Down-Regulation of CEND1 Expression Contributes to The Progression and Temozolomide Resistance of Glioma

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Abstract

Objective: This study was conducted to clarify the expression characteristics of cell cycle exit and neuronal differentiation 1 (CEND1) in glioma and its effects on the proliferation, migration, invasion, and resistance to temozolomide (TMZ) of glioma cells.

Materials and Methods: In this experimental study, CEND1 expression in glioma tissues and its relationship with patients' survival were analyzed through bioinformatics. Quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemistry were performed to detect CEND1 expression in glioma tissues. The cell counting kit-8 (CCK-8) method was adopted to detect cell viability and the effects of different concentrations of TMZ on the inhibition rate of glioma cell proliferation, and the median inhibitory concentration of TMZ (IC_{50} value) was calculated. 5-Bromo-2'-deoxyuridine (BrdU), wound healing and Transwell assays were performed to evaluate the impacts of CEND1 on glioma cell proliferation, migration, and invasion. Besides, the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, Gene Ontology (GO) analysis, and Gene Set Enrichment Analysis (GSEA) were applied to predict the pathways regulated by CEND1. Nuclear factor-kappa B p65 (NF-κB p65) and phospho-p65 (p-p65) expression were detected by Western blot.

Results: CEND1 expression was reduced in glioma tissues and cells, and its low expression was significantly associated with the shorter survival of glioma patients. CEND1 knockdown promoted glioma cell growth, migration, and invasion, and increased the IC50 value of TMZ, whereas up-regulating CEND1 expression worked oppositely. Genes co-expressed with CEND1 were enriched in the NF-κB pathway, and knocking down CEND1 facilitated p-p65 expression, while CEND1 overexpression suppressed p-p65 expression.

Conclusion: CEND1 inhibits glioma cell proliferation, migration, invasion, and resistance to TMZ by inhibiting the NF-κB pathway.

Keywords: CEND1, Glioma, Proliferation, Temozolomide

Introduction

Glioma is a common neurological malignancy, and glioma cases account for 60% of all intracranial tumors (1, 2). Up till now, the treatment options for glioma include surgery, radiotherapy, and chemotherapy, which improve the prognosis of some glioma patients (3). However, due to the biological characteristic of invasive growth of glioma, glioma has a high recurrence rate; the patients' prognosis is still unfavorable (4, 5). With the application of the first-line chemotherapeutic drug temozolomide (TMZ), a growing number of glioma patients have become resistant to TMZ (6). In this context, an in-depth exploration into the drug resistance mechanism of TMZ is highly significant for improving the prognosis of glioma patients.

Cell cycle exit and neuronal differentiation (CEND1) is a specific transmembrane protein highly expressed in the brain and mainly located on the organelle membranes such as the endoplasmic reticulum membrane and mitochondrial outer membrane; reportedly, it regulates the differentiation of neuronal precursors (7, 8). The defect of CEND1 causes impaired cerebellar development and motor coordination disorder in mice (9). Besides, CEND1 can terminate the cell cycle progression and trigger the differentiation of neurons (10). The role of CEND1 in tumors has also been reported. In invasive breast cancer, it is revealed that CEND1 expression is epigenetically inhibited by methylation, which indicates that CEND1 may play a tumor-suppressive role in invasive breast cancer (11). However, the expression characteristics and biological functions of CEND1 in glioma remain unclear.

In this study, bioinformatics analysis indicated that CEND1 expression in glioma tissues was significantly reduced and was linked to the patient's poor prognosis. We investigated the expression pattern of CEND1 in

gliomas, its biological functions, and its underlying mechanism.

Materials and Methods

Clinical samples

From January 2015 to January 2018, 40 cases of tumor samples (glioma) and 20 cases of normal brain tissue (NBT) samples were collected from glioma patients. All tissues, after being surgically removed, were frozen in liquid nitrogen. Every patient signed the informed consent form prior to the surgery. The diagnoses of glioma were histologically confirmed by three independent pathologists. The study got the approval of the Ethics Committee of the Affiliated Hospital of Kunming Medical University (SCXK(Dian)K2019-0004).

Cell culture and transfection

From the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), glioma cell lines (U251, U251/TMZ, U87, and U87/TMZ) and normal human brain astrocyte cell line (HEB) were obtained. U251/TMZ and U87/TMZ were glioma cells that were resistant to TMZ treatment. All the cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C in 5% CO_2 and 100% humidity. CEND1 overexpression plasmid was constructed via cloning the cDNA of the CEND1 sequence into the pcDNA3.1 vector (GenePharma Co., Ltd., Shanghai, China). And the empty pcDNA3.1 vector was used as a negative control (NC). Small interfering RNA (siRNA) normal control (si-NC: 5′-AGGCCAGCGTCTTCCACTCACCCAA-3′) and siRNAs targeting CEND1 (si-CEND1#1: 5′-AGGCCGACCCTGCCCTTCTCAACAA-3′ and si-CEND1 #2: 5′-GACCCTGCCCTTCTCAACAACCACA-3′) were constructed with the Silencer™ siRNA Construction Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. Glioma cells were transferred into 6-well plates (3×10^5 cells/well) and cultured for 24 hours with a serum-free medium. After that, cell transfection was performed employing Lipofectamine® 2000 (Invitrogen, Thermo Fisher Scientific, Inc.) following the supplier's instructions. After 24 hours, the serum-free medium was replaced by a complete medium, followed by the culture for 24 hours. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to detect the transfection efficiency.

