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ELK4 induced upregulation of HOMER3 promotes the proliferation and metastasis in glioma via Wnt/β-catenin/EMT signaling pathway

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Abstract

Glioma is an aggressive brain tumor characterized by its high invasiveness, which complicates prognosis and contributes to patient resistance against various treatment options. The HOMER family, consisting of HOMER1, HOMER2, and HOMER3, has been implicated in various cancers, yet their specific roles in glioma remain inadequately understood. This study conducted a comprehensive pan-cancer analysis to evaluate the expression profiles of HOMER family members across different tumor types, utilizing data from public databases such as TCGA and GTEx. Our findings indicate significant dysregulation of HOMER1, HOMER2, and HOMER3 in multiple cancers, with HOMER3 emerging as a potential prognostic biomarker, particularly for lower-grade glioma. Elevated expression levels of HOMER3 were associated with shorter overall survival and disease-specific survival in LGG patients, supported by Cox regression analysis that confirmed HOMER3 as an independent prognostic factor. Furthermore, HOMER3 expression correlated positively with advanced clinical stages and key tumor markers. To elucidate the mechanisms behind HOMER3 dysregulation, we identified ELK4 as a transcription factor that binds to the HOMER3 promoter, promoting its expression in glioma cells. Functional assays demonstrated that silencing HOMER3 significantly reduced glioma cell proliferation and metastatic potential in vitro and in vivo, highlighting its oncogenic role. Additionally, HOMER3 was found to influence the Wnt/β-catenin/EMT signaling pathway, with knockdown resulting in altered expression of critical EMT markers. Collectively, our results indicated that HOMER3 plays a crucial role in glioma progression and metastasis, underscoring its potential as a therapeutic target and prognostic biomarker in glioma management.

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Keywords HOMER3, Glioma, Wnt/β-catenin pathway, Prognostic biomarker, ELK4, Glioma

Introduction

Gliomas are among the most common primary tumors of the central nervous system, with an annual incidence of approximately 5 to 7 cases per 100,000 people, varying by region and population [1, 2]. They are relatively rare in children, although certain types, such as cerebellar gliomas and oligodendrogliomas, still occur, typically in ages 5 to 10 [3]. The incidence in adults is highest between ages 45 and 70, especially for glioblastoma. The incidence in males is generally higher than in females, particularly for high-grade tumors like glioblastoma, where the male-to-female ratio can reach 2:1 or higher [4, 5]. Gliomas are classified into various types based on their cellular origin and degree of malignancy, including glioblastoma, diffuse astrocytoma, oligodendroglioma, and ependymoma [6]. The incidence is generally higher in developed countries, which may be related to better healthcare facilities and early screening capabilities. As per the World Health Organization's system, gliomas are categorized as either low-grade glioma (LGG) or high-grade glioma (HGG) [7, 8]. Compared to high-grade glioma, the prognosis for LGG is typically better, and it makes up 6% of primary tumors of the central nervous system in adults [9, 10]. However, comprehensive treatment, including surgical resection combined with chemotherapy and radiation therapy, still cannot avoid treatment resistance and tumour recurrence, and greater than half of LGG patients will eventually develop highly aggressive gliomas [11, 12]. Tumor molecular subtypes and prognostic biomarkers are currently receiving a lot of attention in the World Health Organization's (WHO) approach for classifying tumors of the central nervous system. The current state of glioma treatment and prognosis remains unresolved, despite the discovery of several prognostic indicators such as IDH mutation and 1p/19q codeletion status. Consequently, in order to forecast glioma prognosis, it is essential to find new biomarkers in LGG patients.

The HOMER protein family is a crucial group of cellular signaling proteins that are widely present in various organisms and play important roles in numerous physiological and pathological processes [13, 14]. The main members of this family include HOMER1, HOMER2, and HOMER3, which facilitate the assembly and regulation of cellular signaling complexes as scaffolding proteins [15, 16]. HOMER proteins contain an EVH1 (Ena/VASP Homology 1) domain, which can recognize and bind specific ligands, such as various G protein-coupled receptors (GPCRs) and other signaling molecules. Additionally, the C-terminus of HOMER proteins contains a coiled-coil structure, which facilitates the formation of homodimers or heterodimers, promoting the assembly of protein complexes [17, 18]. In the nervous system, Homer proteins modulate calcium signaling and influence neuronal function. They achieve this by binding to calcium ion channels, such as IP3 receptors and NMDA receptors, regulating the flow and concentration of calcium ions within cells. This regulatory role is crucial for synaptic plasticity, learning, and memory processes [19–21]. Homer proteins also participate in the formation and maintenance of postsynaptic density complexes, impacting the efficiency of synaptic signal transmission and, in turn, profoundly affecting neural network function. Beyond their roles in the nervous system, Homer proteins also play important functions in other cell types. They are involved in regulating various physiological processes, including cell proliferation, apoptosis, and metabolism [22, 23]. Homer proteins influence cell growth and survival through interactions with multiple signaling pathways. For instance, HOMER3 may play a regulatory role in certain cancer types by modulating cell cycle and apoptotic pathways, indicating that Homer proteins have potential regulatory effects in tumorigenesis [24–26]. Dysregulation of Homer proteins is closely linked to the development of various diseases. Studies have found that changes in the expression of Homer proteins are significantly associated with the pathophysiological mechanisms of neurodegenerative diseases, such as Alzheimer's disease, depression, and autism. In these conditions, dysfunction of Homer proteins may lead to abnormal neuronal signaling, thereby affecting cognitive and emotional functions [27–29]. Additionally, in cancer research, abnormal expression and functional changes of Homer proteins in various tumors may be associated with tumorigenesis, progression, and metastasis. However, the expression and function of HOMER protein family in glioma remain largely unclear. Future research will further elucidate the specific mechanisms of Homer proteins in cellular signal transduction, helping us better understand their roles in health and disease, and potentially providing new ideas and strategies for the treatment of related conditions.

The purpose of this study was to evaluate the expression differences of the HOMER family genes (including HOMER1, HOMER2, and HOMER3) in various cancer types and their correlation with patient prognosis. The study analyzed data from public databases (TCGA and GTEx) and finds that the HOMER family exhibits different expression patterns in various tumors, particularly highlighting that HOMER3 was significantly associated with prognosis in LGG. Additionally, the article investigated the regulatory role of the transcription factor ELK4 on HOMER3 expression and confirms the binding of

ELK4 to the HOMER3 promoter. Additionally, the study showed that HOMER3 may affect cell migration and proliferation, which could be significant in the development and progression of gliomas. Lastly, the article disclosed that the Wnt/β-catenin/ EMT pathway was used by HOMER3 to enhance the metastatic capacity of glioma cells. The importance of HOMER3 in tumor growth and patient outcomes is demonstrated by this work, which emphasizes its potential as a prognostic biomarker and therapeutic target in LGG.

Materials and methods

Cell culture and transfection

From the National Collection of Authenticated Cell Cultures in Shanghai, China, we procured human glioma cell lines (LN229, U87, TG905, LN308, and U251) as well as normal human astrocytes (NHAs). When the cells arrived, they were placed in RPMI-1640 media that had 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin added to it. A humidified environment containing 5% CO_2 was used to incubate the cells at 37 °C. Cells were subcultured after they reached about 80–90% confluence, and the medium was changed every 2–3 days. General Biological corporation of Xiamen, Fujian, China, cloned the ELK4 and HOMER3 shRNA sequences into the PLVX-shRNA vectors sh-ELK4-1, sh-ELK4-2, sh-HOMER3-1, and sh-HOMER3-2, respectively. Then, using Lipofectamine 2000 reagents, U87 and U251 cells (70% cell confluent) were transfected with pREV, pRRE and pVSVG plasmids, or a control lentivirus vector together with sh-ELK4-1, sh-HOMER3-2, or sh-ELK4-2.

RNA extraction and quantitative real-time PCR analysis

Once the cells attained the correct confluence (about 70 to 80%), they were removed from the culture dish. A reagent called RNAiso Plus (TaKaRa, Japan) was used for total RNA extraction. Before being centrifuged again at 7,500 × g for 5 minutes at 4°C, the RNA pellet was quickly vortexed after being washed with 1 mL of 75% ethanol. Before being stored at -80°C for future analysis, the RNA pellet was allowed to air-dry for 5–10 minutes. It was then resuspended in 20–30 μL of water that did not contain RNase. Pure RNA was deemed acceptable when the A260/A280 ratio was between 1.8 and 2.0. To ensure the RNA was intact, 1 μg of RNA was electrophoresed on a 1% agarose gel and the presence of distinct 18S and 28S rRNA bands was examined using ultraviolet light. We followed the manufacturer's instructions to reverse transcribe messenger RNA (mRNA) into complementary DNA (cDNA) using the PrimeScript RT Master Mix Kit (TaKaRa, Japan). With the help of the SYBR Premix Ex Taq II Kit (TaKaRa, Japan) and the ABI 7500 Real-Time PCR System (Applied Biosystems, USA), the qRT-PCR analysis was performed to determine the levels of mRNAs (ELK4, HOMER3, and GAPDH). A melting curve analysis was carried out after amplification to validate the specificity of the PCR products. Each experiment was performed with three technical replicates per sample to ensure the reliability and consistency of the results. The typical $2^{-\Delta\Delta Ct}$ method was used to determine relative RNA abundances, with GAPDH serving as an internal control for messenger RNA. The specific sequences for the primers used are listed below: ELK4: Forward: 5'-TG GACCTCTAATGATGGGCAG-3' Reverse: 5'- AGGCTT GTTCTTGCGAATCCC – 3' HOMER3: Forward: 5'- AG GGAGCAGCCAATCTTCAG-3' Reverse: 5'- CCCACT GCCCGAACTTCTG-3' GAPDH: Forward: 5'-GGAGC GAGATCCCTCCAAAAT-3' Reverse: 5'-GGCTGTTGT CATACTTCTCATGG-3'.

