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Research article

TMEM2 induces epithelial-mesenchymal transition and promotes resistance to temozolomide in GBM cells

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ABSTRACT

Glioblastoma multiforme (GBM) is the most common intracranial malignant tumor and is notorious for its poor prognosis. An important element in the short overall survival of GBM patients is the lack of understanding the pathogenesis and progression of tumor and deficiency biomarkers that can be used for early diagnosis and therapeutic sensitivity monitoring. Studies have shown that transmembrane protein 2 (TMEM2) is participated in tumorigenesis of various human tumors, including rectal and breast cancers. Although Qiuyi Jiang et al. have reported that TMEM2 combined with IDH1/2 and 1p19q can predict the survival time of glioma patients based on bioinformatics, its expression and biological role of glioma remain unclear. In our study, we investigated the effect of TMEM2 expression level on glioma malignancy in public datasets and an independent internal dataset. We revealed TEMM2 expression was higher in GBM tissues than in non-tumor brain tissues (NBT). Moreover, the increase in TMEM2 expression level was closely related to tumor malignancy. The survival analysis showed that TMEM2 high expression reduces survival time in all glioma patients, including GBM and LGG patients. Subsequent experiments demonstrated that knockdown TMEM2 inhibited proliferation of GBM cells. In addition, we analyzed TMEM2 mRNA levels in different GBM subtypes, and demonstrated that TMEM2 expression was upregulated in mesenchymal subtype. Meanwhile, bioinformatics analysis and transwell assay indicated that knockdown TMEM2 suppressed epithelial-mesenchymal transition (EMT) in GBM. Importantly, Kaplan–Meier analysis demonstrated that TMEM2 high expression reduced the treatment response to TMZ in GBM patients. Knockdown of TMEM2 alone did not reduce apoptosis GBM cells, but significant apoptotic cells were observed in the group treated with additional TMZ. These studies may contribute to improving the accuracy of early diagnosis and evaluating the effectiveness of TMZ treatment in GBM patients.

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1. Introduction

GBM is a WHO grade IV intracranial malignancy, which is one of the most aggressive malignancies with the worst prognosis. Despite standard clinical treatment, the median survival time of patients with GBM is only 12–15 months, and the five-year survival rate is less than 5% [1,2]. In the past few decades, with the rapid development of biotechnology, more and more molecular targets have been detected. Targeted therapy has grown up to be a potential therapeutic approach for GBM. More molecular targets for early diagnosis, classification and monitoring of the treatment effect of glioma need to be found.

TMEM2 was a cell surface transmembrane protein that can directly degrade hyaluronan (HA) in the extracellular matrix, and regulated cell invasion and migration [3,4]. TMEM2 has been indicated to take part in endoplasmic reticulum homeostasis [5]. Recently, it was found that TMEM2 also involved in the development of a variety of tumors. YuzanKudo et al. reported that TMEM2 overexpression predicted poor prognosis in pancreatic ductal adenocarcinoma [6]. Furthermore, knockdown of TMEM2 inhibited cell migration and invasion via suppressed *p*-JAK1 and p-STAT pathways in triple-negative breast cancer [7]. TMEM2 was transcriptional activated by SOX4, which promoted cell invasion and metastasis, and reduced the survival time of breast cancer patients [8]. In another study, TMEM2 combined with IDH and 1p19q was utilized to predict survival in glioma patients [9]. Nevertheless, the potential role of TMEM2 in glioma and tumorigenesis mechanism remains unknown.

In this research, we combined public databases and an independent internal dataset to analysis the expression of TMEM2 and its influence on tumor malignancy. We confirmed that TMEM2 was upregulated in GBM, and its expression was associated with tumor malignancy. Patients with TMEM2 low expression have a better prognosis in all glioma patients, including GBM and LGG patients. Knockdown of TMEM2 not only inhibited cell proliferation, but also suppressed EMT-induced invasion and metastasis in GBM. Importantly, we identified TMEM2 as a biomarker of GBM response to TMZ using Kaplan–Meier analysis in the TCGA database and obtained the consistent results using flow cytometry in U87 and U251 cells.

2. Materials and methods

2.1. Tissue samples

The tissue chip microarray consists of 174 glioma tissues and 8 NBT. All tissues were collected from the Department of Neurosurgery of Renmin Hospital of Wuhan University from July 2015 to Mar 2020. A total of 3 NBT and 7 GB M tissues were used for Western blot analysis. All patients signed informed consent, and the study was approved by the Ethics Committee of Renmin Hospital of Wuhan University (Approval number: 2012LKSZ (010) H).

2.2. Public data acquisition

A total of five public datasets were used in our study, including TCGA-GBM, TCGA-LGG, TCGA-GBMLGG, CGGA and Rembrandt. All datasets were obtained from GlioVis website (http://gliovis.bioinfo.cnio.es/) [10].

2.3. Antibodies and drug

TMEM2 antibody (ab272644) was purchased from Abcam. *E*-cadherin (20874-1-AP), *N*-cadherin (22018-1-AP, 1), β-actin (66009-1-Ig), SNAI1 (13099-1-AP) and MMP2(10373-2-AP) were purchased from Proteintech. Temozolomide (S1237) was obtained from Selleck.

2.4. Cell culture and transfection

GBM cell lines were purchased from Pricella (Wuhan, China). All cell lines were incubated at 37 °C in an incubator containing 5% CO_2 using Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1% Penicillin-Streptomycin. TMEM2-shRNA-1 and TMEM2-shRNA-2 were purchased from Miaolingbio (Wuhan, China). Cells in each well of a 6-well plate were transfected with 2 μg of plasmid by lipo3000 (Invitrogen, USA), and cells were harvested after 48 h of transfection.

2.5. CCK8 and edu assay

For CCK8 assay, cells were harvested and planted into 96-well plates after transfection for 48 h. Then 10 μl of CCK8 (Dojindo) was added to each well at certain time, and the absorbance at 450 nm was measured by a microplate reader after incubation at 37 °C for 60 min. For Edu assay, 5000 cells were harvested and planted into 96-well plates. After 24 h, 100 μl of 50 μM Edu was added to each well and incubated at 37 °C for 60 min, cells were then stained with Apollo® reaction cocktail and Hoechst 33,342, respectively. Fluorescence images were obtained using a fluorescence microscope (Olympus BX51).

2.5.1. Transwell assay

For invasion assay, GBM cells were seeded into the upper chambers precoated with Matrigel (R&D). For migration assay, cells were planted into the upper chambers. Cells in upper chambers were cultured with 200 μl DMEM without FBS, and lower chambers were added with 600 μl cell culture medium supplemented with 10% FBS. After 36 h of culture, cells were fixed for 15 min and then washed

three times with PBS, and 0.2% Crystal Violet was added to stain 10 min. The images were obtained an inverted microscope (Olympus BX51).