Quantitative real-time polymerase chain reaction

Glioma tissues and cells were harvested, and total RNA was extracted using TRIzol reagent (Invitrogen, Shanghai, China). A NanoDrop1000 spectrophotometer (NanoDrop Technologies LLC, DE, USA) was employed to detect the concentration and purity of RNA. A TaKaRa Reverse Transcription Kit (TaKaRa Inc., Japan) was utilized to reversely transcribe RNA into cDNA. The cDNA, as the template, was mixed with SYBR Premix Eaq™ II (Takara, Dalian, China), primers, and DEPC water, and then the amplification was performed. The following are the primer sequences:

CEND1-
F: 5′-ATGGAATCCCGAGGAAAGTCA-3′
R: 5′-GCCTGAGGCACCTTGGTATC-3′

GAPDH-
F: 5′-CATGGGTGTGAACCATGAGA-3′
R: 5′-CAGTGATGGCATGGACTGTG-3′.

The $2^{-\Delta\Delta Ct}$ method was used for the calculation of the relative expression of *CEND1*, with *GAPDH* as the endogenous control.

Immunohistochemistry

The histological sections of the glioma were baked at 56°C for 2 hours before being deparaffinized and rehydrated. Subsequently, antigen retrieval was performed, and the sections were blocked in 3% bull serum albumin for 30 minutes. After that, the sections were incubated with the primary Recombinant Anti-CEND1 antibody (ab113076, 1:500, Abcam, Shanghai, China) at 4°C overnight in a wet box. On the second day, the sections were incubated with the secondary Goat anti-rabbit IgG H&L antibody (ab150077, 1:1000, Abcam, Shanghai, China) at room temperature for 30 minutes in a wet box. Then, the sections were stained with diaminobenzidine and observed under a microscope. The color development was terminated after the color or the tissues turned brown. Eventually, the sections were sealed with neutral gum. The images were analyzed with the Image-Pro Plus 6.0 software, and the intensity of staining and the percentage of stained cells were evaluated and quantified by three independent pathologists. IHC score was calculated based on the staining intensity score (0, no staining; 1, weak staining; 2, moderate staining; and 3, intense staining) and the proportion score (0, no staining; 1, 1-25% of the tumor cells were stained; 2, 26-50%; 3, 51-75%; and 4, more than 75% of the tumor cells were stained). IHC score=staining intensity score × proportion score. IHC score was used to evaluate the expression of CEND1: 0-3 points, negative; 4-7 points, weakly positive; 8-12 points, strongly positive.

Cell counting kit-8 assay

U251/TMZ and U87/TMZ cells during the logarithmic growth phase were transferred to 96-well plates (5×10^3 cells/well), with 3 replicate wells in each group. After culturing cells in the incubator for 3 hours, different concentrations of TMZ (0, 1, 5, 10, 20, 40, and 60 μmol/L) were added, with 3 parallel wells in each group. After another 24 hours, the cells were incubated with 10 μL of CCK-8 reagent (Beyotime Biotechnology, Shanghai, China) for 2 hours. An automatic microplate reader was

used to measure the absorbance (OD) of the cells in each well at 450 nm wavelength. The following equation is for calculating cell survival rate and the inhibition rate of the drug on cells: cell survival rate (%)=(control group OD value-drug group OD value)/control group OD value. Ultimately, SPSS 22.0 software (SPSS Inc.,Chicago, IL, USA) was adopted to calculate the median inhibitory concentration of the drug (IC_{50} value).

5-bromo-2'-deoxyuridine assay

U87/TMZ and U251/TMZ cells were transferred to 24-well plates. After the cells were cultured in serum-free medium for 24 hours, the cells were incubated with 10 μmol/L of BrdU solution (Beyotime, Shanghai, China) in a complete medium for 4 hours at 37°C. After the medium was removed, the cells were rinsed 3 times with phosphate buffer saline (PBS), fixed with 70% ethanol for 10 minutes at 4°C, and then washed 3 times with PBS. Then, 2 mol/L of HCl was added to denature the cellular DNA at 37°C for 60 minutes before the cells were washed 3 times with PBS. After that, the cells were blocked with 3% bovine serum albumin (KPL, Gaithersburg, MD, USA) at room temperature for 1 hour, followed by the rinse with PBS 3 times, 5 minutes for each time. Subsequently, the cells were incubated overnight at 4°C with anti-BrdU monoclonal antibody (ab8152, 1:300, Abcam, Shanghai, China) and then with goat anti-mouse fluorescent secondary Goat Anti-Mouse IgG H&L antibody (ab150113, 1:1000, Abcam, Shanghai, China) at room temperature for 2 hours. After counterstaining the cell nucleus with DAPI, the cells were observed under a fluorescence microscope. In 10 randomly selected non-overlapping fields, the number of BrdU-positive cells was counted, and the average was calculated.

Scratch wound healing assay

After the confluency of U251/TMZ and U87/TMZ cells cultured in a 6-well plate reached about 90%, the cells were scratched with a sterile pipette tip and then cultured with serum-free medium. The width of the scratch was observed at 0 and 24 hours, respectively. Scratch wound healing rate=(scratch width 0 hour after scratching-scratch width 24 hours after scratching)/scratch width 0 hour after scratching×100%.

