with IDH1 mutation status, CDH2 expression level and whether the patient had received chemotherapy. Subsequently, potential prognostic factors associated with OS were evaluated through a multivariate Cox regression model. The results demonstrated that CDH2 expression was an independent prognostic factor for OS [hazard ratio (HR), 1.746;

Table IV. Clinical and pathological characteristics of 275 glioma samples in association with CDH2 expression in the Rembrandt dataset.

	CDH2 expression		
Variables	Low (n=138)	High (n=137)	P-value
Sex			<0.001
Male	47	34	
Female	80	67	
NA	11	36	
TCGA subtype			<0.001
Neural	30	5	
Proneural	37	31	
Mesenchymal	65	53	
Classical	6	48	
WHO grade			<0.001
II	53	14	
III	33	19	
IV	52	104	

CDH2, cadherin 2; TCGA, The Cancer Genome Atlas; WHO, World Health Organization. sex (female and male); TCGA subtype (neural, proneural, mesenchymal and classical); WHO grade (II, III and IV).

95% confidence interval (CI), 1.211-2.518; P=0.003], following adjustment for IDH1 and chemotherapy status (Table III). The same statistical approach was also conducted for 275 glioma samples in the Rembrandt dataset. The results demonstrated that CDH2 expression remained an independent factor for predicting OS following adjustment for sex and WHO grade (HR, 1.397; 95% CI, 1.102-1.770; P=0.006) (Tables IV and V).

Association between CDH2 expression and sensitivity to chemotherapy. To investigate the association between CDH2 level and the sensitivity to chemotherapy, a primary GBM group was enrolled from the CGGA dataset. They were divided into subgroups depending on the median level of CDH2 and whether the patients received TMZ chemotherapy. Kaplan-Meier survival analysis demonstrated that, in patients with low CDH2 expression, TMZ treatment was associated with improved OS and PFS compared with patients not treated with TMZ (P=0.0002 and P=0.0002, respectively) (Fig. 2A). However, no evident survival benefit of TMZ therapy was identified for patients with high CDH2 expression (OS, P=0.2384; PFS, P=0.0600) (Fig. 2B), indicating that low CDH2 expression predicted a better response to TMZ. The results were also corroborated by a Cox regression analysis (Table VI) which indicated that patients benefited from TMZ with low expression of CDH2 after adjusting for age, IDH1 status, sex and radiotherapy. Furthermore, TCGA dataset was analyzed, as described above, which identified that patients with low CDH2 expression and who were treated by TMZ therapy also had better OS and PFS than patients treated without TMZ in the low CDH2 expression group (P=0.0010 and P=0.0029, respectively) (Fig. 3A). However, no evident survival benefit of chemotherapy for patients with high CDH2 expression was identified (OS, P=0.1813; PFS, P=0.0663) (Fig. 3B). Cox regression analysis confirmed these results (Table VII) which further revealed that patients with low expression of CDH2 may benefit from TMZ.

Discussion

Glioma is the most common intracranial malignant tumor in adults. GBM is characterized by its high invasive ability, self-renewal capability and drug resistance. Therefore, the 5-year survival rate of patients with GBM is poor (13). Patients treated with TMZ and radiotherapy have a favorable median survival time of 18.8 months compared with those treated with radiotherapy alone (14.4 months) after complete resection of GBM. Prior to the development of novel targeted drugs for clinical glioma treatment, TMZ was considered to be the most effective chemotherapeutic agent. However, although it has significant effect in prolonging the lifespan of some patients with glioma, the efficacy of TMZ for treating certain GBM patients is limited (14), and TMZ resistance may result in a poor prognostic outcome in patients with GBM. Several mechanisms, including DNA repair mechanisms (15), high expression of epidermal growth factor receptor (16), the mutation of p53 (17) and the deficiency of phosphatase and tensin homolog (18), are involved in TMZ resistance. However, in a previous study, one-third of patients exhibited hypermethylation of methylguanine-DNA methyltransferase promoter, signifying sensitivity toward alkylating agents (19). Effective molecular biomarkers for glioma prognosis must be identified in order to provide a guide for clinical treatment.

EMT-associated molecules have been reported to serve an important role in glioma progression. Cells expressing low levels of ZEB1 demonstrated an increased sensitivity to TMZ in GBM (20). Our previous study demonstrated that GBM patients with low vimentin expression had improved survival rates when treated with TMZ (21). N-cadherin (encoded by the CDH2 gene) is a 99.7-kDa glycoprotein and is widely distributed throughout the central nervous system in neuronal and glial cells (22). N-cadherin appears to be upregulated and downregulated according to the requirements of cells and developing tissues (23). Comparable to vimentin and matrix metallopeptidase 9, N-cadherin is accompanied by the downregulation of epithelial cell-surface markers, such as CDH1 (E-cadherin) (24). N-cadherin is broadly expressed in a number of tumor types (25), including neuroblastoma (26), melanoma (27) and multiple myeloma (28). Consequently, we hypothesized that N-cadherin had a potential value to guide the clinical application of chemotherapy.