2.6. Apoptosis assay

Annexin V-PE/7- ADD kit (Becton Dickinson) was utilized to detect cell apoptosis. GBM cells were treated with TMZ (400 μM) or the

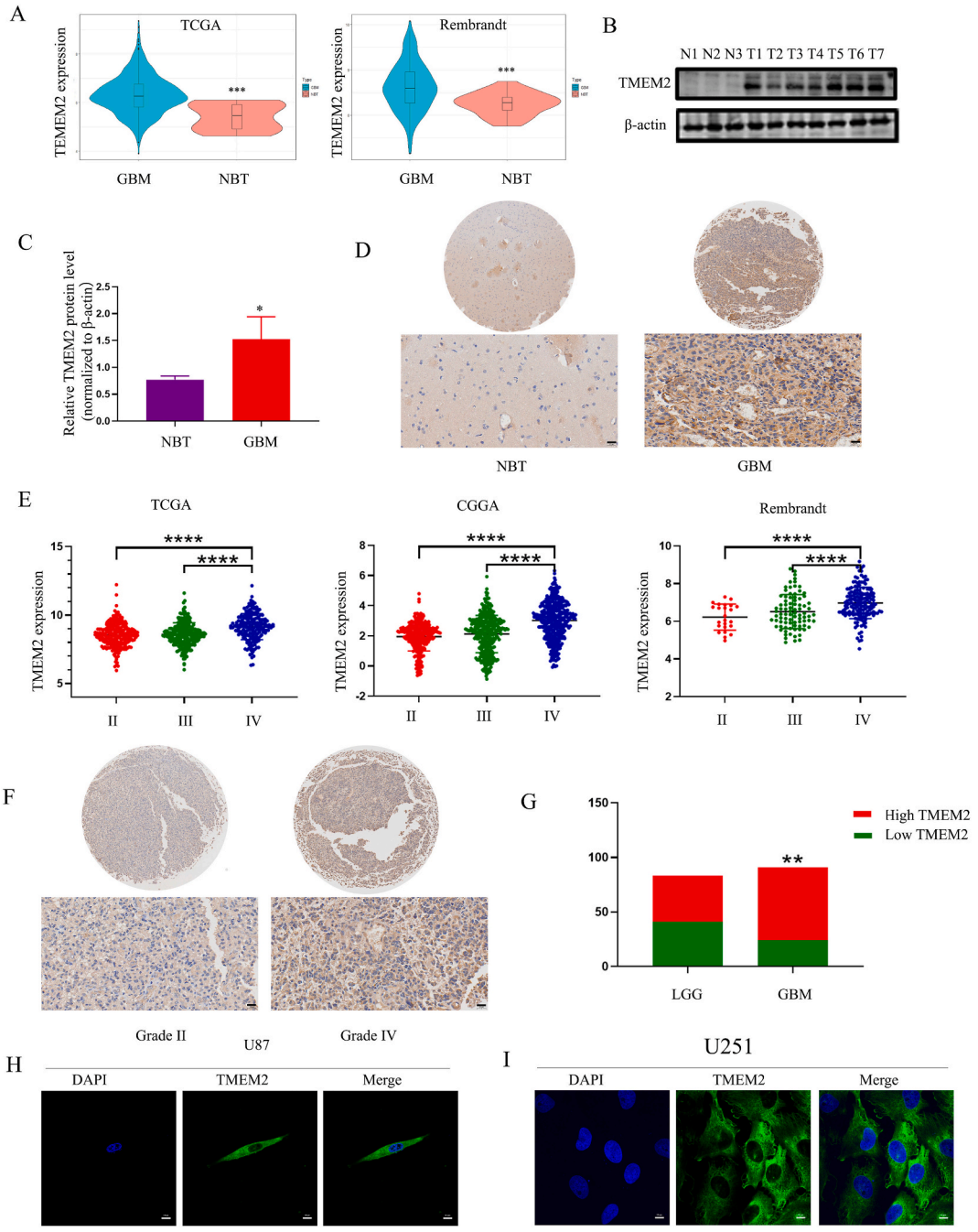

Fig. 1. TMEM2 is overexpressed in GBM and is associated with glioma malignancy. **(A)** Relative mRNA expression of TMEM2 in NBT and GBM from public datasets. (**B–C**) The expression of TMEM2 in NBT and GBM tissues was analyzed by Western blot. (**D**) Representative images of IHC staining of TMEM2 in NBT and GBM tissues. (**E**) Relative mRNA expression of TMEM2 in different glioma grades from public datasets. (**F-G**) IHC staining of TMEM2 in LGG and GBM tissues. (H–I) Immunofluorescence staining of TMEM2 in U87 and U251 cells. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. NBT, non-tumor brain tissue. N, non-tumor, T, tumor.

same volume of DMSO for 24 h, then harvested and added 500 μl 1*X* binding buffer, followed by PE/7- ADD staining for 15 min, and apoptosis cells were measured by FACS Calibur flow cytometer (BD).

2.7. Immunohistochemical (IHC) staining and evaluation

Glioma tissue microarray chip was heated at 65 °C for 60 min. Tissue microarray was deparaffinized and hydrated in xylene, xylene, xylene,100%, 95% and 75% ethanol, respectively. Then 10 mM sodium citrate was employed to antigen repair, and removed endogenous peroxidase by 3% H_2O_2. The tissue microarray was blocked with 1% BSA for half hour, and incubated with TMEM2 antibody (1:200) overnight. The next day, the tissue microarray chip was incubated with HRP rabbit IgG for 60 min. Then DAB kit and hematoxylin reagent were used for staining. Finally, 1% hydrochloric acid ethanol was used to separate the colors. Air dried and observed under an Olympus BX40 microscope (Tokyo). The expression intensity of TMEM2 was assessed by the proportion of positive cells (0, <10%; 1, 10–25%; 2, 26–50%; 3, 51–75%; and 4, >75%). An immune score of 0–2 was defined as low TMEM2 expression, and a score of 3–4 was defined as high TMEM2 expression.

2.8. Immunofluorescence (IF) staining

Sterile slides were placed in 6-well plates, and 8000 calls were planted per well and cultured for 24 h. The medium was removed and the cells were fixed with 4% paraformaldehyde for half hour. After washing with PBS buffer for 3 times, block with 1% BSA (ServiceBio) for 30 min. Then, an appropriate amount of TMEM2 antibody was added to the slides and placed on a shaker at 4 °C overnight. The next day, cells were incubated with Alexa Fluor®488 Donkey anti-rabbit IgG (AntGene) for 1 h under dark conditions. Finally, cell nuclei were stained with DAPI. The images were captured under a laser confocal microscope (Olympus).

