European Review for Medical and Pharmacological Sciences 2019; 23: 2476-2485

HOXB7 promotes proliferation and metastasis of glioma by regulating the Wnt/β-catenin pathway

X.-Y. HUO[1], X.-Y. ZHANG[2], F. YUAN[3], X.-Y. ZHAO[4], B.-A. YOU

[1]Heart Centre, Qilu Hospital of Shandong University (Qingdao), Qingd China
[2]Laboratory Medicine, Qingdao Oncology Hospital, Qingdao, China
[3]Department of Geriatric Neurology, Qilu Hospital of Shandong Univ (Qi Qing China
[4]Medical Examination Center, Qilu Hospital of Shandong Univers Qingdao), China

Abstract. – OBJECTIVE: The purpose of this study was to investigate the expression level of HOXB7 in gliomas and its effect on the proliferation and metastasis of gliomas, as well as its regulatory mechanism of promoting the malignant progression of glioma.

PATIENTS AND METHODS: In this st pairs of glioma tumor tissue specimens jacent ones were collected and the HOX x-pression levels in these tissues were det d using quantitative Real Time Polymerase C Reaction (qRT-PCR), and the interplay betwe HOXB7 level and clinical para of gliom was analyzed. QRT-PCR was her ver-ify the expression of HOXB gliom ll lines. The sh-HOXB7 knockdo model con-structed in glioma cell and t of HOXB7 on the bio gic of glioma cells was examined ell C Kit-8 (CCK-8) and transwell a Meanwhi stern blot was applied to e whether HO n pro-mote the progr lioma throug he Wnt/β-catenin pa y.

RESULTS: QRT-PCR showed that the level of B7 in glioma tissue speci-mens w onspicuously high than that in the adjac ormal s. The occurrence of lymph nod distan etastasis was higher and the pro worse atients with higher HOXB sion. I dition, compared with sh-NC ce liferation, invasiveness migrati of the sh-HOXB7 group dec ed co iously. Subsequently, the Wes blot resu revealed that the expression of in the Wnt/β-catenin signaling p onspicuously reduced in the sh-B7 group, thereby promoting the malignant ression of glioma.

CLUSIONS: HOXB7 may promote the in-vas ess and migration of glioma cells via reg-ing the Wnt/β- atenin signaling pathway, and onspicuousl sociated with lymph node or ant metastas nd poor prognosis.

Key
HO catenin signaling pathway, Glioma, Metastasis, Proliferation.

Introduction

Glioma is the most common primary malignant central nervous system tumor in adults, with prevalence of 40%-50% in all intracranial tumors[1-3]. Although the current treatment methods (surgery, radiotherapy, chemotherapy) are constantly improving, the efficacy has not been conspicuously improved[4,5]. However, the risk factors for its incidence have been studied intensively, including genetics, diet, unhealthy lifestyle and precancerous lesions. More than half of clinical glioma patients have undergone radical surgery; however, micrometastasis has emerged as a direct cause of postoperative metastasis and recurrence of gliomas[6,7]. The pathogenesis of glioma has not been fully elucidated, so the difficulties in its diagnosis and treatment are one of the important reasons for its high morbidity and mortality[7,8]. Therefore, elucidation of the molecular mechanism, prediction, diagnosis and prognosis of glioma metastasis and proliferation are important aspects of colorectal cancer research[9].

The Wnt/β-catenin signaling pathway is a highly conserved signal transduction pathway in the evolution of organisms, regulating and con-

Corresponding Author: Beian You, MD; email: youbeian@sina.com

trolling many life processes[10]. In the process of embryonic development, it does not only determine the growth and differentiation of cells, but also regulates the differentiation and formation of important organs such as cardiovascular and central nervous cells. *In vivo*, the Wnt/β-catenin pathway directly controls tumor cell proliferation, differentiation, polarization, apoptosis and anti-apoptosis[11]. The dysregulation of this pathway has been confirmed to be closely related to a variety of tumors, such as cervical cancer, breast cancer, melanoma, glioma, etc[12,13]. The abnormality in this pathway can cause an accumulation of intracellular β-catenin, which can enter the nucleus and regulate downstream gene expression, thereby promoting tumorigenesis[14,15].