Transwell assays

The density of cell suspension was diluted to $1×10^5$ cells/ml with a serum-free medium. The upper compartment of the Transwell chamber (Corning, NY, USA) was added with 200 μL of cell suspension, and 500 μL of complete medium was added to the lower compartment. After 24 hours, the cells in the upper compartment were wiped off with cotton swabs, and the cells attaching to the below surface of the membrane were washed twice with PBS and then fixed with paraformaldehyde for 30 minutes. Subsequently, the cells were stained for 30 minutes with 0.1% crystal violet, followed by being washed with PBS. Under an inverted microscope (DMI4000, Leica, Germany), the number of cells passing through the membrane was counted, and the cells were photographed. Three random fields were observed, and the average of the cell number was calculated. Matrigel-coated Transwell chamber was used in the invasion assay, and the remaining steps were the same as those in the migration assay.

Western blot

For each sample, 100 mg of glioma tissues or $2×10^7$ cells were added to 1 ml of RIPA lysis buffer (Pierce, Rockford, IL, USA). The tissue or cells were lysed on the ice for 30 minutes. Next, the total cell protein was extracted, and a BCA kit (Rockford, IL, USA) was applied for determining the protein concentration. The protein, after electrophoresis, was transferred to the polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), and the membranes were then blocked with 5% skimmed milk for 1 hour at room temperature. Subsequently, the membranes were incubated with primary antibodies at 4°C overnight and then with secondary Goat Anti-Rabbit IgG H&L antibody (HRP) (ab6721, 1:5000) for 2 hours at room temperature. Besides, the protein bands were detected by a Bio-Rad chemiluminescence apparatus (Bio-Rad, Berkeley, CA, USA), with GAPDH as the internal control, and the Image J software was used for the quantitative analysis of the protein bands. The primary antibodies included: the anti-nuclear factor-kappa B p65 (NF-κB p65) antibody (ab32536, 1:2000) and the anti-phospho-p65 (p-p65) antibody (ab97726, 1:2000). All antibodies were bought from Abcam (Shanghai, China).

Statistical analysis

The statistical analysis was conducted employing SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). All the experiments were repeated 3 times. All measurement data were expressed by "mean ± standard deviation". The comparison between the two groups was performed with the t test. P<0.05 represented that a difference was of statistical significance.

Results

### CEND1 expression is downregulated in glioma tissues and cell lines

First of all, GEPIA (http://gepia.cancer-pku.cn/) and UALCAN (http://ualcan.path.uab.edu/cgi-bin/ualcan-res.pl) databases were utilized to analyze CEND1 expression in glioma, and it was revealed that CEND1 expression was downregulated in both low-grade glioma and glioblastoma (Fig.1A, B). Moreover, the GEPIA database indicated that low CEND1 expression was also linked to the shorter overall survival of the patients (Fig.1C). Additionally, qRT-PCR was performed to detect the CEND1 mRNA expression in 20 cases of NBT samples and 40 cases of glioma tissue samples, and it was shown that CEND1 mRNA expression was significantly upregulated in glioma tissues (Fig.1D). Furthermore,

CEND1 protein expression in glioma tissues was detected by IHC, the results of which showed that there was a lower positive rate of CEND1 protein expression in the glioma tissues compared with NBT (P=0.044, Chi-square=4.07, Fig.1E).

The effects of CEND1 on glioma cell viability, sensitivity to TMZ, migration, and invasion

Additionally, we performed the western blot to detect the expression of CEND1 in different cell lines. It showed that the expression of CEND1 in immortalized glial cell line HMC3 was lowly expressed, while its expression in primary human neuron cells was highly expressed, and its expression was reduced in glioma cell lines (U251 and U87); the results also indicated that CEND1 expression was downregulated in U251/TMZ and U87/TMZ cells as opposed to U251 and U87 cells (Fig.2A). The expression of CEND1 was lower in U87 cells, so U87 cells were selected for subsequent CEND1 overexpression experiments; the expression of CEND1