CCK8 assay

To measure the number of U87 and U251 glioma cells, an automated cell counter was used during the exponential growth phase. In 100 μL of complete RPMI-1640 medium supplemented with 10% FBS and antibiotics (penicillin and streptomycin), 1,000 cells were seeded into each well of a 96-well plate. At 0, 24, 48, 72, and 96 h following cell seeding, cell proliferation was measured using the CCK8 test kit (Dojindo, Japan). Ten microliters of CCK8 reagent were added to each well, including the blank wells, at each predetermined time point (e.g., 24 h, 48 h, 72 h, 96 h). To ensure even distribution of the CCK8 reagent across the well surface, the plates were gently shaken. After reagent addition, the plates were incubated in a CO_2 incubator for 1 to 2 h at 37 °C. The incubation time was standardized for all experiments. After adding the CCK8 reagent, all plates were incubated for 2 h at 37 °C in a CO_2 incubator. The color change of the reagent, which typically turns orange when reacting with metabolically active cells, was monitored as a guide. However, a fixed 2-hour incubation time was used for all wells to ensure consistency and reproducibility across the experiments with U87 and U251 glioma cells. Absorbance at 450 nm was measured using a microplate reader (Thermo Fisher, USA) after the incubation period.

Colony formation assay

Glioma cells (U87 and U251) were transfected with specific shRNA or control vectors, then gathered, numbered, and seeded onto 6-well plates at a density of 1,000 cells/well. A 2 mL volume of RPMI-1640 media with 10% FBS and penicillin/streptomycin was placed in each well. For 10–14 days, or until colonies with 50 cells or more were visible to the human eye, the cells were cultured in an incubator set at 37 °C with 5% CO_2. The culture medium was delicately removed from the wells after the incubation time, when colonies had formed. Each well was incubated at room temperature for 15 min after 1 mL of

4% paraformaldehyde (Thermo Scientific, Rockford, IL, USA) was added to fix the cells. A 1 mL solution of 0.2% crystal violet (Solarbio, China) was added to each well to stain the fixed colonies. For 20 min, the plate was gently rocked at room temperature to make sure the stain was evenly distributed across all the colonies. Colony counting was performed using ImageJ software with a colony-counting plugin. The colonies were manually outlined in the software, and the number of colonies was automatically counted. This method ensured a consistent and objective analysis of the colony formation in the experiments.

Cell migration and invasion assays

The upper chamber of a transwell insert was seeded with 2×10^4 cells for migration tests, while the lower chamber was given 10% FBS medium. Chamber inserts were sterilised and coated with BD Matrigel and medium the night before invasion experiments. In the upper chamber of every transwell insert, 1×10^5 cells were plated in 200 μL of serum-free DMEM. To serve as a chemoattractant, 600 μL of RPMI-1640 media with 10% FBS was introduced to the lower chamber. Cell invasion through the Matrigel-coated membrane was achieved by incubating the plate at 37 °C for 24 h. The transwell inserts were delicately removed from the 24-well plate after incubation had been completed for 24 h. Using a cotton swab, the cells that had not penetrated or migrated were delicately scraped off, leaving only the cells on the top of the insert membrane. Methanol was used to fix the cells that had invaded or migrated to the lower side of the membrane. After 20 min of room temperature staining with a 0.2% crystal violet solution, the inserts were removed. Under a light microscope, the transwell membranes that had been stained were examined. Either by hand or with the use of image analysis tools like ImageJ, the quantity of cells that invaded or moved through the membrane was determined.

Western blotting

Up until 70–80% confluence, the cells were grown according to the normal protocol. The cells were washed with PBS before lysis to eliminate any serum components and residual growth medium that could contaminate the study. For half an hour, cells were suspended in a radioimmunoprecipitation assay buffer (RIPA; Beyotime Institute of Biotechnology, Haimen, China) that contained protease inhibitors (1% PMSF) and phosphatase inhibitors (1% NaF). After lysis, the cell lysates were centrifuged at max speed for 10 min at 4 °C to separate the insoluble and cell debris. The soluble proteins were extracted by meticulously collecting the supernatant. The 5× loading buffer(Bio-Rad Laboratories, Inc. Hercules, California, USA), which contained bromophenol blue and β-mercaptoethanol, was combined with 30–50 μg of protein in equal parts. Before loading the materials onto a 10–12% polyacrylamide gel(Homemade) for electrophoresis, they were denatured by heating at 95 °C for 5 min. A steady voltage, typically between 80 and 120 V, was applied to the gel until the dye front reached the gel's base. Through a wet transfer procedure, proteins were moved to PVDF membranes (Millipore, Billerica, MA, USA) by applying a voltage of 100 V for one to two hours at 4 °C or, alternatively, by leaving the membranes at a lower voltage of 20–30 V overnight. To avoid nonspecific antibody binding, the membranes were incubated at room temperature for 1 h with 5% bovine serum albumin (BSA) in Tris-buffered saline with Tween-20 (TBST). Primary antibodies diluted in blocking buffer were incubated with the membranes at 4 °C for 10–12 h. The antibodies included ELK4, HOMER3, CDK4, CDK6, Cyclin D1, P27, β-catenin, E-cadherin, N-cadherin, Slug, Snail, Twist1, Twist2, and β-actin. The membranes were rinsed three times with TBST for 10 min each after the primary antibody incubation to eliminate any unbound antibodies. The membranes were left to incubate at room temperature for 2 h with the corresponding secondary antibodies that were horseradish peroxidase (HRP) conjugated and diluted in blocking buffer. Following the directions provided by the manufacturer, protein bands were detected using ECL-Plus kits from Anhe Biotech in Qingdao, Jinan, China. We bought all of the antibodies from Proteintech in Wuhan, China.

In vivo tumor xenograft model

The animal experiment was approved by the Animal Care Committee. All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Huizhou Central People's Hospital, Guangdong Medical University. The Beijing Vital River Laboratory Animal Technology Company supplied the female BALB/c nude mice, which were 6–8 weeks old. Under specified pathogen-free (SPF) settings, the mice were housed in a 12-hour light/dark cycle with controlled temperature (22–24 °C) and humidity (40–60%). Prior to the trial, the mice were given ample time to adjust to their new environment and were given free access to food and water. Until 70–80% confluence was achieved, U251 glioma cells were grown in [specified media, for example, DMEM with 10% FBS] under standard conditions (37 °C, 5% CO_2). The cells were concentrated to 5×10^6 cells per 100 μL in PBS before being trypsinized, counted, and resuspended for injection. Each mouse had a sterile 27-gauge needle inserted subcutaneously into its flank, and U251 cells at a concentration of 5×10^6 were used for tumor induction. To ensure the injection site was free of infection,

70% ethanol was used to disinfect it before the injection. Injecting the cell suspension into each mouse was done just once. Six mice each were given the sh-HOMER3-1 or sh-NC therapy, and the mice were then randomly placed into one of the two groups. The purpose of randomization was to level the playing field in terms of tumor size distribution and reduce the possibility of bias. Each mouse received a total of five injections of sh-HOMER3-1 or sh-NC given intratumorally every three days. A transfection reagent (Entranster™-in vivo, Engreen, China) was used to administer each injection in accordance with the manufacturer's instructions. To make sure the medicine was distributed evenly, the injections were done at several sites inside the tumor. In accordance with ethical rules, mice were euthanized using CO_2 inhalation and cervical dislocation for confirmation at the conclusion of the treatment period or when tumors reached a preset size limit. After tumors were removed and weighed, they were partially frozen in liquid nitrogen and sent on for additional analyses, such as Western blotting and RT-PCR.

Immunohistochemistry analysis

After a 24-hour fixation in 4% paraformaldehyde at room temperature, the tissue samples were embedded in paraffin. The microtome was used to cut sections that were 4–5 μm thick. After that, the sections were placed on glass slides and left to dry overnight at 37 °C to make sure they adhered properly. After soaking the slides in xylene for 10 min, they were deparaffinized. They were then washed twice more with fresh xylene. After that, the slides were then rehydrated by running them through a succession of progressively more diluted ethanol solutions (100%, 95%, 70%) for 5 min each. Slides were incubated in a 0.3% H_2O_2 in methanol solution for 30 min to block the action of endogenous peroxidase. The slides were washed three times with PBS after treatment to eliminate any excess H_2O_2. For the purpose of allowing antibodies to penetrate the tissue, tissue sections were permeabilized by allowing them to sit in a solution of 0.5% Triton X-100. The slides were heated in a citrate buffer (pH 6.0) in a microwave or pressure cooker for 10–20 min to facilitate antigen retrieval, which involved making the relevant antigens accessible to the antibodies. Three washes with PBS were performed after the slides had cooled to room temperature. The slides were left to incubate for one hour with 5% normal goat serum diluted in PBS to avoid non-specific binding. For two hours, the slides were exposed to primary antibodies (HOMER3, Ki-67, and PCNA) that were diluted at a ratio of 1:100 in antibody dilution buffer, such as PBS with 1% BSA. We obtained the antibodies from Proteintech in Wuhan, China. To eliminate any unbound antibodies, the slides were washed three times with PBS, each wash lasting 5 min, after the primary antibody incubation. The next step was to dilute the HRP-conjugated anti-rabbit IgG (H + L) secondary antibody per the manufacturer's instructions and let the slides sit at room temperature for 30 min. By incorporating a DAB (3,3'-diaminobenzidine) substrate solution onto the slides, the enzyme activity could be observed. The presence of the target antigens was confirmed when a brown hue emerged, which was achieved by allowing the reaction to progress. The ideal duration of incubation for DAB was observed under a microscope. Nuclei of cells were shown by 5 min of counterstaining with DAPI (4',6-diamidino-2-phenylindole). The slides were then washed with PBS to eliminate any remaining DAPI. The slides were subsequently mounted with an anti-fade mounting media (such as glycerol or a commercially available option) after being dehydrated using a series of graduated ethanol washes.