In the present study, the level of CDH2 was identified to be associated with glioma grade and outcome in the CGGA and Rembrandt datasets. Patients with high-grade glioma had high CDH2 expression compared with patients with low-grade glioma, and patients with high CDH2 expression exhibited a worse outcome. Statistical analysis revealed that CDH2 was an independent prognostic factor in glioma. These results suggested that CDH2 may serve a vital role in the molecular and pathological classification of gliomas and may become a predictive indicator for glioma treatment. Furthermore, patients with GBM

Table V. Univariate and multivariate Cox regression analyses for overall survival in the 275 glioma specimens of the Rembrandt dataset.

	Univariate			Multivariate		
Variable	HR	CI	P-value	HR	CI	P-value
Sex	1.673	1.461-1.916	<0.001	1.620	1.415-1.856	<0.001
TCGA subtype	1.592	1.374-1.844	<0.001	1.027	0.862-1.223	0.768
WHO grade	1.818	1.525-2.167	<0.001	1.297	1.045-1.610	0.018
CDH2 expression	2.034	1.654-2.503	<0.001	1.397	1.102-1.770	0.006

TCGA, The Cancer Genome Atlas; WHO, World Health Organization; CDH2, cadherin 2; HR, hazard ratio; CI, confidence interval; sex (female and male); TCGA subtype (neural, proneural, mesenchymal and classical); WHO grade (II, III and IV); CDH2 expression (low and high expression).

Table VI. Univariate and multivariate Cox regression analyses of overall and progression-free survival for the low CDH2 expression group of the Chinese Glioma Genome Atlas dataset.

A, Overall survival

	Univariate			Multivariate		
Variable	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.463	0.796-2.691	0.221	1.11	0.582-2.115	0.752
IDH1 status	0.429	0.196-0.943	0.035	0.312	0.133-0.737	0.008
Sex	0.943	0.521-1.707	0.846	1.17	0.629-2.178	0.620
Chemotherapy	0.522	0.284-0.958	0.036	0.322	0.161-0.643	0.001
Radiotherapy	1.273	0.500-3.241	0.613	3.622	1.051-12.490	0.042

B, Progression-free survival

	Univariate			Multivariate		
Variable	HR	CI	P-value	HR	CI	P-value
Age	1.246	0.687-2.259	0.211	1.026	0.546-1.927	0.936
IDH1 status	0.622	0.298-1.299	0.206	0.459	0.206-1.026	0.058
Sex	0.925	0.514-1.665	0.795	1.193	0.643-2.213	0.576
Chemotherapy	0.519	0.283-0.954	0.035	0.338	0.169-0.676	0.002
Radiotherapy	1.138	0.500-2.588	0.759	3.011	0.958-9.458	0.059

CDH2, cadherin 2; HR, hazard ratio; CI, confidence interval; IDH1, isocitrate dehydrogenase 1; age (<45 vs. ≥45 years); IDH1 status (mutant and not mutant); sex (female and male); chemotherapy (yes, no and NA); radiotherapy (yes, no and NA).

expressing a lower level of CDH2 may benefit to a greater extent from TMZ therapy.

In conclusion, the present study demonstrated that CDH2 expression is significantly associated with glioma grade, and that high CDH2 expression is an unfavorable prognostic factor for patients with glioma and may have an important value for glioma patients receiving TMZ. These results suggest that CDH2 may serve as a prognostic and predictive molecular biomarker for the grading and treatment of glioma.

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Table VII. Univariate and multivariate Cox regression analyses of overall and progression-free survival for the low CDH2 group of The Cancer Genome Atlas dataset.

A, Overall survival

	Univariate			Multivariate		
Variable	HR	CI	P-value	HR	CI	P-value
Age	2.022	1.153-3.546	0.014	1.845	1.030-3.305	0.040
Sex	0.529	0.292-0.958	0.036	0.905	0.449-1.824	0.779
Chemotherapy	0.343	0.198-0.592	<0.001	0.495	0.249-0.983	0.045
Radiotherapy	0.230	0.121-0.439	<0.001	0.292	0.131-0.650	0.003

B, Progression-free survival

	Univariate			Multivariate		
Variable	HR	CI	P-value	HR	CI	P-value
Age	1.880	0.877-4.030	0.104	1.928	0.799-4.654	0.144
Sex	0.907	0.372-2.210	0.829	0.640	0.247-1.653	0.356
Chemotherapy	0.204	0.065-0.641	0.006	0.240	0.072-0.802	0.020
Radiotherapy	0.488	0.123-1.937	0.308	0.321	0.068-1.518	0.152

CDH2, cadherin 2; HR, hazard ratio; CI, confidence interval; Age (<45 vs. ≥45 years); sex (female and male); chemotherapy (yes, no and NA); radiotherapy (yes, no and NA).