2.9. Western blot

Cells were placed on ice and lysed using RIPA buffer (Beyotime, China) for 30 min. The lysate was collected and centrifuged, and the protein concentration was detected by the BCA method (Beyotime). Then add the protein loading buffer to the lysate and heated. The same amount of protein was added to SDS-PAGE for electrophoresis and then transferred to the PVDF membrane by electrolysis. The membrane was blocked in 5% non-fat milk for 60 min after being washed with PBST buffer. The PVDF membrane was trimmed into strips of suitable size, and incubated with the target antibody at 4 °C overnight. The following day, the strips were incubated with HRP anti-rabbit/mouse IgG for 1 h, and then visualized using ECL method.

2.10. Statistical analysis

All data were expressed as means ± standard deviations (SD). Student's *t*-test was used to measure the differences between two groups, and for more than two groups, one-way ANOVA was performed. Spearman correlation analysis was used to detect the correlation between two genes. Differences in survival between groups were analyzed using Kaplan-Meier survival analysis. Statistical analysis was performed using GraphPad Prism 8 software, and a p-value <0.05 was considered significant.

Table 1
Association between TMEM2 and clinical features.

Variables		TMEM2	expression	P value
	Number	Low (n = 70)	High (n = 104)	
Age(years)				>0.05
<60	104	42	62	
≥60	70	28	42	
Gender				>0.05
Female	77	34	43	
Male	97	36	61	
Location				>0.05
Supratentorial	142	57	85	
Subtentorial	32	13	19	
KPS				>0.05
≥80	114	46	68	
<80	60	24	36	
Grade				0.03
LGG	84	41	43	
GBM	90	29	61	

Low TMEM2: 0–2(IHC score). High TMEM2:3-4 (IHC score).

3. Results

3.1. TMEM2 is overexpressed in GBM

To explore the expression of TMEM2 in gliomas, we analyzed TMEM2 expression levels in GBM and non-tumor brain tissues using TCGA and Rembrandt databases, and results revealed that TMEM2 was overexpressed in GBM tissues (Fig. 1A). We further compared TMEM2 expression in GBM and non-tumor brain tissues using our in-house cohort. Western blot and IHC revelated that TMEM2 was overexpressed in GBM tissues (Fig. 1B–D, Supplementary file). Furthermore, immunohistochemical staining analysis of our independent internal tissue chip microarray revealed that TMEM2 was mainly distributed in cytoplasm, and immunofluorescence staining of GBM cells acquired consistent results (Fig. 1H–I). We further analyzed the expression of TMEM2 in gliomas of different grades, and results showed that the TMEM2 expression raised with the grade in multiple public datasets (Fig. 1E). Immunohistochemical staining in the in-house cohort demonstrated that TMEM2 was upregulated in GBM tissues (Fig. 1F–G). The correlation between TMEM2 and clinicopathological features in patients with gliomas in our internal cohort, TCGA and CGGA was shown in Tables 1,2 and 3 respectively.

3.2. TMEM2 high expression was associated with poor prognosis in glioma patients

Survival analysis was employed to investigate the impact of TMEM2 expression on the prognosis of glioma patients in public datasets. We ranked the expression levels of TMEM2, with the top 50% defined as the high expression group and the bottom 50% as the low expression group. Survival analysis indicated that patients with high TMEM2 expression have a worse prognosis compared with patients with low TMEM2 expression in all gliomas, including GBM and LGG patients (Fig. 2A-2 F).

3.3. The function analysis of TMEM2 in GBM

The LinkedOmics database was employed to investigate the biological function of TMEM2 in GBM (http://www.linkedomics.org/) [11]. The Pearson correlation test was used for statistical analysis of TMEM2 co-expression in TCGA-GBM, and the results showed a volcano plot (Fig. 3A). The heat map showed the top 50 genes positively and negatively correlated with TMEM2 (Fig. 3B–C). Furthermore, the GSEA was utilized to explore GO analysis (biological process) of TMEM2 co-expression genes. GO analysis indicated that TMEM2 mainly joins in extracellular structure organization, cell-substrate adhesion, angiogenesis, bone development, collagen metabolic process, tissue migration, positive regulation of cell motility, etc. (Fig. 3D). KEGG analysis illustrated enrichment in ECM-receptor interaction, protein digestion and absorption, focal adhesion, amoebiasis, proteoglycans in cancer, arrhythmogenic right ventricular cardiomyopathy, etc. (Fig. 3E).

Table 2
Correlation between TMEM2 and clinicopathological characteristics in patients with gliomas in TCGA.

Clinicopathological characteristics	TMEM2 expression		*P* value
	Low (n = 332)	High (n = 332)	
Age			<0.001
≥60	42	102	
< 60	252	210	
Gender			<0.001
Male	167	126	
Female	127	186	
WHO grade			<0.001
II-III	277	192	
IV	25	123	
IDH status			<0.001
Mutant	259	168	
Wild-type	72	158	
Chr.1p19q			<0.001
Codeletion	111	57	
Non-codeletion	219	271	
MGMT promoter			<0.001
Methylation	269	207	
Unmethylation	56	101	
TERT status			>0.05
Mutant	84	70	
Wild-type	85	81	
ATRX status			>0.05
Mutant	106	89	
Wild-type	223	236	

Table 3
Correlation between TMEM2 and clinicopathological characteristics in patients with gliomas in CGGA.

Clinicopathological characteristics	TMEM2 expression		*P* value
	Low (n = 489)	High (n = 490)	
Age			>0.05
≥60	441	424	
< 60	48	66	
Gender			>0.05
Male	278	296	
Female	211	194	
WHO grade			<0.001
II-III	376	229	
IV	113	261	
IDH status			<0.001
Mutant	314	198	
Wild-type	127	291	
Chr.1p19q			<0.001
Codeletion	143	62	
Non-codeletion	281	422	
Chemotherapy			>0.05
Yes	300	328	
No	148	123	
Radiotherapy			>0.05
Yes	377	382	
No	81	75	