ChIP analysis

For accurate and repeatable results, we used Millipore EZ ChIP analysis kits(.

Millipore, Merck Group, Darmstadt, Germany) to perform chromatin immunoprecipitation (ChIP) studies. To summarize, glioma cells U251 and U87 were cultivated in 6-centimeter dishes and cross-linked to DNA by treating them with 1% formaldehyde for 15 min at 27 °C. After this fixation phase, 125 nM of glycine was added to stop the cross-linking and quench the formaldehyde. Following the treatment, the cells were collected and broken down using a specialized cell lysis buffer(Pplygen, Beijing, China) that was developed to maintain the integrity of the chromatin. We used a needle sonicator to sonicate the chromatin extracts, generating DNA fragments with lengths ranging from 200 to 400 base pairs. The sonication was performed using the following parameters: 50% amplitude and 10-second sonication pulses with 20-second intervals between pulses, which effectively fragmented the chromatin for subsequent ChIP analysis. For successful immunoprecipitation and analysis, this size range is ideal. The following step was to extract the DNA fragments linked to ELK4 from the lysate using an anti-ELK4 antibody(Cat no: 14666-1-AP, Proteintech, Wuhan, China). To ensure that the immunoprecipitation method did not involve any nonspecific binding, a negative control consisting of normal rabbit IgG (Abcam, Cambridge, MA, USA) was utilized. The precipitated DNA was extracted from the complexes bound to the antibodies during the immunoprecipitation process. After elution, the DNA was analyzed using real-time PCR to determine the amount of the target DNA fragments and evaluate ELK4's binding affinity to specific genomic areas. By taking an all-encompassing strategy, we are able to derive significant conclusions regarding ELK4's function in glioma cell gene expression control.

Luciferase activity detection

To investigate the regulatory mechanisms governing HOMER3 expression, we cloned specific promoter sequences of the HOMER3 gene into pGL3-basic luciferase reporter vectors. These sequences included both the wild-type (WT) and a mutant-type (MUT) version, which contained predicted binding sites identified through the JASPAR database. The resulting constructs were designated as WT and MUT, respectively, allowing us to compare the luciferase activity based on the presence of these binding sites. For the luciferase activity assay, we plated U251 and U87 glioma cells into 96-well plates at a density of 4,000 cells per well. Once the cells adhered to the plate, we performed transient transfections using a mixture of the designated reporter plasmids, including both the WT and MUT constructs to assess their effects on HOMER3 expression. After a 48-hour incubation period to allow for sufficient expression of the transfected constructs, we measured luciferase activity using the Promega luciferase reporter assay kit (Kejia, Hangzhou, Zhejiang, China). The experiment was performed in triplicate to ensure the reliability and reproducibility of the results.

Download data

Clinical and mRNA sequencing data were acquired from two large databases: The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) and the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn/). The genes with consistently low expression levels were filtered out of the raw expression data. Genotype-Tissue Expression (GTEx) was the source for the RNA sequencing data related to normal brain tissues. Genes with counts below a set threshold (e.g., counts per million (CPM) < 1 in at least 50% of samples) were excluded to minimize background noise. Gene expression data were normalized to account for differences in sequencing depth and gene length using the EDASeq R package. To address potential batch effects arising from different sequencing platforms or study cohorts, we applied the ComBat function from the sva R package. This step standardizes data distributions across different batches, ensuring more accurate downstream analysis. Log2 transformation was applied to the normalized expression values to stabilize the variance and improve data interpretability.

Bioinformatics analysis

Gene expression data and corresponding clinical information, including survival time, survival status, and other clinical traits, were analyzed using R software. The data were first filtered using ROC curve analysis to identify significant thresholds for HOMER3 expression associated with survival outcomes. To assess the effect of HOMER3 on patient survival, Kaplan-Meier survival curves were plotted using the survival and survminer packages in R. Patients were stratified into high and low expression groups based on the predetermined cutoff values. The survival curves visualized the difference in overall survival (OS) between these groups, and the log-rank test was applied to compare survival distributions. p-values < 0.05 were considered statistically significant. Univariate Cox regression analysis was conducted to quantify the association between HOMER3 expression and survival outcomes (overall survival, progression-free survival). Hazard ratios (HR) with 95% confidence intervals (CI) were calculated to determine whether high or low expression of HOMER3 influenced patient prognosis. Subsequently, multivariate Cox regression was performed to adjust for confounding variables, including patient age, tumor grade, treatment status, and other clinical covariates. To evaluate the predictive performance of HOMER3 as a biomarker for glioma survival, the survivalROC package was employed to generate time-dependent ROC curves. ROC curves were plotted for survival at 1-year, 3-year, and 5-year intervals, with the AUC values used to gauge the discriminative ability of HOMER3 expression in predicting patient survival. Statistical significance was determined with p-values < 0.05, and results were presented through visual plots to illustrate the relationship between HOMER3 expression and patient outcomes in glioma.

Statistical analysis

We used R 3.4.3 and GraphPad Prism 5.0 for Windows (GraphPad Software Inc., San Diego, CA, USA) to conduct our statistical analyses. To compare different groups and see if there were any variations in cell viability, migration, invasion, and other quantitative measurements, we utilized one-way analysis of variance (ANOVA) with Newman-Keuls post hoc test. A paired two-tailed Student's t-test was used for comparisons between two related samples. To analyze the survival data and determine how EKL4 and HOMER3 expression affected survival times, we used Kaplan-Meier survival analysis, which includes log-rank testing. Furthermore, chi-squared tests were used to examine the connection between gene expression levels and clinical outcomes in tumor specimens from humans. For all tests, a p-value less than 0.05 was considered statistically significant. Cell viability, migration, invasion, and other quantitative measurements were performed in three independent biological experiments ($n = 3$). Each experiment was conducted with three technical replicates to ensure the reliability and reproducibility of the results.

Results

Pan-cancer analysis of HOMER family

The HOMER family includes HOMER1, HOMER2, and HOMER3, which may play important biological roles in

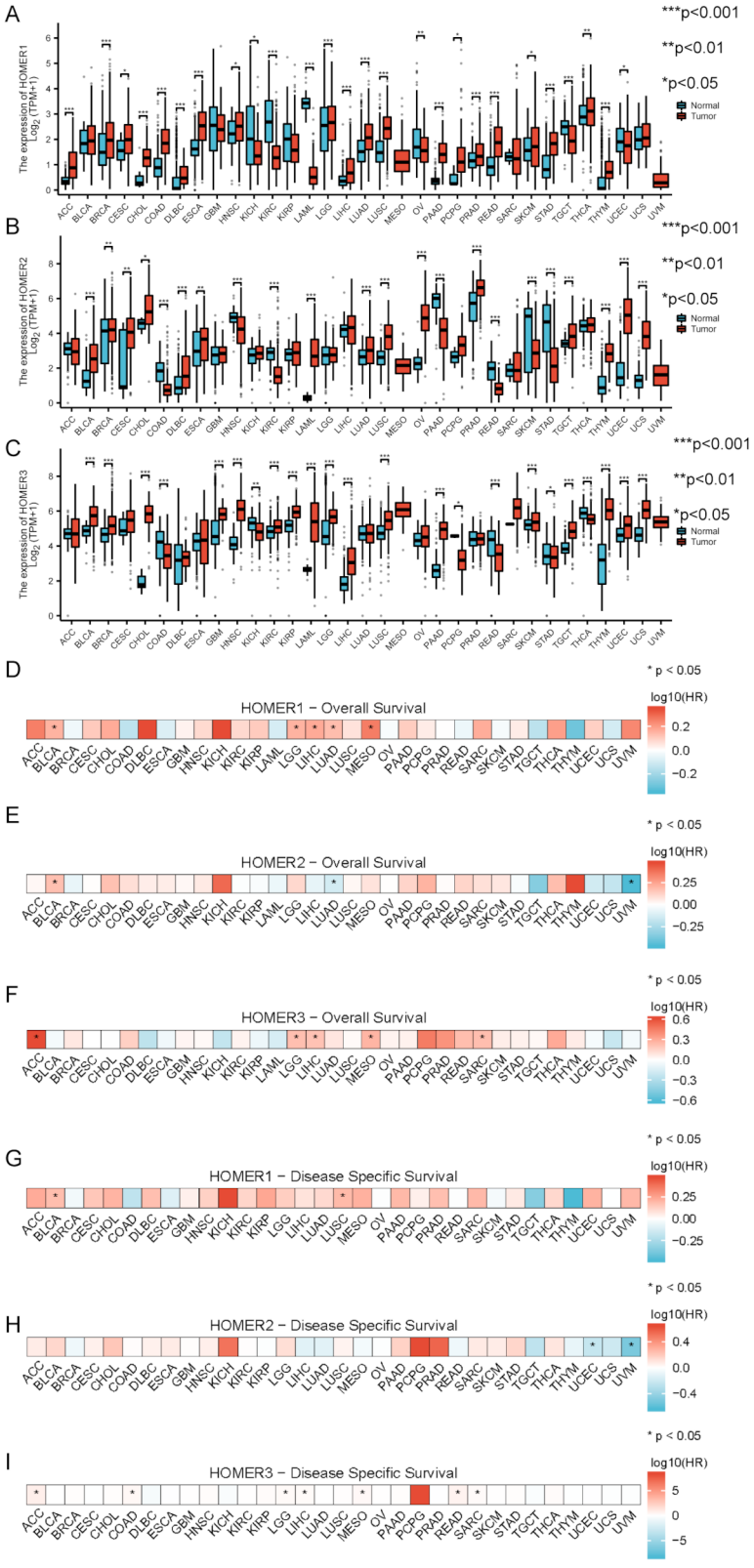

Fig. 1 (See legend on next page.)