was higher in U251 cells, so U251 cells were selected for subsequent CEND1 knockdown experiments. Western blot (Fig.2B) and immunofluorescence (Fig.2C) were performed to confirm the successful construction of the CEND1 overexpression model and CEND1 knockdown model. Since transfection with si-CEND1#2 exhibited a greater silencing effect, si-CEND1#2 was used for subsequent experiments. CCK-8 assay revealed that CEND1 knockdown markedly promoted U251/TMZ cell viability and increased the IC_{50} value of TMZ (Fig.2D), and CEND1 overexpression remarkably suppressed U251/TMZ cell viability and decreased the IC_{50} value of TMZ (Fig.2E). BrdU assay suggested that CEND1 knockdown facilitated U251/TMZ cell proliferation, and CEND1 overexpression observably inhibited U87/TMZ cell proliferation (Fig.3A, B). Scratch wound healing and Transwell assays showed that knocking down CEND1 promoted U251/TMZ cell migration and invasion, and CEND1 overexpression suppressed U87/TMZ cell migration and invasion (Fig.3C-F).
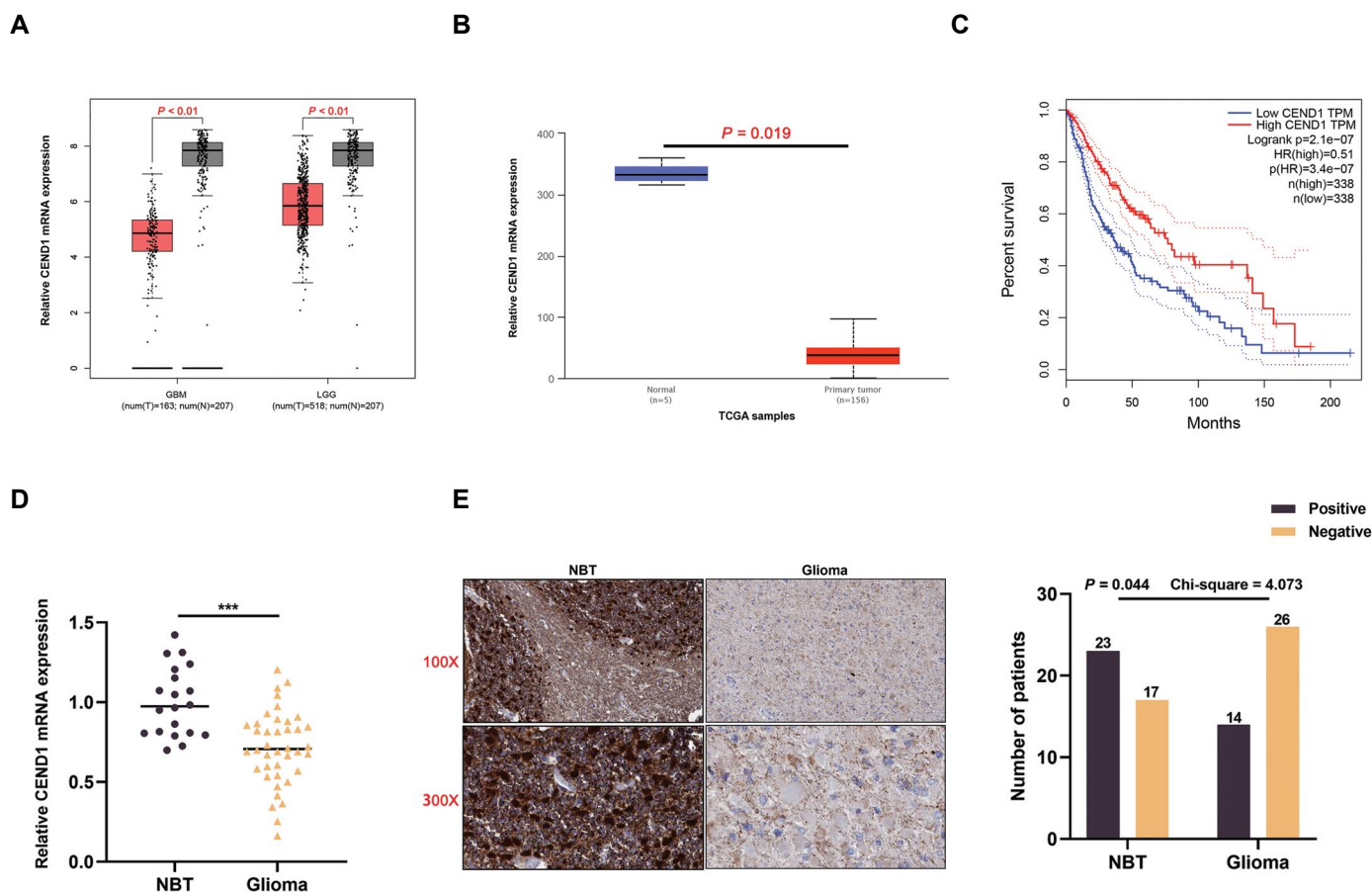
Fig.1: Expression of CEND1 in glioma tissues and cell lines. **A.** CEND1 mRNA expression in glioma tissues (red column) and normal brain tissues (grey column) was analyzed by the GEPIA database (log2|FC|>1.0 and P<0.05 as the selection criteria). **B.** CEND1 mRNA expression in glioma tissues (red column) and normal brain tissues (blue column) was analyzed by the UALCAN database (log2|FC|>1.0 and P<0.05 as the selection criteria). **C.** The relationship between CEND1 expression and the glioma patients' overall survival was analyzed by the GEPIA database. **D.