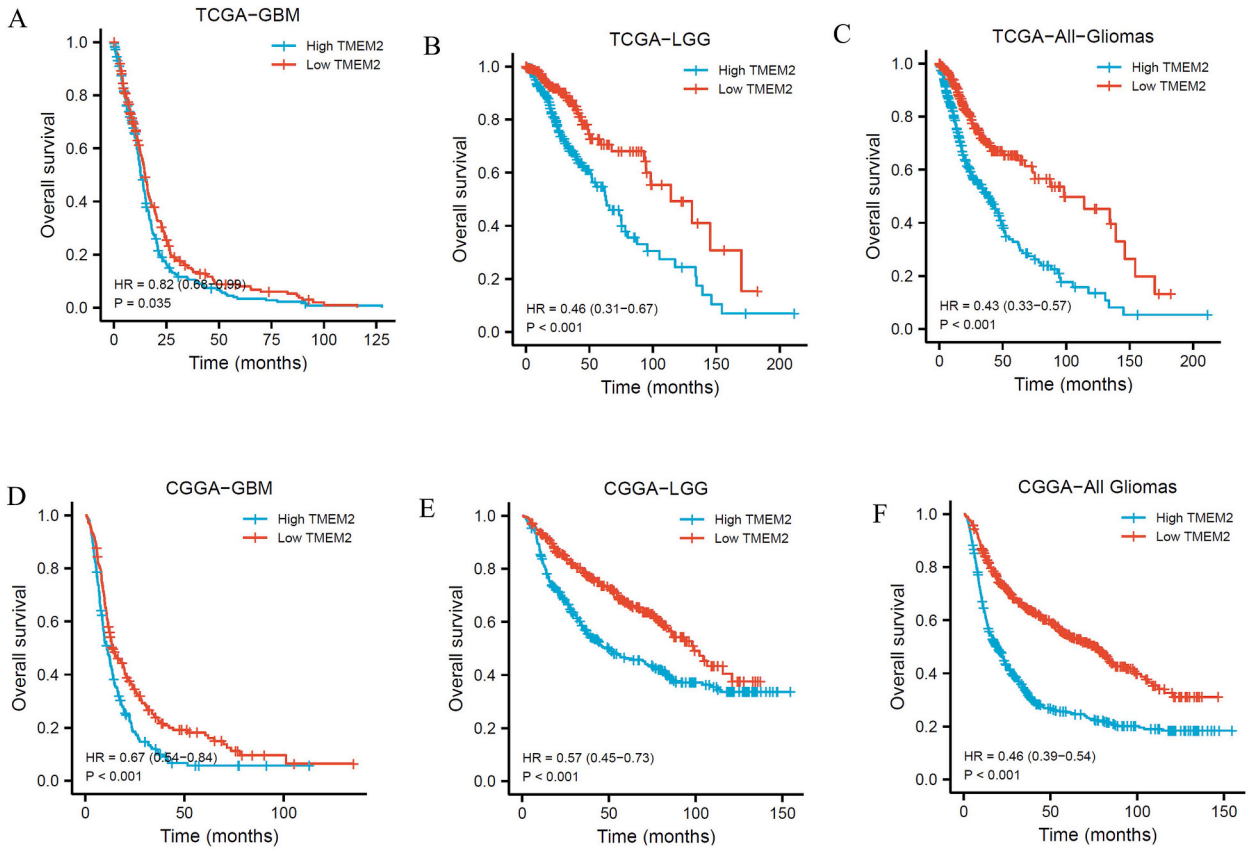

Fig. 2. High TMEM2 expression of TMEM2 was associated with poor prognosis in gliomas. (**A-F**) Kaplan–Meier method was employed to investigate the effect of TMEM2 on the prognosis of All-gliomas, LGG and GBM patients in TCGA and CGGA datasets. HR, hazard ratio.

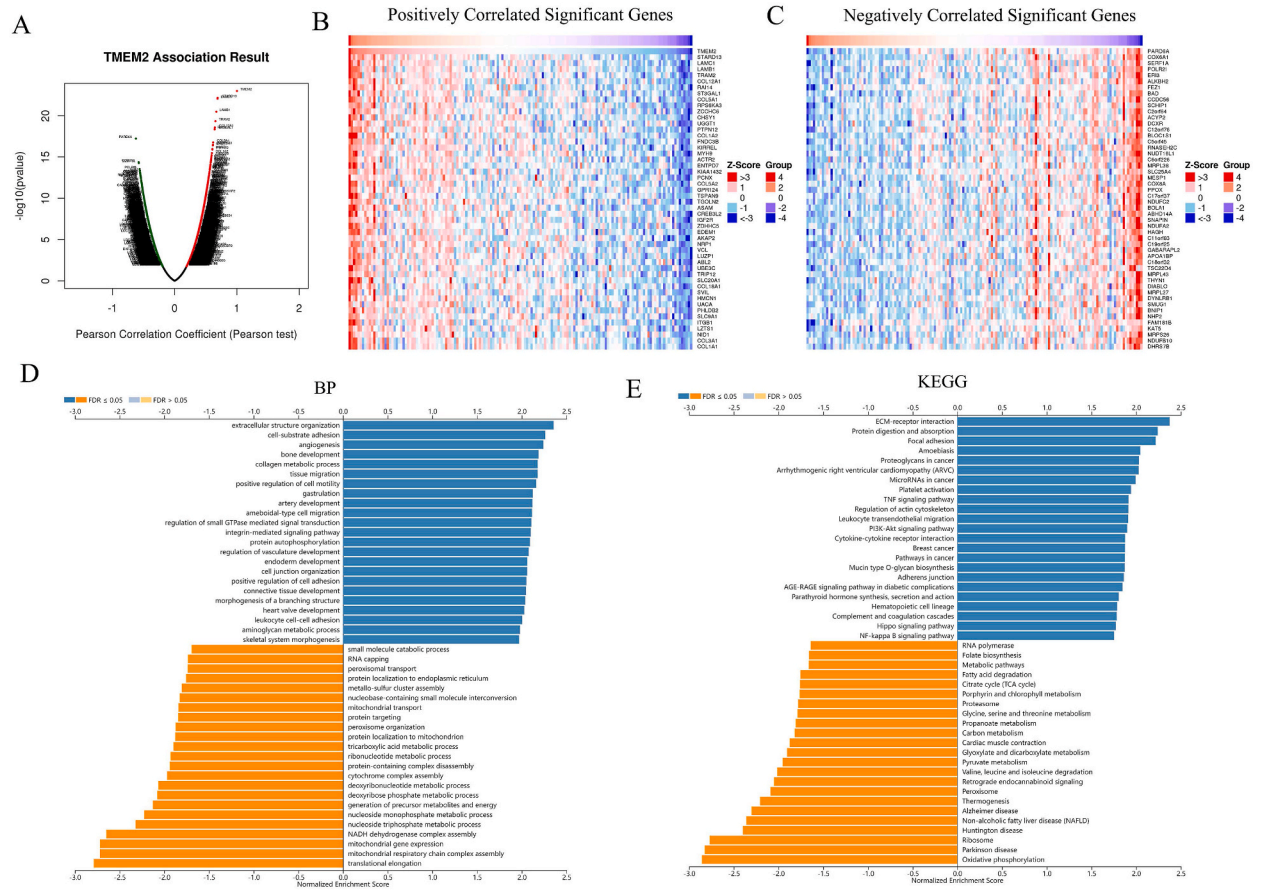

Fig. 3. The function analysis of TMEM2 in GBM. (**A**)The TMEM2 co-expression genes in TGCA-GBM. (**B, C**) The top 50 genes positively and negatively correlated to TMEM2. (D, E) GO and KEGG analysis of TMEM2 co-expression genes in GBM.