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Fig. 1 Pan-cancer analysis of HOMER family gene expression and prognostic value. (**A**-**C**) Differential expression levels of HOMER1, HOMER2, and HOMER3 across various cancer types compared to normal tissues, illustrating the dysregulation of these genes in tumors. Statistical analysis was performed using Wilcoxon rank-sum test, with $P < 0.05$ considered significant. (**D**-**I**) Correlation of HOMER family gene expression with overall survival (OS) and disease-specific survival (DSS) in different cancers. Specifically, (**D**) HOMER1 in bladder cancer (BLCA), lower-grade glioma (LGG), liver cancer (LIHC), and mesothelioma (MESO); (**E**) HOMER2 in BLCA and lung adenocarcinoma (LUAD); (**F**) HOMER3 in adrenocortical carcinoma (ACC), LGG, LIHC, MESO, and sarcoma (SARC). (**G**) HOMER1 associated with DSS in BLCA and lung squamous cell carcinoma (LUSC); (**H**) HOMER2 in uterine corpus endometrial carcinoma (UCEC) and uveal melanoma (UVM); and (**I**) HOMER3 in ACC, colon adenocarcinoma (COAD), LGG, LIHC, MESO, rectum adenocarcinoma (READ), and SARC

various cancers. We aim to evaluate the expression differences of HOMER family members in different types of cancers and their correlation with patient prognosis. We used public databases such as TCGA and GTEx to collect expression data of HOMER family genes in various cancer tissues and normal tissues. Additionally, bioinformatics tools (such as R, Python, and related biostatistical packages) were used for data analysis. Firstly, we performed pan-cancer analysis of HOMER Family including HOMER1, HOMER2 and HOMER3. As shown in Fig. 1A and C, HOMER1, HOMER2 and HOMER3 exhibited a dysregulated level in many types of tumors. We next looked at their predictive power in pan-cancer. Figure 1D shows that BLCA, LGG, LIHC, and MESO survival rates were correlated with HOMER1 expression. Figure 1E shows that BLCA and LUAD overall survival was correlated with HOMER2 expression. Overall survival of ACC, LGG, LIHC, MESO, and SARC was correlated with HOMER3 expression (Fig. 1F). Furthermore, we found that the HOMER1 gene was correlated with BLCA and LUSC DSS (Fig. 1G). There was a correlation between HOMER2 expression and the DSS of UCEC and UVM, as shown in Fig. 1H. Researchers found that the DSS of ACC, COAD, LGG, LIHC, MESO, READ, and SARC were linked with HOMER3 expression (Fig. 1I). Among the HOMER family members, only HOMER3 showed a significant association with OS and DSS in LGG patients, suggesting that it may be useful as a predictive biomarker for this particular cancer subtype. Our results provide more evidence that HOMER3 has a distinct function in LGG development and clinical outcome.

The expression and prognostic value of HOMER family in LGG patients

Two separate but related types of brain tumors, LGG and GBM, were the subjects of our extensive study of HOMER family gene expression. Figure 2A and C show that our results showed that, compared to normal tissue samples, only HOMER3 showed a substantial overexpression in LGG and GBM samples, suggesting that it may have a role in the pathogenesis of gliomas. Among the three HOMER genes that were examined, it was found that increased expression of HOMER3 was the only one linked to reduced OS and DSS in LGG patients (Fig. 2D and E). The correlation prompted us to focus our investigation specifically on HOMER3, as it may serve as a critical biomarker in these conditions. To further elucidate the prognostic significance of HOMER3 in glioma, we performed Cox regression analysis. Our analysis confirmed that HOMER3 expression is an independent prognostic factor for both OS and DSS in LGG patients (Fig. 2F and G), reinforcing the notion that higher levels of HOMER3 may reflect a more aggressive tumor phenotype. Additionally, survival analyses utilizing data from the CGGA demonstrated a compelling relationship between HOMER3 expression levels and patient outcomes. Specifically, we found that low levels of HOMER3 expression were associated with improved overall survival, whereas high levels correlated with poorer prognoses in glioma patients (Fig. 2H). To further characterize the impact of HOMER3 on patient survival, we conducted a univariate Cox analysis, which indicated that HOMER3, in conjunction with several clinical variables such as age, tumor grade, and treatment history, constituted a significant risk factor for poor outcomes (Fig. 2I). In addition, multivariate assays confirmed that HOMER3 expression was a significant predictor of overall survival, highlighting its possible use as a biomarker or therapeutic target (Fig. 2J). We found that high levels of HOMER3 expression correspond with advanced clinical stages in LGG patients, which suggests a possible link with tumor aggressiveness and progression (Fig. 3A and B), in addition to its prognostic relevance. The importance of studying HOMER3 and its function in tumor biology, as well as the mechanisms that drive its expression, is highlighted by our association. Furthermore, our analysis of glioma samples from the CGGA database revealed that differential expression of HOMER3 was significantly related to various clinical and molecular characteristics, including patient age, tumor grade, 1p19q codeletion status, IDH mutation status, and histological type (Fig. 3C and D). These results demonstrate how diverse HOMER3 is in its impact on patient prognosis and how intricate glioma biology is.

ELK4 bound to HOMER3 promoter and stimulated its expression in glioma cells

To investigate the potential mechanism underlying the aberrant dysregulation of HOMER3 expression in glioma, we focused on identifying transcription factors (TFs) that could activate its promoter. Given that HOMER3 was found to be upregulated in glioma tumor samples, we aimed to uncover the factors responsible for its upregulation. Previous studies have demonstrated