** CEND1 mRNA expression in 20 NBT and 40 glioma tissues was detected by qRT-PCR. **E.** CEND1 protein expression in 20 NBT and 40 glioma tissues was detected by IHC assay. Then, the samples were diagnosed by pathologists. CEND1; Cell cycle exit and neuronal differentiation 1, ***; P<0.001, GBM; Glioblastoma multiforme, LGG; Lower grade glioma, NBT; Normal brain tissue, and qRT-PCR; Quantitative real-time polymerase chain reaction.
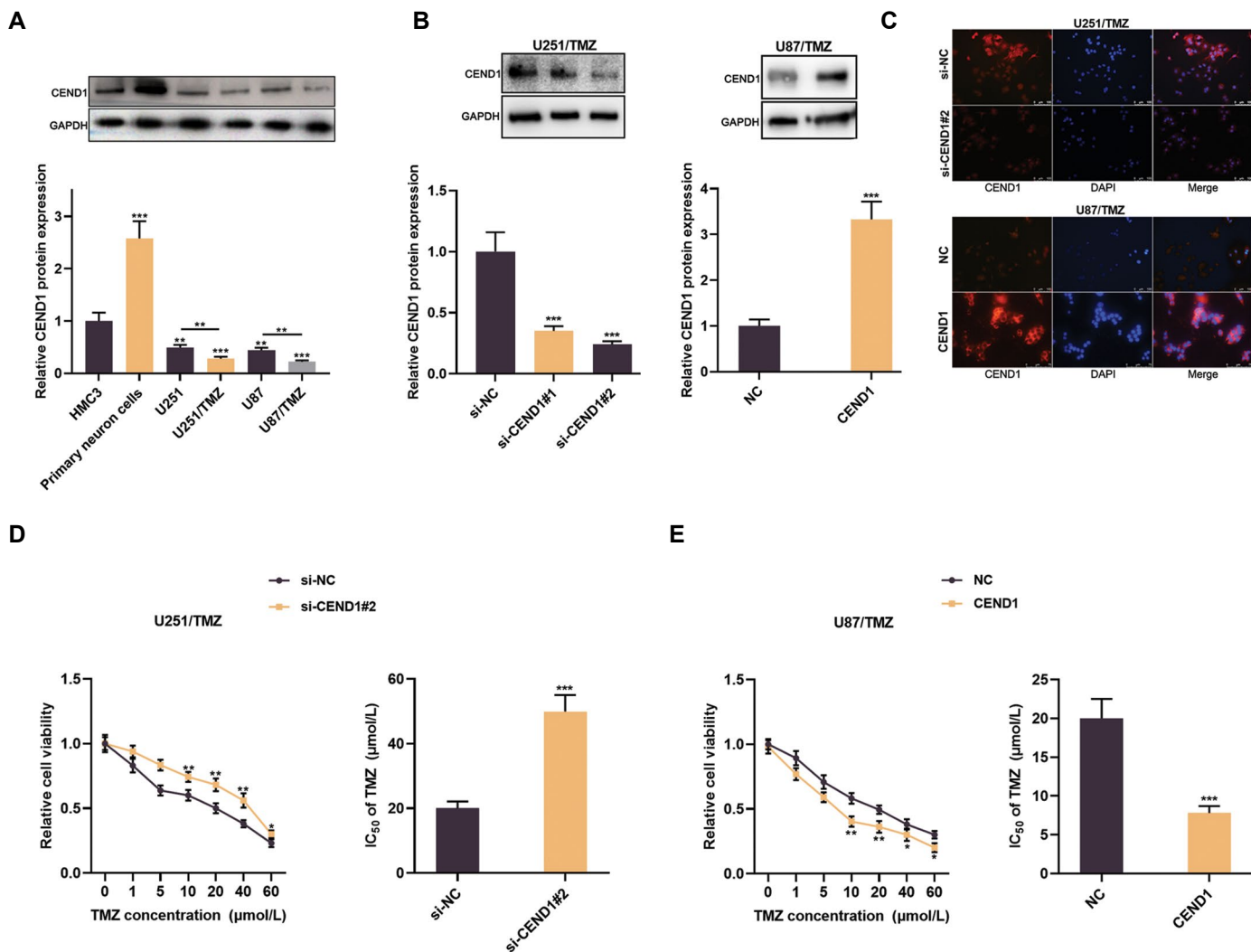
Fig.2: Effects of CEND1 on the resistance of glioma cells to TMZ. **A.** CEND1 protein expression in glioma cell lines, glial cell line HMC3, and primary neuron cells was detected by Western blot. **B, C.** After transfecting si-NC, si-CEND#1, and si-CEND#2 into U251/TMZ cells and transfecting NC and CEND1 into U87/TMZ cells, Western blot and immunofluorescence were utilized to confirm the successful construction of the CEND1 knockdown model and the CEND1 overexpression model. **D.** After transfecting si-NC or si-CEND#2 into U251/TMZ cells, the CCK-8 assay was conducted to detect the effects of knocking down CEND1 on the IC$_{50}$ value of TMZ. **E.** After transfecting NC or CEND1 overexpression plasmid into U87/TMZ cells, the CCK-8 assay was performed to detect the effects of CEND1 overexpression on the IC$_{50}$ value of TMZ. ***; P<0.001, CEND1; Cell cycle exit and neuronal differentiation 1, TMZ; Temozolomide, si-NC; Negative control siRNA, si-CEND1; CEND1 siRNA, and IC$_{50}$; Half maximal inhibitory concentration.