3.4. Knockdown of TMEM2 inhibits cell proliferation of GBM cells

ShRNA is employed to specifically inhibit TMEM2 expression in GBM cells. The result demonstrated that TMEM2 was significantly downregulated in GBM cells (Fig. 4A–B, Supplementary file). Whereafter, we detected the expression of TMEM2 on cell proliferation by CKK8 assay, and results confirmed that TMEM2 downregulated prevented cell proliferation in GBM cells (Fig. 4C–D). Furthermore, Edu staining assay was also used to further detect the effect of TMEM2 on cell proliferation. Edu is a thymidine kinase nucleoside analog that can be inserted into DNA in place of thymidine kinase, and then detect DNA activity based on a specific fluorescent reaction to detect cell proliferation. Edu assay indicated that proportion of Edu-positive cells (Red) in TMEM2 knockdown group was decreased (Fig. 4E–F). Our results indicated that down-regulating TMEM2 expression inhibits cell proliferation of GBM cells.

3.5. Knockdown of TMEM2 inhibits EMT process of GBM

Clinically, GBM is classified as proneural, mesenchymal and classical subtypes. The patients with mesenchymal subtype have a worse prognosis and greater metastatic ability compared with patients with proneural and classical subtypes [12,13]. We compared the expression of TMEM2 in three subtypes of GBM patients in public datasets, and analysis showed that TMEM2 expression was upregulated in mesenchymal subtype (Fig. 5A). Whereafter, Spearman method was employed to measure the correlation between TMEM2 and mesenchymal-related genes, and analysis indicated that TMEM2 was positively associated with CDH2, Vimentin, SNAI1, SNAI, CD44, TWIST1, MMP2, RELB and CHI3L1 in TCGA and CGGA datasets, and negatively correlated with ZEB1 in TCGA, but positively associated with ZEB1 in CGGA (Fig. 5B–C). Therefore, we speculated that TMEM2 might be involved in the EMT processes of GBM. To test our conjecture, transwell assay was used to investigate the cell invasion and metastasis of U87 and U251 cells after TMEM2 knockdown, and experimental results showed that down-regulation of TMEM2 inhibited cell migration and invasion in GBM cells (Fig. 5D–G). We further examined the key molecules involved in the EMT process by Western blot analysis to determine the mechanism by which TMEM2 affects cell invasion and migration in GBM cells. Western blot analysis demonstrated that the expression of *N*-cadherin, MMP2 and SNAI1 was downregulated, and *E*-cadherin was upregulated in TMEM2 knockdown group (Fig. 5H–J, Supplementary file). These results revealed that TMEM2 facilitated EMT in GBM cells.

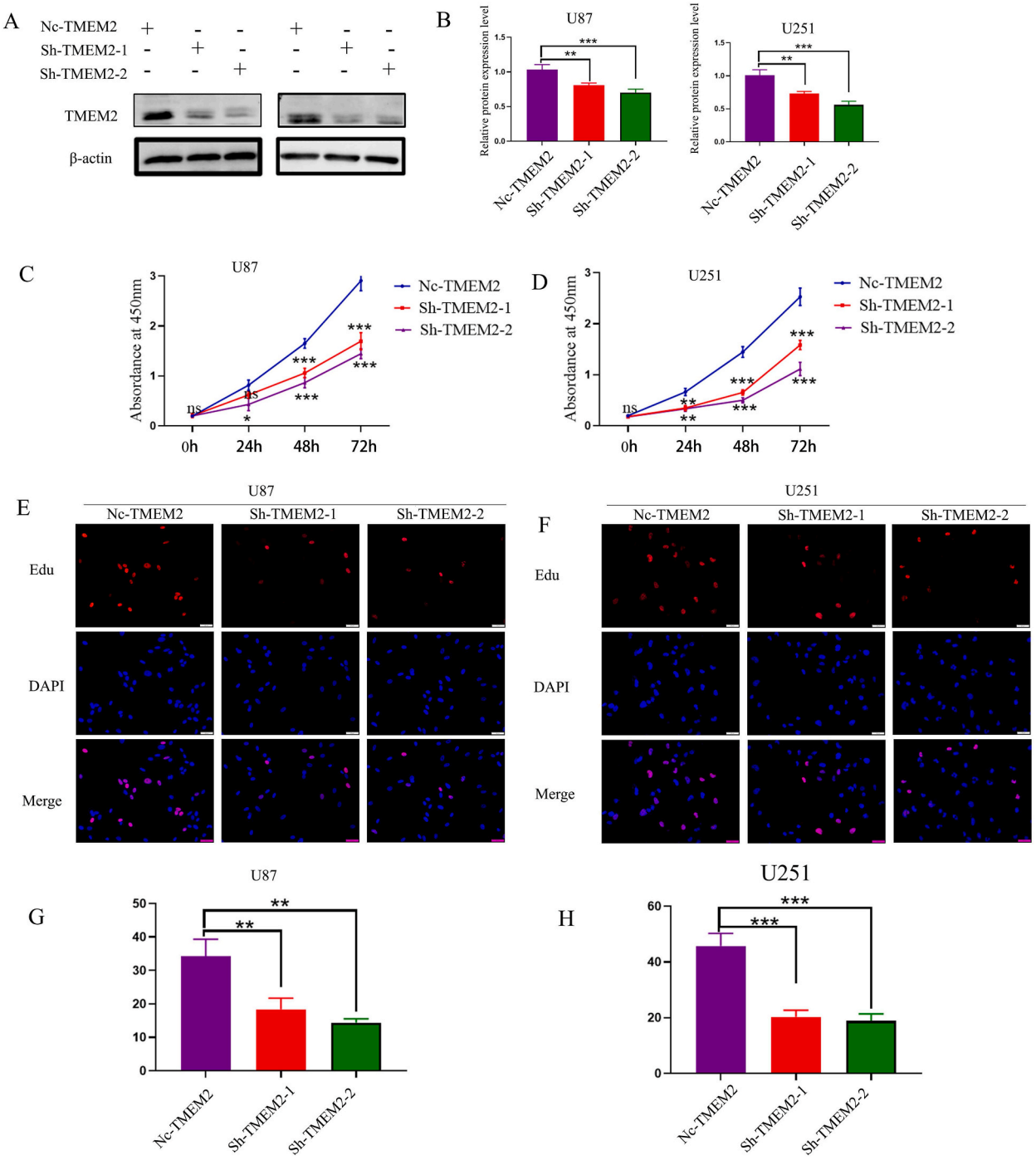

Fig. 4. Down-regulation of TMEM2 inhibits cell proliferation of GBM cells. (**A, B**) The knockdown of TMEM2 protein level was displayed. (**C, D**) Cell viability of GBM cells in TMEM2 downregulated group and control group was measured at certain time. (***E*-H**) Edu assay was performed to assess cell proliferation. Red represents the Edu positives cell and blue represents the nucleus. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, no significance.