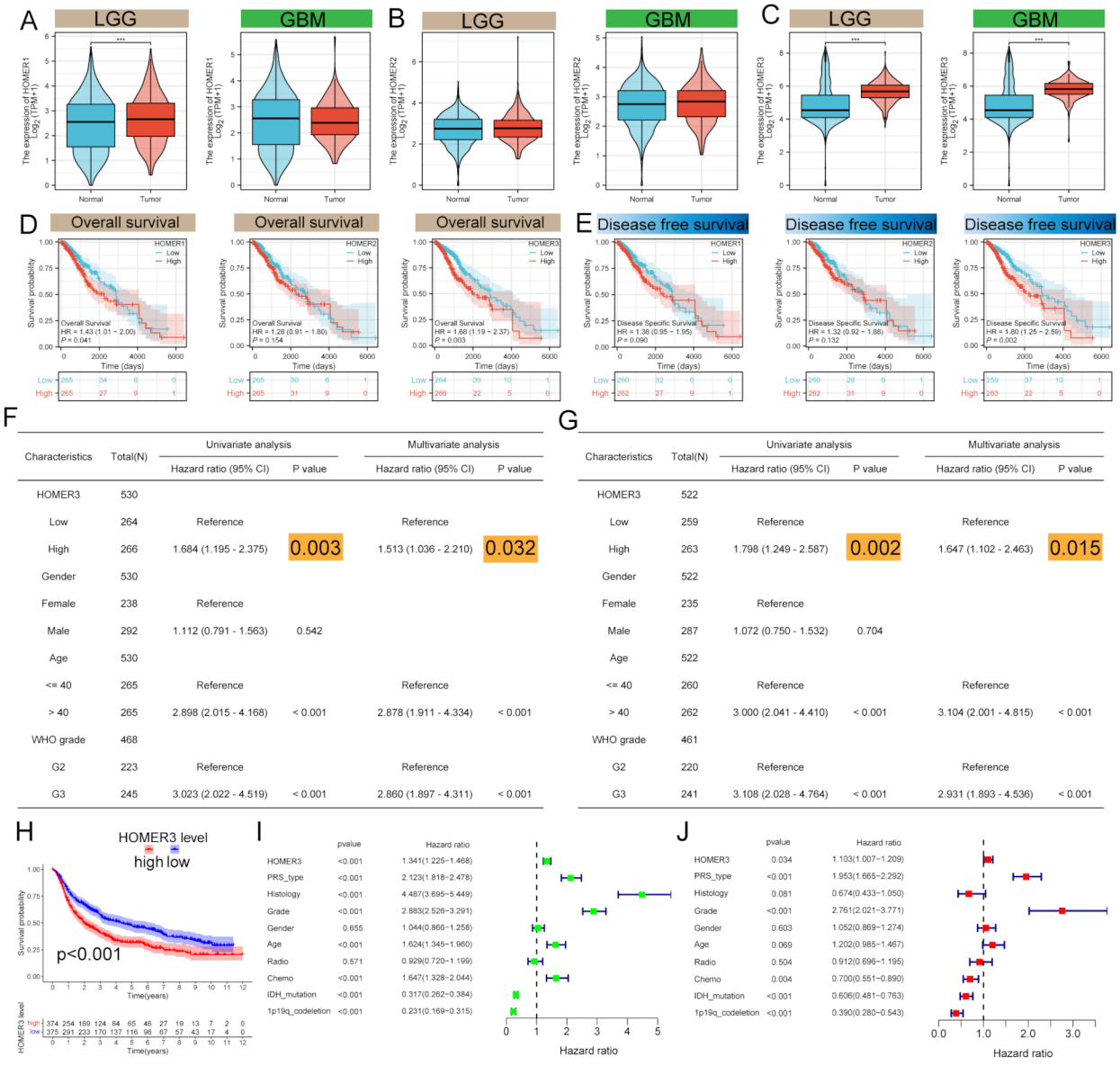

Fig. 2 Expression and prognostic significance of HOMER3 in glioma patients. (**A**-**C**) Expression levels of HOMER family genes in lower-grade glioma (LGG) and glioblastoma (GBM) compared to normal tissue samples, showing significant upregulation of HOMER3. (**D**) Correlation between HOMER3 expression and overall survival (OS) in LGG patients. (**E**) Disease-specific survival (DSS) correlation with HOMER3 expression. (**F**-**G**) Cox regression analysis confirming HOMER3 as an independent prognostic factor for both OS and DSS in LGG patients. (**H**) Survival analysis from the CGGA, demonstrating that low HOMER3 expression is associated with better outcomes, while high expression correlates with poorer prognosis. (**I**-**J**) Univariate and multivariate Cox analyses indicating that HOMER3 is a significant risk factor for poor outcomes, independent of other clinical variables

that transcription factors can promote gene transcription via binding directly to the corresponding gene promoters [30, 31]. To begin the exploration, we retrieved the HOMER3 promoter sequence from the UCSC Genome Browser, a widely used resource for genomic data. We then utilized the JASPAR database, a tool for predicting TF binding sites, to identify potential transcription factors that could regulate HOMER3 expression. Our analysis revealed three transcription factors—SP1, CREB1, and ELK4—with high predictive scores for binding to the HOMER3 promoter (Fig. 4A). We then evaluated these transcription factors' predictive power using TCGA expression data. According to our results, glioma patients had a better chance of survival when their SP1, CREB1, and ELK4 expression levels were high (Fig. 4B). Further investigation into the expression patterns of SP1, CREB1, and ELK4 in LGG and GBM revealed that SP1 and ELK4 were distinctly upregulated in both tumor

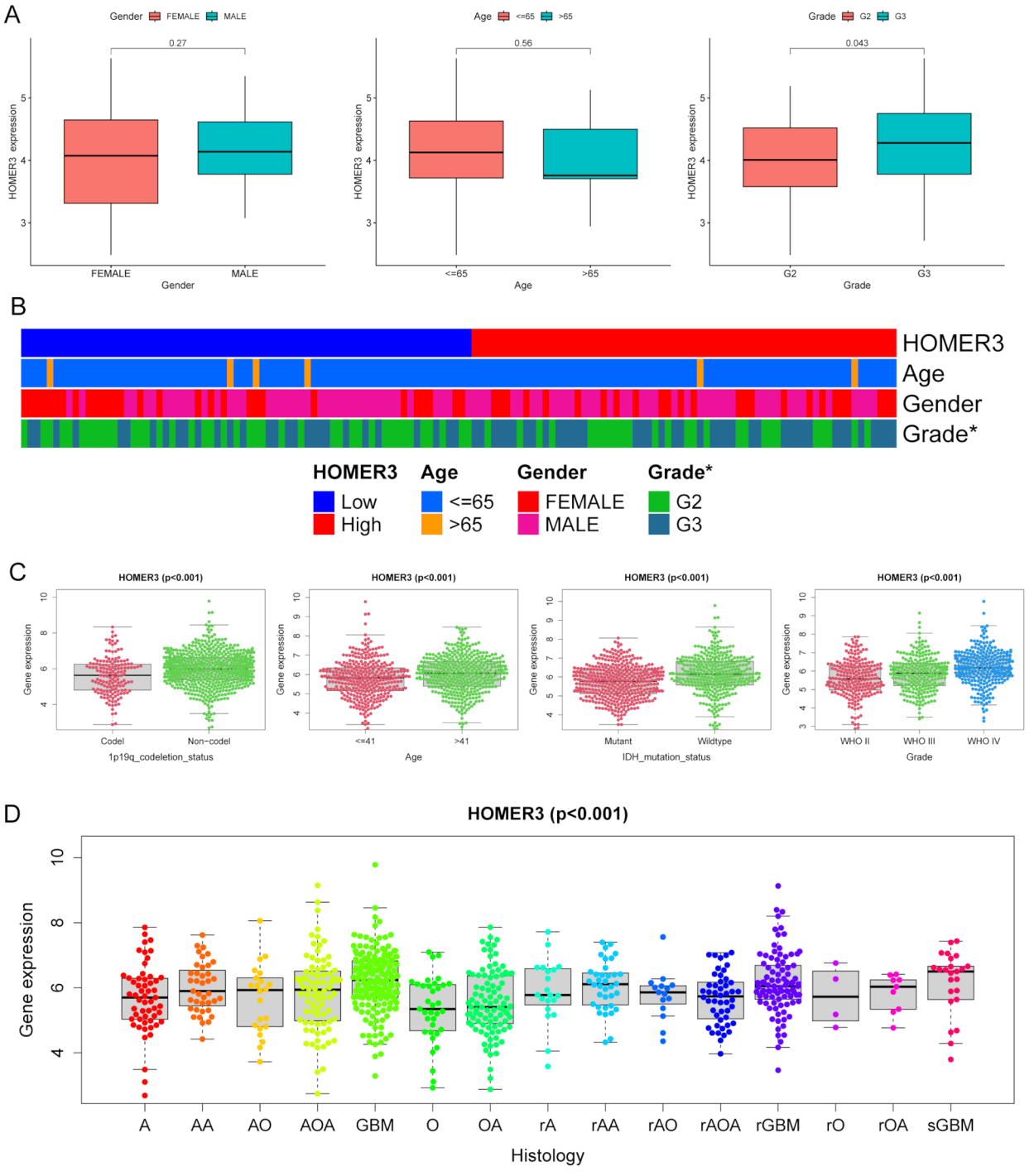

Fig. 3 The association between HOMER3 expression and clinical factors. (**A**-**B**) Correlation of HOMER3 expression with advanced clinical stages in LGG based on TCGA datasets. (**C**-**D**) Associations of HOMER3 expression with various clinical and molecular characteristics in glioma samples from CGGA

types when compared to normal tissue samples (Fig. 4C). Given the established role of SP1 in tumor progression, our study chose to concentrate on the transcription factor ELK4 for further analysis. Utilizing the JASPAR algorithm, we predicted the specific binding sites for ELK4 on the HOMER3 promoter, which are illustrated in Fig. 4D. To validate our predictions, we performed chromatin immunoprecipitation (ChIP) analysis, which confirmed that ELK4 could interact with the designated binding sites within the HOMER3 promoter (Fig. 4E).