CEND1 knockdown activates the NF-κB pathway

To expound the downstream mechanism by which CEND1 functions in glioma, the cBioPortal database was adopted to screen the genes co-expressed with CEND1 (Spearman's r < -0.56, P<0.05) The enrichment analysis was conducted using the Kyoto Encyclopedia of Genes, Genomes (KEGG), and Gene Ontology (GO) databases. GO analysis revealed that the genes co-expressed with CEND1 were associated with multiple biological processes (BP), cellular components (CC), and molecular functions (MF), including the toll-like receptor 7 signaling pathway, SCAR complex, lamellipodium, and toll-like receptor 2 binding and so on (Fig.4A).

KEGG analysis implied that the genes co-expressed with CEND1 were associated with the NF-κB pathway (Fig.4B). Then, Gene Set Enrichment Analysis (GSEA) analysis was performed using the LinkedOmics database (http://www.linkedomics.org/). The results revealed that NF-κB pathways positively correlated with low expression of CEND1 (Fig.4C). After transfected with si-NC, si-CEND1#2, control plasmids, and CEND1 overexpression plasmids for 24 hours, the U251/TMZ and U87/TMZ cells were collected and followed with the Western blot analysis. It was revealed that knocking down CEND1 enhanced p-p65 expression while up-regulating CEND1 reduced p-p65 expression in