3.6. TMEM2 promotes resistance to temozolomide in GBM

In clinical treatment, patients with MGMT promoter methylation were found to be sensitive to TMZ treatment and had longer median survival time [14]. In order to examine the role of TMEM2 in response to TMZ, we divided glioma patients into two groups according to the methylation status of the MGMT promoter and then performed survival analysis. Our results revealed that GBM patients with MGMT promoter methylation had a better prognosis in TMEM2 low expression group. However, TMEM2 expression did

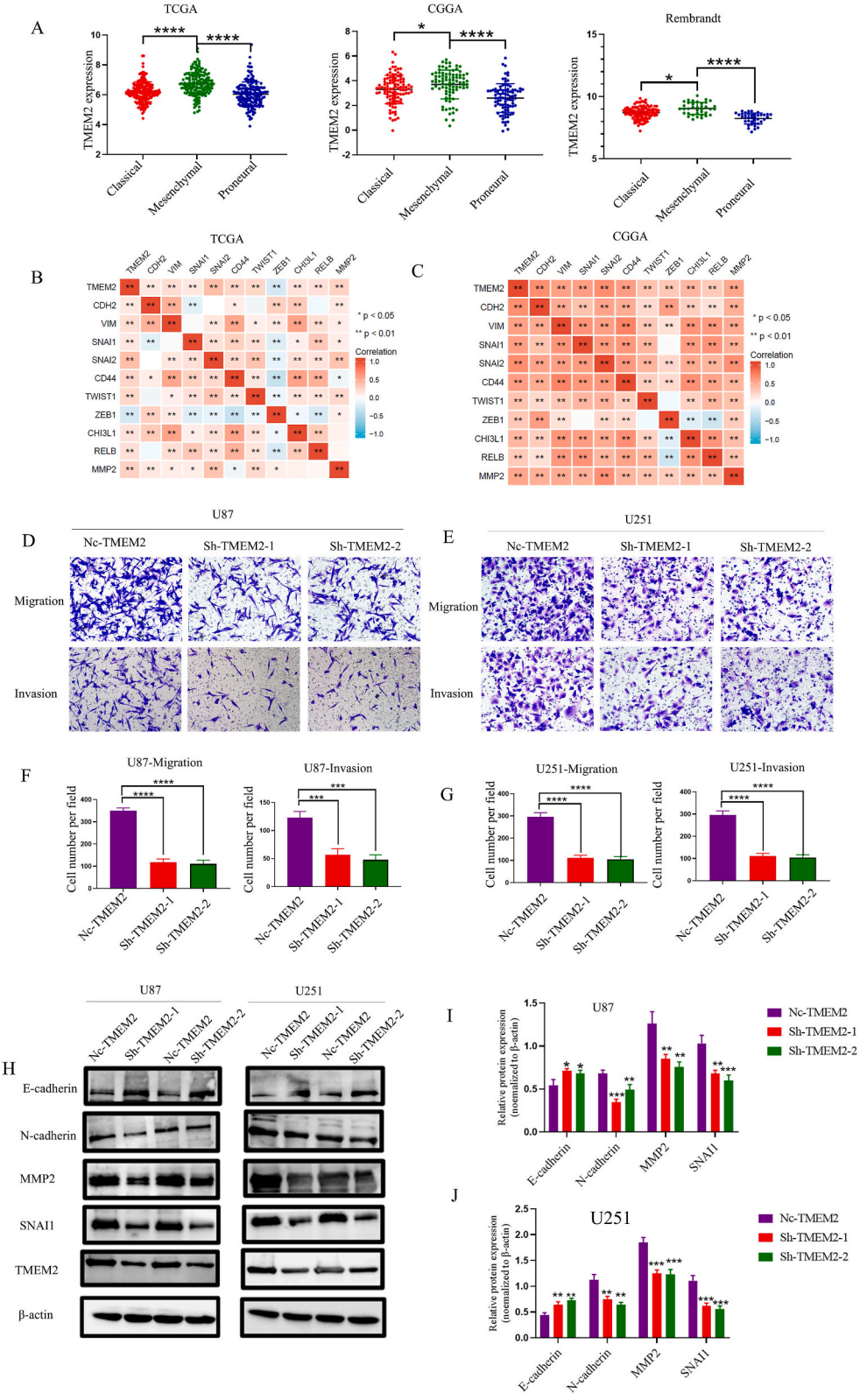

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Fig. 5. Down-regulation of TMEM2 inhibits EMT process of GBM. (A)TMEM2 expression in three subtypes of GBM in the public datasets. (**B, C**) Correlation analysis between TMEM2 and mesenchymal-related genes in TCGA and CGGA. (**D-G**) Down-regulation of TMEM2 suppressed cell migration and invasion in GBM cells. (**H-J**) EMT-related proteins were detected by western bolt. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

not separate significant survival differences in the MGMT promoter unmethylated group (Fig. 6A). We further investigated and found that TMEM2 expression did not affect the survival of GBM patients in patients treated with IR alone. However, for patients treated with TMZ alone, TMEM2 expression separated GBM patients into different survival groups (Fig. 6B). Surprisingly, TMEM2 did not affect the prognosis of LGG patients in MGMT promoter methylation and TMZ-only chemotherapy group (Fig. 6C–D). Next, we further detected the effect of TMEM2 on TMZ treatment in vivo, and flow cytometry demonstrated that the expression of TMEM2 had no significant effect on cell apoptosis in GBM cells treated with DMSO. However, knockdown of TMEM2 significantly upregulated the proportion of apoptotic cells in GBM cells treated with TMZ (Fig. 6G–L). These results indicated that TMEM2 expression promoted resistance to TMZ in GBM.