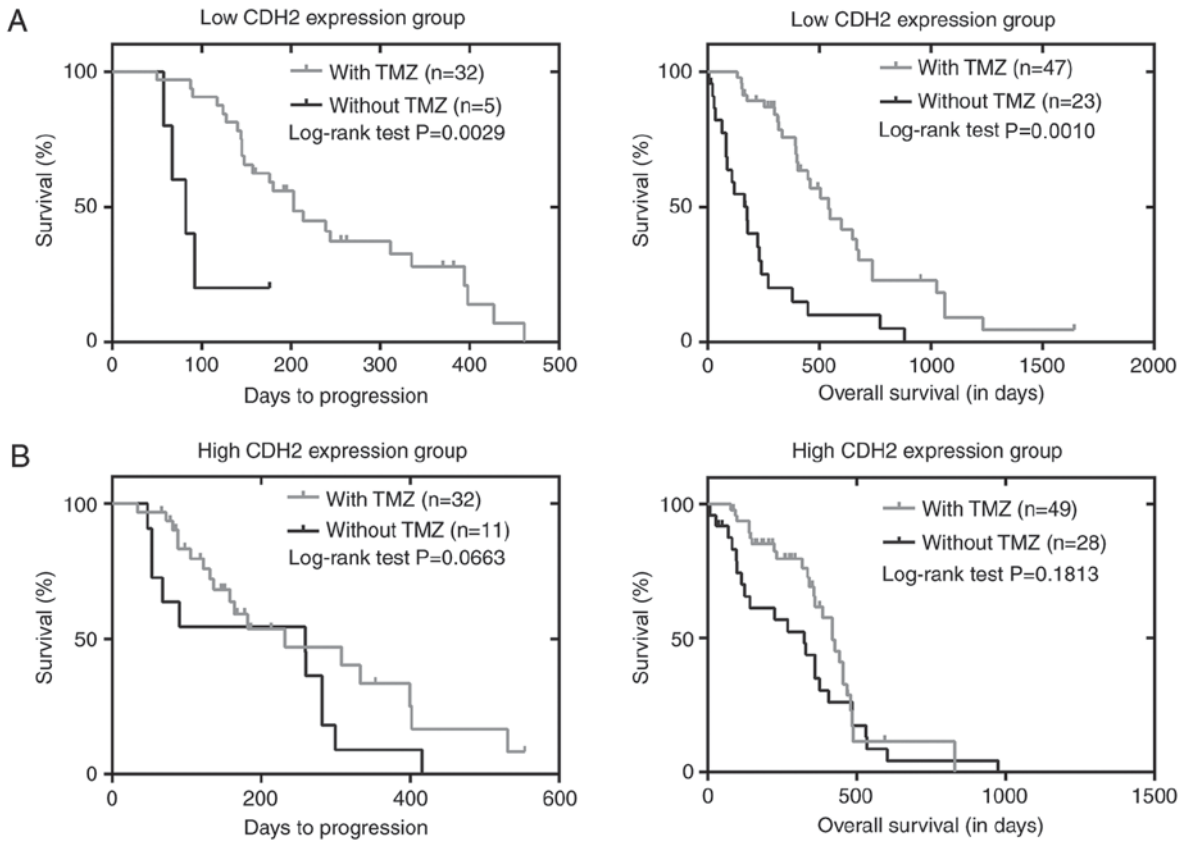

Figure 3. Survival analysis of patients with high-grade glioma treated with or without TMZ in The Cancer Genome Atlas dataset. (A) Kaplan-Meier analyses of PFS (left) and OS (right) in patients in the low CDH2 expression group treated with or without TMZ. (B) Kaplan-Meier analyses of PFS (left) and OS (right) in patients in the high CDH2 expression group treated with or without TMZ. TMZ, temozolomide; PFS, progression-free survival; OS, overall survival; CDH2, cadherin 2.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

QC performed development of methodology, acquisition of data, analysis and interpretation of data and writing, review and revision of the manuscript, and administrative, technical and material support (i.e., reporting or organizing data, constructing databases). JC and CJ performed the conception and design of the study, along with study supervision.

Ethics approval and consent to participate

All patients provided written informed consent, and all human experiments were approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University.

Consent for publication

Consent to publish has been obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

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