glioma cells. Additionally, after CEND1 was depleted, the expression levels of CD133 and CD15 were upregulated, while after CEND1 was overexpressed, those were downregulated (Fig.4D), which suggested that reduced CEND1 expression in glioma cells contributed to increased stemness of glioma cells. The aforementioned findings suggested that CEND1 could probably regulate glioma cell proliferation, migration, invasion, and sensitivity to TMZ by modulating the NF-κB pathway.
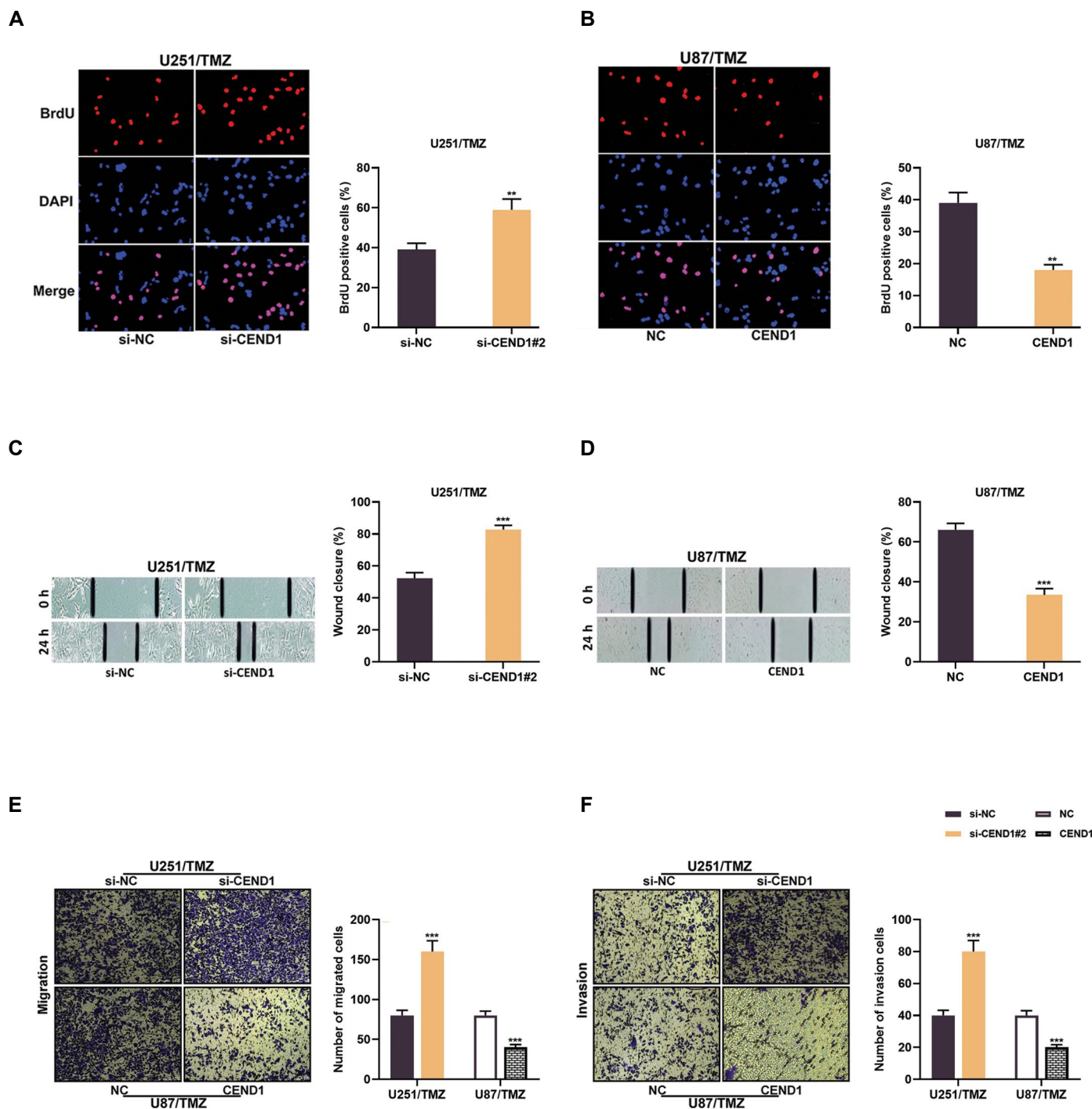
Fig.3: Effects of CEND1 on glioma cell proliferation, migration, and invasion. **A, B.** After transfecting si-NC or si-CEND#2 into U251/TMZ cells and transfecting NC or CEND1 overexpression plasmid into U87/TMZ cells, BrdU assay was performed to detect the effects of CEND1 overexpression or knockdown on U251/TMZ and U87/TMZ cell proliferation. **C, D.** A scratch wound-healing assay was conducted to detect the effects of CEND1 overexpression or knockdown on U251/TMZ and U87/TMZ cell wound healing. **E.** The effects of CEND1 overexpression or knockdown on U251/TMZ and U87/TMZ cell migration were detected by the Transwell migration assay. **F.** Transwell invasion assay was conducted to determine the effects of CEND1 overexpression or knockdown on U251/TMZ and U87/TMZ cell invasion. **; P<0.01, ***; P<0.001, CEND1; Cell cycle exit and neuronal differentiation 1, TMZ; Temozolomide, si-NC; Negative control siRNA, and si-CEND1; CEND1 siRNA.
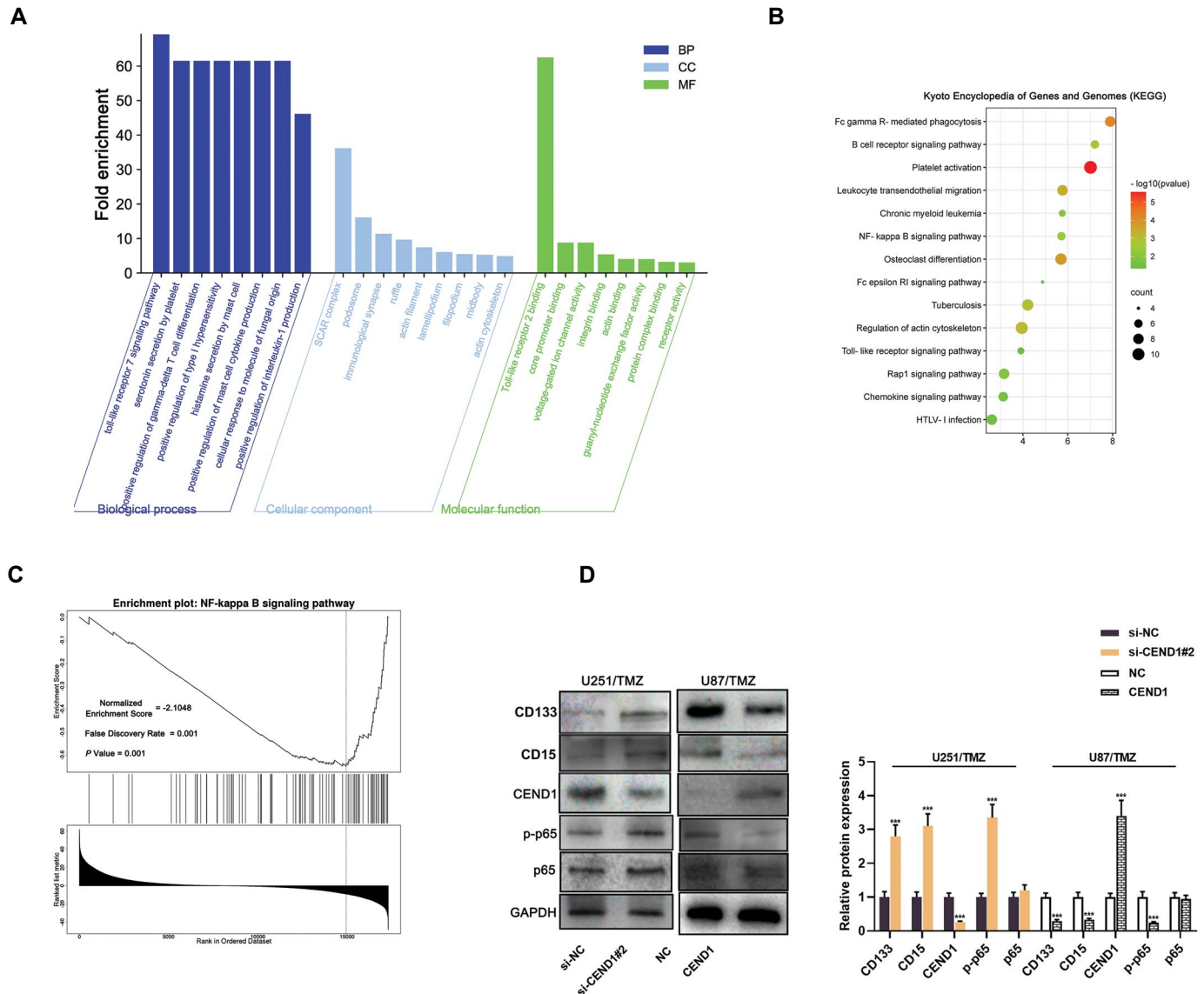
Fig.4: CEND1 knockdown can activate the NF-κB pathway. **A.** cBioPortal database was adopted to screen 200 genes that were co-expressed with CEND1 (Spearman's r<-0.56, P<0.05). The GO database was adopted to analyze the pathways in BP, CC, and MF where these genes were enriched. **B.** KEGG database was used to perform pathway enrichment analysis of these genes. **C.** Through the LinkedOmics database, CEND1 was analyzed for Gene Set Enrichment Analysis (GSEA). **D.** The effects of CEND1 knockdown or overexpression on p65, p-p65, CD133, and CD15 were detected by Western blot. ***; P<0.001, CEND1; Cell cycle exit and neuronal differentiation 1, NF-κB; Nuclear factor-kappa B, GO; Gene Ontology, BP; Biological processes, CC; Cellular components, MF; Molecular functions, TMZ; Temozolomide, si-NC; negative control siRNA, and si-CEND1; CEND1 siRNA.