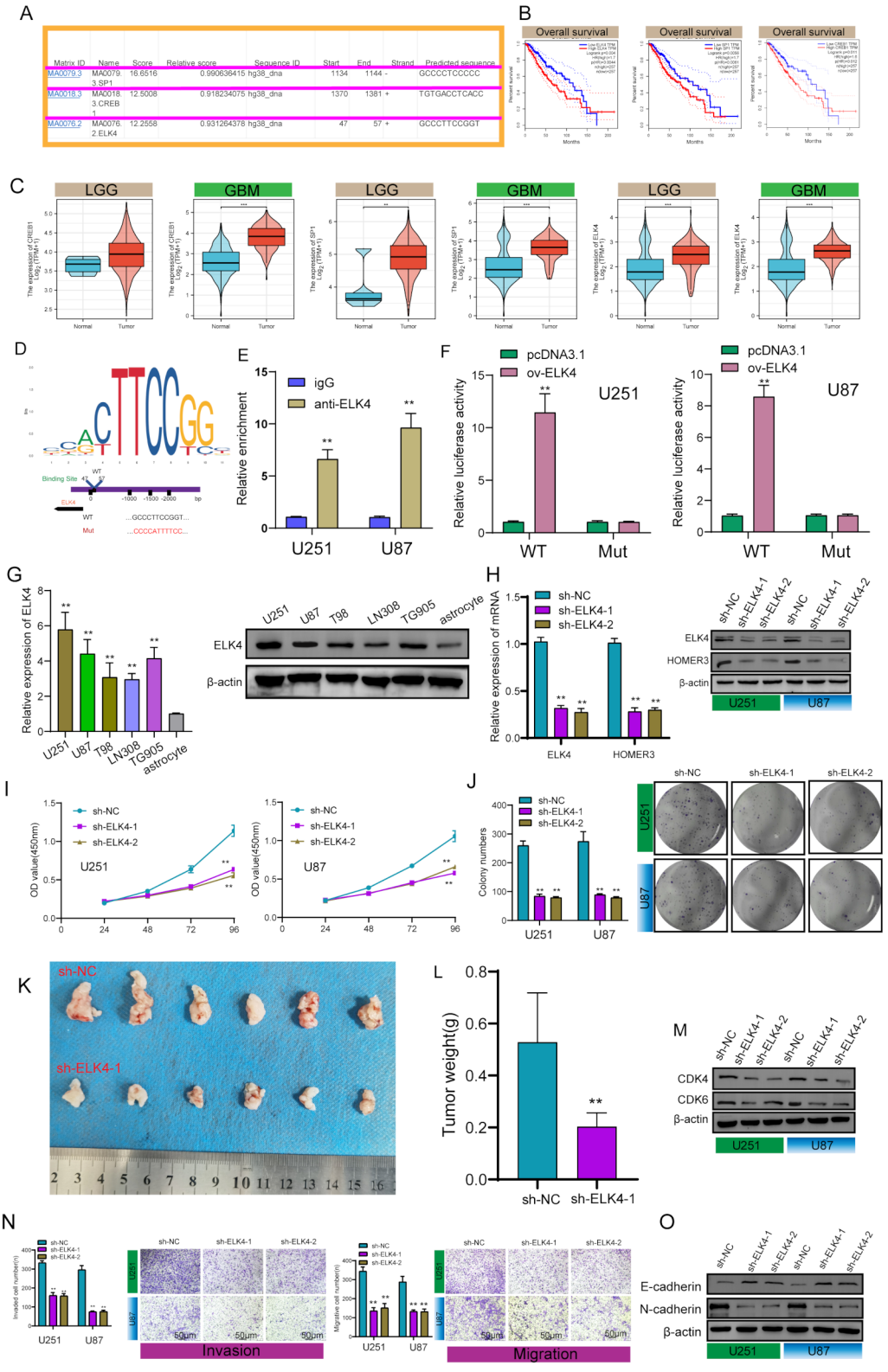

Fig. 4 (See legend on next page.)

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Fig. 4 Identification of transcription factors regulating HOMER3 expression in glioma. (**A**) Prediction of transcription factor binding sites in the HOMER3 promoter using JASPAR, highlighting ELK4, SP1, and CREB1 as potential regulators. (**B**) Association of expression levels of SP1, CREB1, and ELK4 with the overall survival of glioma patients from TCGA datasets. (**C**) Expression patterns of SP1 and ELK4 in LGG and GBM tissues compared to normal tissues. (**D**) ELK4 binding site predictions on the HOMER3 promoter illustrated. (**E**) Chromatin immunoprecipitation (ChIP) analysis confirming ELK4 binding to the HOMER3 promoter. (**F**) Luciferase reporter assays demonstrating increased luciferase activity in the presence of wild-type ELK4 binding sites in U251 and U87 glioma cells. (**G**) Elevated expression levels of ELK4 in glioma cell lines compared to normal cells as assessed by RT-PCR and western blot analysis. (**H**) Knockdown of ELK4 via shRNAs, which was confirmed by RT-PCR and western bot. (**I** and **J**) The effect of ELK4 knockdown on glioma cell proliferation assessed by CCK-8 and clonogenic assays. (**K** and **L**) In vivo analysis of ELK4 knockdown effects on tumor growth. Each group contained $n = 6$ mice, and the experiment was repeated three times independently. (**M**) Western blot for the expression of CDK4 and CDK6 after ELK4 knockdown. (**N**) The effects of ELK4 knockdown on the migration and invasion of U251 and U87 cells. (**O**) The effects of ELK4 knockdown on the expression of EMT-related proteins by western blot. All experiments were performed in three independent biological replicates ($n = 3$) to ensure the reproducibility and reliability of the results

To delve further into the functional influence of ELK4 on HOMER3 expression, we also performed luciferase activity experiments. In Fig. 4F, we can see that luciferase reporter cells having the wild-type ELK4 binding site showed much higher luciferase activity in ectopically expressed U251 and U87 glioma cells compared to cells with a mutant binding site. We used RT-PCR and western blotting to examine ELK4 expression in five different glioma cell lines in an effort to better understand its function in glioma biology. These glioma cells showed significantly higher levels of ELK4 compared to normal cells, according to the data (Fig. 4G and Figure S1A). To elucidate the functional effects of ELK4 in glioma progression, we employed two short hairpin RNAs (shRNAs) targeting ELK4 to decrease its expression in glioma cells. As shown in Fig. 4H and Figure S1B, both shRNA-ELK4-1 and shRNA-ELK4-2 effectively reduced ELK4 levels in these glioma cells. Following this, clonogenic and CCK-8 tests proved without a reasonable doubt that ELK4 knockdown dramatically reduced U251 and U87 cell proliferation, indicating that ELK4 is essential for glioma cell growth (Fig. 4I and J). Furthermore, to explore the in vivo effects of ELK4 knockdown on tumor growth, we utilized a subcutaneous xenograft mouse model. Our findings demonstrated that tumors in the ELK4 knockdown group were significantly smaller and exhibited lower final tumor weights compared to the negative control groups (Fig. 4K and L). Additionally, we observed that the knockdown of ELK4 led to a marked suppression of cell cycle regulators, specifically CDK4 and CDK6, indicating a potential mechanism through which ELK4 promotes glioma cell proliferation (Fig. 4M and Figure S1C). Transwell assays were used to evaluate the impact of ELK4 knockdown on glioma cell migratory and invasive capacities. According to the findings, the migration and invasion of both U87 and U251 cells were significantly reduced when ELK4 was silenced (Fig. 4N). Lastly, we conducted western blot analysis on proteins related to the EMT pathway to study the underlying mechanisms of ELK4's oncogenic role in glioma cell metastasis. Importantly, our results showed that knocking down ELK4 considerably reduced N-cadherin expression while increasing E-cadherin expression; this lends credence to the idea that ELK4 is essential for glioma metastasis promotion by regulating the EMT process (Fig. 4O and Figure S1D).

Knockdown of HOMER3 suppressed the proliferation of glioma in vitro and in vivo

To explore the functional implications of HOMER3 in glioma, we began by examining its expression levels in five glioma cell lines compared to normal cells. Using RT-PCR and western blot analysis, we found that HOMER3 expressions were distinctly elevated in all five glioma cell lines, indicating its potential role in tumor biology (Fig. 5A and Figure S2A). To investigate the impact of HOMER3 on glioma cell proliferation, we employed short hairpin RNA (shRNA) plasmids specifically designed to knock down HOMER3 expression in U251 and U87 glioma cells. Our experiments achieved an efficient knockdown of HOMER3, exceeding 50% at both mRNA and proteins, as demonstrated in Fig. 5B and Figure S2B, confirming the effectiveness of our shRNA approach. After HOMER3 was successfully knocked down, we tested its effect on glioma cell growth in multiple ways. Figure 5C and D indicated that after HOMER3 knockdown, the CCK-8 tests and clonogenic assay demonstrated a marked decrease in the proliferation rates of U251 and U87 cells. Based on our findings, HOMER3 is an essential factor in glioma cell lines that promote cell proliferation. Using a subcutaneous xenograft mouse model, we expanded our findings to an in vivo context in order to better understand the function of HOMER3 in tumor growth. In comparison to the NC groups, those whose HOMER3 was knocked down had much smaller tumors and a lot lower final tumor weight (Fig. 5E). Additionally, immunohistochemistry (IHC) analysis of HOMER3 expression confirmed successful knockdown in vivo, validating our in vitro findings. To assess the effect of HOMER3 knockdown on cellular proliferation, we performed Ki-67 and PCNA staining. The results indicated that tumors from the HOMER3 knockdown group exhibited impaired cellular proliferation, further corroborating our hypothesis about HOMER3's role in glioma growth (Fig. 5F). Additionally, we utilized correlation analyses to explore the connections between HOMER3 expression and important regulators of the cell cycle.

While cyclin D1 and the cyclin-dependent kinase inhibitor p27 showed a negative correlation with HOMER3 expression, cyclin-dependent kinases CDK4 and CDK6 showed a positive link based on TCGA datasets (Fig. 5G and H). HOMER3 may influence the expression of critical regulators involved in cell cycle progression. Moreover, our analysis confirmed that HOMER3 expressions were positively associated with tumor proliferation signatures, indicating its involvement in driving glioma cell proliferation (Fig. 5I). Finally, we performed additional western blot analysis, which showed that the knockdown of HOMER3 resulted in a marked suppression of CDK4 and CDK6 expression, while concurrently promoting the expression of cyclin D1 and p27 (Fig. 5J and Figure S2C). This shift in expression patterns highlights the complex regulatory role of HOMER3 in glioma cell proliferation and suggests potential pathways through which HOMER3 may exert its oncogenic effects.

Knockdown of HOMER3 suppressed the metastasis of glioma via Wnt/β-catenin/EMT pathway

To investigate the potential role of HOMER3 in the metastasis of glioma cells, we conducted transwell assays to assess the invasive and migratory capabilities of U251 and U87 cells following HOMER3 knockdown. We observed that the knockdown of HOMER3 significantly suppressed both the invasion and migration of these glioma cell lines, as evidenced in Fig. 6A and B. Our observation suggested that HOMER3 may play a crucial role in promoting glioma metastasis. To further elucidate the underlying mechanisms through which HOMER3 exerts its oncogenic functions in glioma progression, we performed correlation analyses using data from TCGA datasets. The findings revealed a positive association between HOMER3 expression and several critical biomarkers involved in the Wnt/β-catenin signaling pathway and EMT. Specifically, we identified that higher levels of HOMER3 were correlated with increased expression of key proteins, including β-catenin, E-cadherin, N-cadherin, Slug, Snail, Twist1, and Twist2 (Fig. 6C and D). These findings implied that HOMER3 may influence the activation of the Wnt/β-catenin/EMT signaling cascade, which is known to be pivotal in promoting metastasis in various cancers [32–34]. Moreover, we confirmed the association of HOMER3 levels with the expression of EMT markers through additional analyses (Fig. 6E). We used western blotting to determine how HOMER3 knockdown affected the expression of these important signaling molecules in order to provide protein-level support for our findings. Figure 6F and Figure S3 showed that when HOMER3 was knocked down, the expression of β-catenin, N-cadherin, Slug, Snail, Twist1, and Twist2 was all significantly reduced, whereas E-cadherin expression was increased. This shift in the expression of EMT-related proteins further supports the notion that HOMER3 is integral to the modulation of the Wnt/β-catenin/EMT pathway, thereby facilitating glioma cell metastasis. Taken together, these findings provide compelling evidence that HOMER3 not only promotes the proliferation of glioma cells but also plays a critical role in enhancing their metastatic potential via the Wnt/β-catenin/EMT signaling pathway.