4. Discussion

Previous studies have found that TMEM2 combined with IDH and 1p19q can be utilized to predict survival in glioma patients [9]. These findings were obtained only through bioinformatics analysis, and the potential biological role of TMEM2 needs to be verified in more glioma samples and in vivo experiments. In our study, mRNA data and clinical information from public datasets, as well as our internal dataset containing 174 glioma tissues and 8 NBT were used. TMEM2 was obviously overexpressed in GBM tissues compared with NBT, and its expression was increased with the grade of glioma. We further found that TMEM2 knockdown suppressed the proliferation of GBM cells, which revealed that TMEM2 may be related to the malignancy of glioma and may affect the survival of glioma patients. Subsequent studies identified TMEM2 as a prognostic marker for all gliomas, including LGG and GBM.

EMT was a biological process that involves cancer progression and metastasis. Epithelial cells undergoing EMT transition lose their cell polarity and expression of epithelial markers and undergo phenotypic transition. These transformed epithelial cells obtain mesenchymal cell-like properties, acquire expression of mesenchymal markers, and show decreased intercellular adhesion and increased motility. EMT is also considered to be one of the fundamental mechanisms driving glioma invasion and metastasis [15–17]. Our research found that TMEM2 overexpress in GBM mesenchymal subtype, and which is positively associated with mesenchymal markers of VIM, SNIA1, CDH2, CD44, etc. In TCGA and CGGA datasets. Subsequent research confirmed that TMEM2 downregulated inhibits cell migration and invasion, and decreases SNAI1, MMP2, and *N*-cadherin protein expression. These results indicated that TMEM2 induced EMT in GBM. Extracellular stroma (ECM) is a major physical barrier to maintaining homeostasis and preventing cell migration. Hyaluronic acid (HA) is one of the most influential non-protein components in ECM, which can form a gel-like anti-adhesion barrier to hinder cell migration. Previous studies have revealed that TMEM2 can mediate the degradation of HA, which results in the reduction of cell adhesion and the enhancement of cell metastasis ability [4,18]. Furthermore, tumor cells can also degrade ECM protein through MMPs to facilitate the invasion and migration of tumor cells [19]. Taking into account these results, we hypothesized that the mechanism of TMEM2 promoting cell invasion and metastasis may be related to the induction EMT of GBM, the promotion of MMP2 degradation of ECM protein and the degradation of HA in ECM.

TMZ is currently the first-line chemotherapeutic drug for the clinical treatment of gliomas, and the chemoresistance of TMZ is one of the major clinical challenges [20].

Clinically, MGMT methylation status is often used to evaluate the sensitivity of glioma patients to TMZ. However, some studies suggest that MGMT status is not universally related to TMZ responsiveness, and its accuracy in predicting glioma patients' response to TMZ therapy is controversial [21]. In this study, we found that TMEM2 could be used as a complementary marker to predict the responsiveness of GBM patients to TMZ treatment in combination with MGMT methylation status. Interestingly, TMEM2 does not possess such properties in LGG patients. There is increasing evidence that activating EMT in tumor cells increases cellular resistance to drug therapy [22,23]. Therefore, we hypothesized that TMEM2 reduces the responsiveness of GBM patients to TMZ chemotherapy by promoting the EMT of GBM.

Although our study is helpful to understand the malignant behavior and drug resistance mechanism of glioma, there are still some limitations. First of all, we only explored the effect of TMEM2 on malignant phenotype of glioma from in vitro experiments, without further verification in vivo. In addition, the mechanism by which TMEM2 regulates glioma EMT and the resistance of glioma to TMZ is still unknown, and our subsequent research will focus on the molecular mechanisms involved.

5. Conclusion

TMEM2 is a biomarker associated with glioma malignancy, which can effectively predict the prognosis of LGG and GBM patients. In addition, TMEM2 also promotes GBM cell proliferation, induces the EMT process, promotes tumor metastasis and reduces the responsiveness of GBM patients to TMZ treatment.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Renmin Hospital of Wuhan University.