Discussion

CEND1 expression is significantly elevated during the differentiation of neural precursor cells into neurons, and this process is accompanied by an increase in p53 protein expression, which suggests that CEND1 can activate the p53-p21 signaling pathway to prolong the G1 phase of the cells, thus arresting the cell cycle (10, 12, 13). It is reported that in a mouse model, after CEND1 knockdown, the proliferation of granule cell precursors is promoted, radial granule cell migration is delayed, and the differentiation of Purkinje cells is blocked (14). Some studies indicated that there are many genes involved in the development of glioma. The downregulation of ABCE1 expression significantly reduces the protein expressions of p-PI3K, p-Akt, and NF-κB in glioma cells, thus inhibiting glioma

cell growth (15). CRNDE knockdown represses the expressions of LC3 II/I, Beclin1, and Atg5 and increases the p62 expression to inhibit autophagy due to the activation of PI3K/Akt/mTOR pathway, thus promoting TMZ chemosensitivity in glioma (16). In neuroblastoma, CEND1 is an important molecular target for histone deacetylase (HDAC) inhibition, and CEND1 knockdown reduces the impacts of trichostatin-A (TSA) on inhibiting tumor cell proliferation and inducing cell differentiation (17). Another study reports that the functional interaction among CEND1, RanBPM, and Dyrk1B affects the balance between the proliferation and the differentiation of neuroblastoma cells in mice; CEND1 restrains neuroblastoma cell proliferation through modulating cyclin D1 pathway (12). Consistently, our study revealed

that CEND1 probably functioned as a tumor suppressor in glioma. We demonstrated that CEND1 expression was downregulated in glioma tissues and was associated with the increased tumor grade and shorter survival time of the patients. In addition, knocking down CEND1 significantly facilitated cell multiplication, migration, and invasion, and enhanced the TMZ resistance, whereas CEND1 overexpression induced the opposite effects. The above-mentioned evidence supported that CEND1 was a potential prognostic marker for glioma patients, and the restoration of CEND1 might be a novel strategy to reduce the aggressiveness of glioma and prolong the survival of glioma patients.

Known as a kind of imidazole tetrazine derivative, TMZ is an oral alkylating agent and is distributed throughout the body without liver metabolism, and it can easily penetrate the blood-brain barrier and enter the cerebrospinal fluid to exert its pharmacologic efficacy; these properties allow TMZ to be a chemotherapy drug extensively used for glioma treatment (18-21). TMZ effectively inhibits postsurgical glioma recurrence, and it has become the first-line chemotherapy agent to treat high-grade glioma (21). TMZ transforms into the active compound 5-(3-methyltriazene-1-yl) imidazole-4-carboxamide (MTIC), thus suppressing the growth of various tumor cells and inducing apoptosis (22, 23). O6 methylguanine induced by TMZ is pivotal in the anti-tumor activity of TMZ, and the methylated guanine cannot bind to the thymine and can induce mismatch repair during DNA replication, leading to breaks of DNA, blocking DNA replication, and ultimately arresting the cells in the G2/M phase (24). The mechanism of glioma resistance to TMZ is complex, and reportedly, O-6-methylguanine-DNA methyltransferase (MGMT) mediates TMZ resistance (25). Additionally, there are some studies reporting that DNA damage repair is also implicated in TMZ resistance (26, 27). The present study revealed that CEND1 knockdown facilitated the resistance of glioma cells to TMZ while CEND1 overexpression worked oppositely. Interestingly, MGMT protein is not expressed in U87 and U251 cells (28-30), which are used in the present study, so the effects of CEND1 on TMZ chemosensitivity may not depend on the MGMT pathway.

NF-κB is first discovered in B cell extracts in 1986, and it is a nucleoprotein factor that can specifically bind to the enhancer sequence of the gene of immunoglobulin K light chain (31). The activation of NF-κB can up-regulate the expressions of multiple immune and inflammatory factors and induce an inflammatory response and other BP (32, 33). NF-κB family has 5 members: Rela (p65), RelB, C-Rel, NF-kappa B1 (p50/p105), and NF-kappa B 2 (p52/p100); they form a complex in the form of homodimers or heterodimers, among which p50/p65 heterodimer is the most important form (34). In tumorigenesis and development, p65 partakes in the regulation of gene transcription and modulates downstream target genes such as vascular endothelial growth factor (VEGF), chemokine receptors (such as CXCR4), matrix metalloproteinase 2/9, (MMP2/9), etc. (35). NF-κB is also involved in glioma progression. Specifically, lncRNA-ATB overexpression

can activate the NF-κB pathway to promote the aggression of glioma cells (36). Moreover, knocking down WWP1 can activate the NF-κB pathway to increase the p65 phosphorylation expression level, thereby facilitating the malignant behaviors of glioma cells (37). The present study authenticated that CEND1 knockdown could activate NF-κB signaling, which indicated that CEND1 suppressed glioma cell proliferation and migration via regulating the NF-κB pathway.

Conclusion

To sum up, CEND1 expression is reduced in glioma and is associated with unfavorable prognosis; CEND1 overexpression inhibits the aggressiveness of glioma cells and reduces their resistance to TMZ via regulating NF-κB signaling. In the following studies, more patients are required to be enrolled to further validate the value of CEND1 as a prognostic biomarker.

Acknowledgements

Authors' Contributions

Z.H., B.P.; Conceived, designed the experiments, performed the experiments, and wrote the manuscript. Z.H.; Analyzed the data. All authors read and approved the final manuscript.

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