Discussion

Gliomas, particularly glioblastoma multiforme (GBM), are among the most aggressive and deadly brain tumors [10, 35]. Most patients have a poor prognosis, with survival typically less than 15 months even after surgery, chemotherapy, and radiation therapy. Currently, the main prognostic markers for gliomas include MGMT promoter methylation, 1p/19q co-deletion, IDH mutation, ATRX loss, and TERT promoter mutation [36–38]. IDH mutation is generally associated with a better prognosis, especially in lower-grade gliomas, but its application in glioblastoma is limited. The 1p/19q co-deletion is predictive of better outcomes in oligodendrogliomas, but its use is also limited in scope [39]. MGMT promoter methylation can predict a better response to temozolomide chemotherapy, primarily in glioblastoma. ATRX loss is common in astrocytomas and indicates a better prognosis for IDH-mutant gliomas, while TERT promoter mutations are often associated with poorer outcomes, particularly in glioblastoma [40, 41]. Although these markers provide valuable information about prognosis and treatment response, their use is typically restricted to specific subtypes. Thus, it is necessary to identify more sensitive biomarkers for glioma patients.

HOMER3 is a member of the Homer protein family, which is primarily involved in regulating synaptic signaling and plasticity in the nervous system. It functions as a scaffold protein, interacting with various receptors and signaling molecules to facilitate communication between the cell surface and intracellular pathways [42–44]. HOMER3 has been studied in the context of neuronal function, where it plays a role in modulating calcium signaling and receptor clustering, particularly in excitatory synapses. Recent studies have begun to explore HOMER3's potential involvement in cancer. For example, in non-small cell lung cancer, Sun et al. found that HIGH expression of HOMER3 is associated with a bad prognosis. Lung cancer cell proliferation and metastasis are inhibited by mitochondrial malfunction, which is caused by low HOMER3 levels. One possible treatment for non-small cell lung cancer is mitochondrial targeting, which is regulated by HOMER3 and platelet-activating factor acetylhydrolase 1b catalytic subunit 3. GABPB1 is an important transcription factor for mitochondrial biogenesis [26]. According to research by Liu et al.,

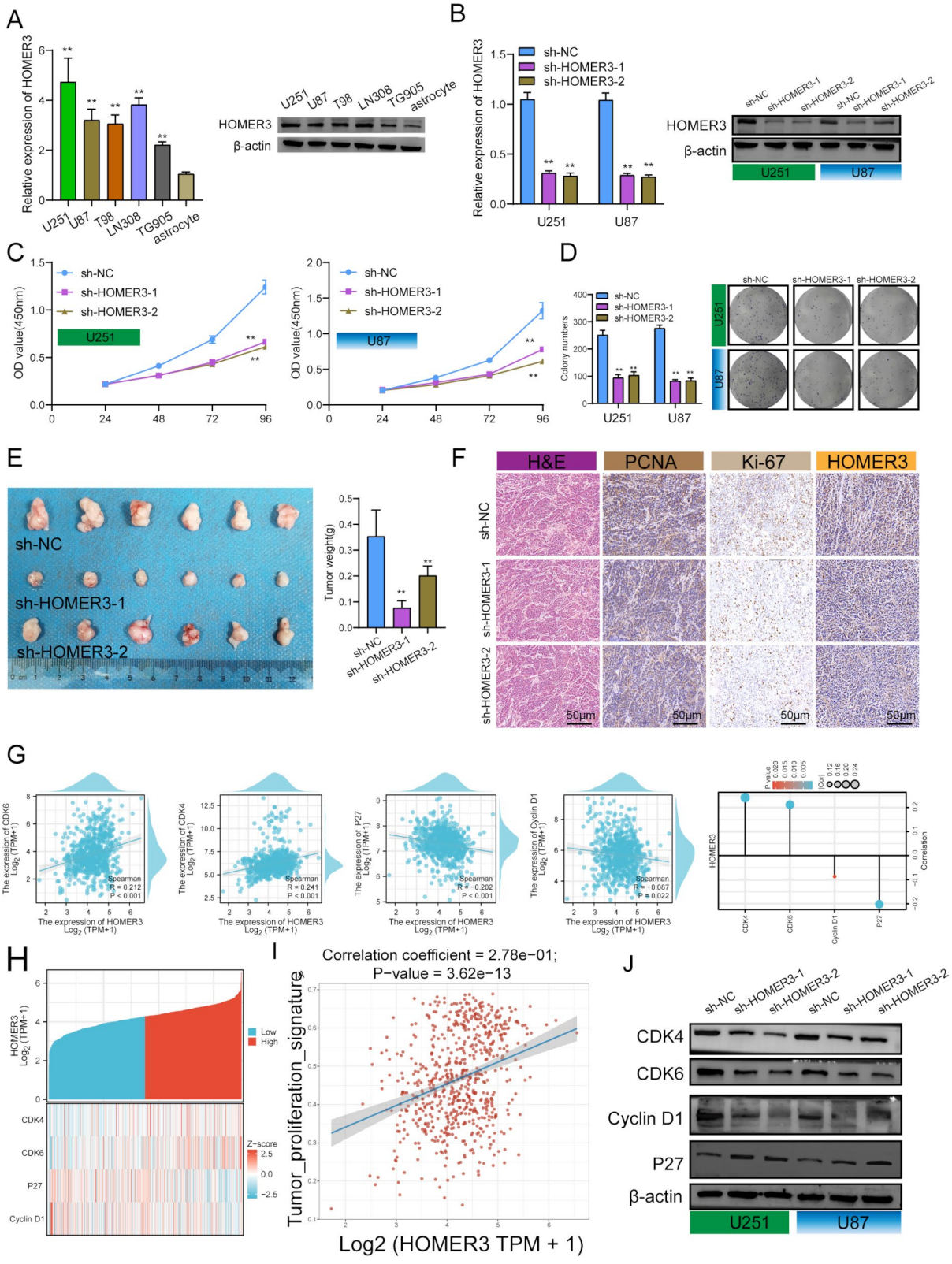

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Fig. 5 Impact of HOMER3 knockdown on glioma cell proliferation and cell proliferation regulators. (**A**) Elevated HOMER3 expression levels in five glioma cell lines compared to normal cells, confirmed by RT-PCR and western blot analysis. (**B**) Efficacy of HOMER3 knockdown using shRNA plasmids. (**C**-**D**) Cell proliferation assessment via CCK-8 assays demonstrating significant reductions in proliferation rates post-HOMER3 knockdown in U251 and U87 glioma cells. (**E**) In vivo effects of HOMER3 knockdown using a subcutaneous xenograft mouse model, illustrating reduced tumor size and weight compared to negative control groups. Each group contained $n=6$ mice, and the experiment was repeated three times independently. (**F**) IHC staining of Ki-67 and PCNA confirming diminished proliferation in HOMER3 knockdown tumors. (**G**-**I**) Correlation analyses revealing relationships between HOMER3 expression and key cell proliferation regulators, indicating HOMER3's influence on cell cycle progression. (**J**) Western blot analysis showing shifts in expression patterns of CDK4, CDK6, cyclin D1, and p27 following HOMER3 knockdown. All experiments were performed in three independent biological replicates ($n=3$) to ensure the reproducibility and reliability of the results

HOMER3 is associated with accelerated tumor spread and worse survival rates in triple negative breast cancer, which is characterized by overexpression of the gene. The nuclear translocation and activation of β-Catenin are promoted by HOMER3, which promotes the aggressiveness and metastasis of TNBC by facilitating c-Src-induced β-Catenin tyrosine phosphorylation. Based on these results, it seems that targeting HOMER3 could be a promising strategy for treating TNBC [24]. But how HOMER3 is expressed and what it does in gliomas is still not well understood. Our in-depth investigation in this work showed that HOMER3 is elevated in GBM and LGG samples, suggesting a possible function for it in glioma pathology. It has been found that higher levels of HOMER3 are associated with shorter OS and DSS in LGG patients, which implies that it may be an important biomarker for prognosis. By confirming HOMER3 as a separate prognostic factor, Cox assays further established its link to a more aggressive tumor phenotype. There was a correlation between advanced clinical stages, high HOMER3 levels, and a number of molecular and clinical variables, such as tumor grade and age. These findings emphasize the need for more research into the regulation of HOMER3 expression and its function in glioma biology and patient prognosis.