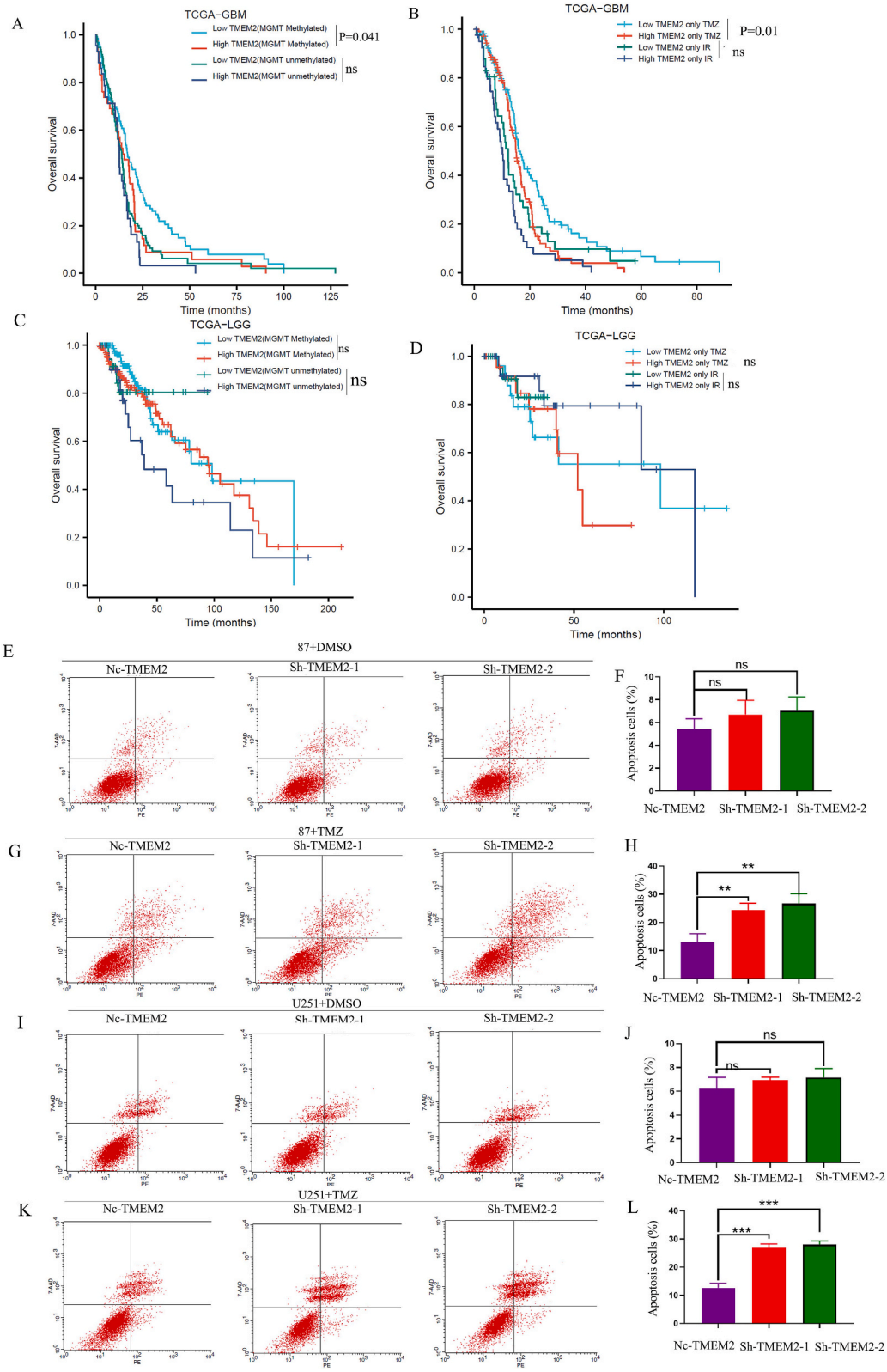

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Fig. 6. TMEM2 promotes resistance to temozolomide in GBM. (**A, C**) Effect of TMEM2 on prognosis of GBM and LGG patients separated by MGMT promoter methylation status, respectively. (**B, D**) Effect of TMEM2 on the prognosis of GBM and LGG patients who received IR or TMZ alone treatment. (***E*-L**) U87 and U251 cells were treated with DMSO or TMZ (400 μM). Flow cytometry analysis was used to investigate cell apoptosis. $^{*}P < 0.01$, $^{***}P < 0.001$, ns, no significance.

Author contribution statement

Lun Gao: Performed the experiments; Wrote the paper.
Shiao Tong: Performed the experiments.
Junhui Liu: Conceived and designed the experiments; Wrote the paper.
Jiayang Cai; Zhang Ye; Long Zhou; Pan Lei; Zhiyang Li: Analyzed and interpreted the data.
Ping Song; Qiuwei Hua; Hangyu Wei: Contributed reagents, materials, analysis tools or data.
Daofeng Tian; Qiang Cai: Conceived and designed the experiments.

Data availability statement

Data included in article/supp. Material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16559.

References

[1] W. Zheng, et al., SDC1-dependent TGM2 determines radiosensitivity in glioblastoma by coordinating EPG5-mediated fusion of autophagosomes with lysosomes, Autophagy (2022) 1–19.
[2] J. Liu, et al., Gasdermin D is a novel prognostic biomarker and relates to TMZ response in glioblastoma, Cancers 13 (22) (2021).
[3] Y. Tobisawa, et al., The cell surface hyaluronidase TMEM2 is essential for systemic hyaluronan catabolism and turnover, J. Biol. Chem. 297 (5) (2021), 101281.
[4] F. Irie, et al., The cell surface hyaluronidase TMEM2 regulates cell adhesion and migration via degradation of hyaluronan at focal adhesion sites, J. Biol. Chem. 296 (2021), 100481.
[5] R.T. Schinzel, et al., The hyaluronidase, TMEM2, promotes ER homeostasis and longevity independent of the UPR(ER), Cell 179 (6) (2019) 1306–1318.e18.
[6] Y. Kudo, et al., Overexpression of transmembrane protein 2 (TMEM2), a novel hyaluronidase, predicts poor prognosis in pancreatic ductal adenocarcinoma, Pancreatology 20 (7) (2020) 1479–1485.
[7] L. Gan, et al., miR-518a-3p suppresses triple-negative breast cancer invasion and migration through regulation of TMEM2, Technol. Cancer Res. Treat. 19 (2020), 1533033820977523.
[8] H. Lee, et al., TMEM2 is a SOX4-regulated gene that mediates metastatic migration and invasion in breast cancer, Cancer Res. 76 (17) (2016) 4994–5005.
[9] Q. Jiang, et al., TMEM2 combined with IDH and 1p19q in refining molecular subtypes for predicting survival of patients with glioma, DNA Cell Biol. 40 (11) (2021) 1381–1395.
[10] R.L. Bowman, et al., GlioVis data portal for visualization and analysis of brain tumor expression datasets, Neuro Oncol. 19 (1) (2017) 139–141.
[11] S.V. Vasaikar, et al., LinkedOmics: analyzing multi-omics data within and across 32 cancer types, Nucleic Acids Res. 46 (D1) (2018) D956–d963.
[12] E. Lee, et al., Comparison of glioblastoma (GBM) molecular classification methods, Semin. Cancer Biol. 53 (2018) 201–211.
[13] M.Z. Han, et al., TAGLN2 is a candidate prognostic biomarker promoting tumorigenesis in human gliomas, J. Exp. Clin. Cancer Res. 36 (1) (2017) 155.
[14] S. Wu, et al., PARP-mediated PARylation of MGMT is critical to promote repair of temozolomide-induced O6-methylguanine DNA damage in glioblastoma, Neuro Oncol. 23 (6) (2021) 920–931.
[15] S. Li, et al., Tumor-associated neutrophils induce EMT by IL-17a to promote migration and invasion in gastric cancer cells, J. Exp. Clin. Cancer Res. 38 (1) (2019) 6.
[16] M. Suarez-Carmona, et al., EMT and inflammation: inseparable actors of cancer progression, Mol. Oncol. 11 (7) (2017) 805–823.
[17] C. Tao, et al., Genomics and prognosis analysis of epithelial-mesenchymal transition in glioma, Front. Oncol. 10 (2020) 183.
[18] Y. Yamaguchi, et al., TMEM2: a missing link in hyaluronan catabolism identified? Matrix Biol. 78–79 (2019) 139–146.
[19] A. Jablonska-Trypuc, M. Matejczyk, S. Rosochacki, Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs, J. Enzym. Inhib. Med. Chem. 31 (sup1) (2016) 177–183.
[20] R. Shrestha, et al., HIT'nDRIVE: patient-specific multidriver gene prioritization for precision oncology, Genome Res. 27 (9) (2017) 1573–1588.
[21] V.J. Herbener, et al., Considering the experimental use of temozolomide in glioblastoma research, Biomedicines 8 (6) (2020).
[22] D. Babu, et al., Rabeprazole has efficacy per se and reduces resistance to temozolomide in glioma via EMT inhibition, Cell. Oncol. 44 (4) (2021) 889–905.
[23] N. Erin, et al., Tumor microenvironment and epithelial mesenchymal transition as targets to overcome tumor multidrug resistance, Drug Resist. Updates 53 (2020), 100715.