Transcription factors are a class of proteins that can bind to specific DNA sequences and are key regulators of gene expression [45–47]. They influence the recruitment and activity of RNA polymerase by interacting with regulatory elements such as promoters and enhancers, thereby promoting or inhibiting gene transcription. The function of transcription factors is typically regulated by various signals, including intracellular and extracellular signaling molecules, post-translational modifications, and interactions with other proteins [48, 49]. These regulatory mechanisms enable transcription factors to play important roles in cell differentiation, proliferation, and stress responses. In tumors, transcription factors affect tumor initiation and progression by regulating the expressions of genes related to cell growth, apoptosis, metabolism, and migration. Some transcription factors may act as oncogenic drivers, such as c-Myc and NF-κB, which are often overexpressed in tumor cells, enhancing cell proliferation and anti-apoptotic signaling [30, 50, 51]. Conversely, the inactivation or downregulation of certain transcription factors, such as the p53 transcription factor, is associated with tumor development, as their loss can lead to the failure of cell cycle regulation and DNA repair mechanisms, promoting tumor progression. Additionally, transcription factors play a crucial role in the tumor microenvironment, influencing the interactions between tumor cells and surrounding cells, which can impact tumor aggressiveness and metastatic potential. In this study, we found that ELK4 directly binds to the HOMER3 promoter and stimulates its expression in glioma cells, highlighting its crucial role in the dysregulation of HOMER3 in glioma. Elevated levels of ELK4 correlate with poorer overall survival in glioma patients, suggesting its potential as a prognostic marker. Functional assays demonstrated that knockdown of ELK4 significantly impairs glioma cell proliferation, migration, and invasion, indicating its importance in tumor aggressiveness. Furthermore, ELK4 knockdown alters the expression of cell cycle regulators and EMT markers, implicating its role in mediating these processes. In addition, we investigated the possible role of HOMER3 in the development of gliomas. Crucially, we found that glioma cell growth and metastasis were inhibited by HOMER3 knockdown. According to our research, glioma cell growth was aided by ELK4-induced overexpression of HOMER3.

When it comes to cancer and progression, the Wnt/β-catenin signaling pathway is extremely important, especially when it comes to EMT. This system controls the proliferation, migration, invasion, and stemness properties of tumor cells, which in turn significantly affects their behavior [52, 53]. The Wnt signaling pathway plays a pivotal role in regulating cell proliferation and differentiation under typical physiological settings. The activation of β-catenin's transcriptional activity occurs when Wnt ligands attach to Frizzled receptors on the surface of the cell. This enables β-catenin to enter the nucleus and interact with T-cell factor/lymphoid enhancer factor (TCF/LEF), which in turn promotes the production of genes associated with cell proliferation and survival [34, 54]. The EMT process, which involves a drop in intracellular E-cadherin levels and an increase in the expression of mesenchymal markers like N-cadherin and fibronectin, is strongly linked to the activation of the Wnt/β-catenin signaling pathway during tumor growth. This change makes tumor cells more capable of spreading from

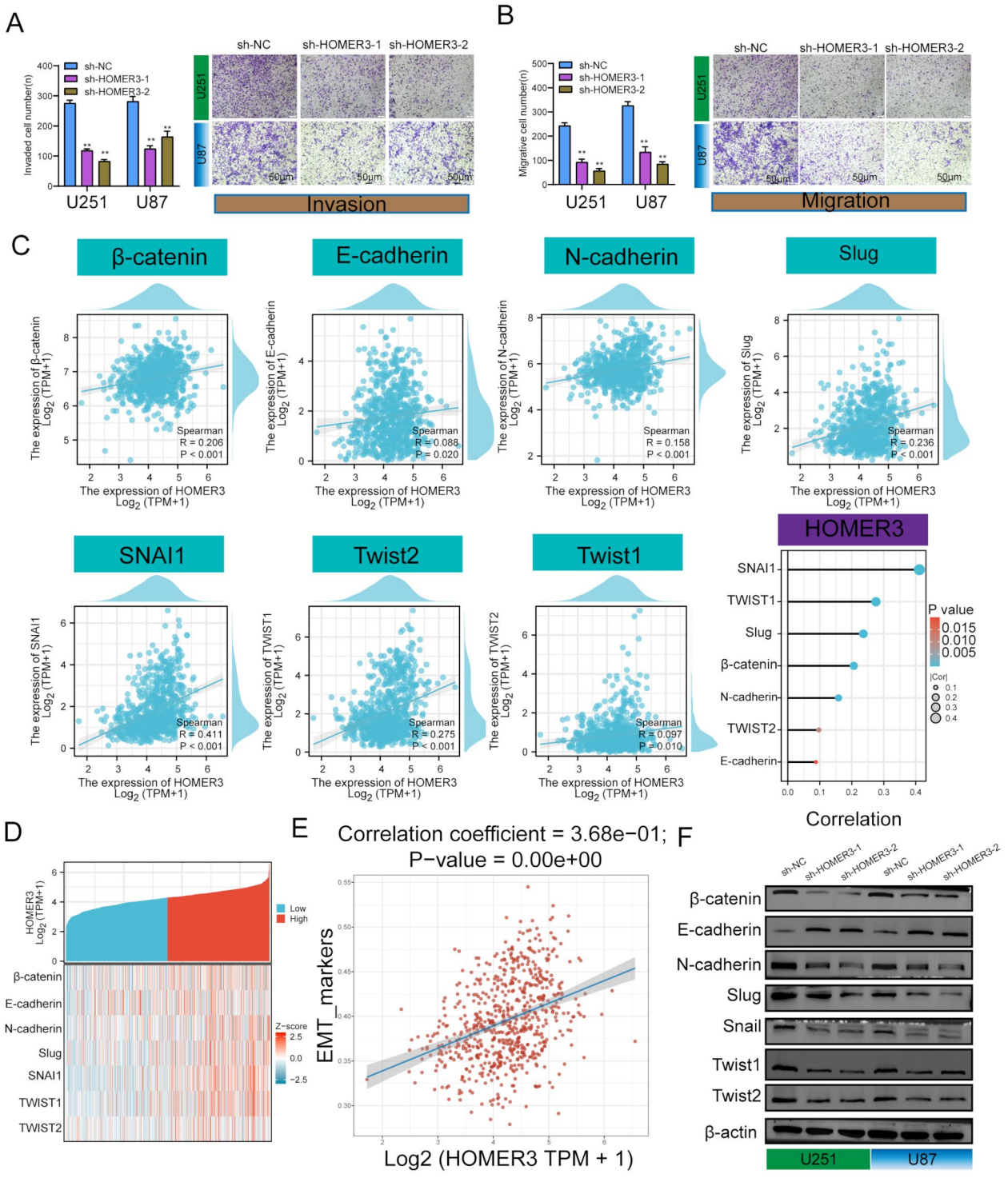

Fig. 6 Role of HOMER3 in glioma metastasis via the Wnt/β-catenin/EMT pathway. (**A**-**B**) Transwell assays assessing migration and invasion capabilities of U251 and U87 cells post-HOMER3 knockdown, revealing significant suppression of both. (**C**-**E**) Correlation analyses demonstrating associations between HOMER3 expression and key biomarkers in the Wnt/β-catenin signaling pathway and epithelial-mesenchymal transition (EMT). (F) Western blotting results confirming the impact of HOMER3 knockdown on the expression of β-catenin, N-cadherin, and EMT markers, reinforcing the role of HOMER3 in promoting glioma metastasis through the modulation of the Wnt/β-catenin/EMT pathway. All experiments were performed in three independent biological replicates ($n = 3$) to ensure the reproducibility and reliability of the results

their original sites to new locations, a process known as metastasis [34, 53, 55]. Moreover, this pathway's activation is associated with tumor stem cell feature maintenance, which in turn contributes to tumor resistance and recurrence. Our analysis of TCGA datasets revealed a strong correlation between HOMER3 expression and the expression of genes relevant to the Wnt/β-catenin signaling pathway, such as β-catenin, N-cadherin, Slug, Snail, Twist1 and Twist2. After that, we verified using western blotting that β-catenin, N-cadherin, Slug, Snail, Twist1 and Twist2 expression was reduced when HOMER3 was knocked down. Initiating the Wnt/β-catenin signaling pathway, our results indicated that HOMER3 aided in the advancement of glioma cells.

Conclusion

Our comprehensive cancer study reveals that LGG patients have a poor prognosis due to the dysregulation of HOMER family genes, especially HOMER3. Highlighting its potential as a predictive biomarker and therapeutic target in glioma, the overexpression of HOMER3 corresponds with aggressive tumor characteristics. In addition, we proved that ELK4 activates HOMER3 expression by binding directly to its promoter, thus linking HOMER3 to the growth and spread of glioma cells. By mainly modulating the Wnt/β-catenin/EMT pathway, the downregulation of HOMER3 considerably decreases glioma cell proliferation and invasiveness. The results of this study highlight the importance of HOMER3 in glioma biology and provide hope for a new approach to treating this disease by focusing on this pathway. The development of targeted treatments for glioma patients should be the focus of future research into understanding the molecular processes of HOMER3 regulation and its connections with other signaling pathways.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13062-025-00643-w.

Supplementary Material 1

Supplementary Material 2

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Not applicable.

Author contributions
Furong Wang, Haining Zhen and Li-wen Li developed a major research plan. Furong Wang, Hui Zhou and Yu Tian performed experiments analyze data and write manuscripts. Xiaoling Wang, Youcai Huang and Yanyang Tu helped collect data and references and provided technical support. All authors contributed to the article and approved the submitted version.

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Data availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The animal experiment was approved by the Animal Care Committee. All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Huizhou Central People's Hospital, Guangdong Medical University.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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