HOXB7 belongs to the homeobox gene HOX family. The homeobox gene was first discovered in *Drosophila* and is a type of gene that plays a pivotal role in the embryonic development and cell differentiation of animals including humans. Evidence suggests that disorders in HOX gene expression may induce the occurrence of lots of tumors[15,16]. It is known to be abnormally expressed in tumors such as pancreatic and colorectal c r and is associated with tumor grading a rentiation[17-19]. In recent years, researchers[1 ve analyzed the role of HOXB7 in tumors and that HOXB7 is involved in tumor cell prolifera and invasiveness, and its expression is closely ass ciated with clinicopathological

Therefore, this study sep y ill ated the possible role of HOXB7 he pro sion of glioma through the activ f the in pathway and explo its regulation mechanism to brin w ideas e diagnosis and treatment of a.

Patients and Methods

Patients and Glioma Samples

Tu and p ncerous tissues from 32 patie ho u went ma radical resection were None patients received any radiother ch herapy before surgery. tholog ification and staging criteria glioma performed according to the Inte onal Union against cash-NCer (UICC) gli criteria. Patients and their fami- n this study have been fully informed and d informed consent. This study was appro- the Ethics Committee of Qilu Hospital of Shan g University.

Cell Lines and Reagents

The human glioma cell lines (U T98-G, A172) and HEB, the hum am mal glial cell, were purchased f the Ameri- can Type Culture Collection (Manassas, VA, USA). High glucose Dulbe Modified Eagle Medium (DMEM) dium a l bo vine serum (FBS) wer rchased fr Technologies (Gaithe g, M USA). were cultured in hi ucose EM medium containing 10% F 3 Incuba with 5% CO_2.

Transfecti ssay

Negativ (sh-NC) and RNA containing HO inte e sequence (sh-HOXB7) were purchased fro ghai Jima Company (Sh China). The were seeded in a 6 plate and grown to a cell density of 70%; siRNA tran fection was conducted using ofectamine 2 (Invitrogen, Carlsbad, CA,). After that cells were collected 48 h late quantita Real Time-Polymerase Chain Reac R) analysis and function experiments.

iferation Assay

The roliferation of the three cell lines was examined using the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan). The main steps were as follows: first, 100 μL of cell suspension was added (containing 2000 cells) in each well, then 10 μL of CCK-8 solution was added and the incubation continued for 1 h in the cell culture incubator. After that, a microplate reader was used to analyze cell proliferation at 450 nm. Wells containing the corresponding amount of cell culture medium and CCK-8 solution but no cells were considered as a blank control.

Transwell Assay

Cells were seeded in a 6-well plate when the density reached to 3 × 10^5/well. Liposomal transfection experiment was performed when cell fusion reached 80%. Positive clones were selected for expanded culture for subsequent transwell experiment. The specific steps were as follows: first, the Matrigel and the serum-free medium were diluted as 1:100, and the dilution was used to soak the transwell chamber. 100-mesh diluted Matrigel was added in the chamber, and the whole device was placed on a clean bench and sterilized by ultraviolet ray overnight before proceeding to the

next experiment. Subsequently, 200 μL was added to the upper chamber, and 600 mL of serum-free medium was added to the lower chamber to balance the pressure. After the invading chamber was taken out, the cells on the membrane were fixed with absolute ethanol and stained with crystal violet staining. Tumor cells that did not invade the stroma were gently wiped off with a cotton swab, and those had successfully invaded the Matrigel were retained and counted under a high-power microscope (x 200). The number of invading cells in 10 high power fields was counted, and the count was repeated three times.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was performed to analyze the mRNA levels of HOXB7, β-catenin and β-actin in glioma tissues and cells. Total RNA was extracted in one step using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse transcribed into the first strand of complementary deoxyribose nucleic acid (cDNA) using Primescript RT R[illegible]t (TaKaRa, Otsu, Shiga, Japan) reverse t[illegible] tion kit, and primers were designed by Pri[illegible]5.0 software. The qRT-PCR reaction was perfo[illegible]d using SYBR® Premix Ex Taq™ (TaKaRa, O[illegible] Shiga, Japan) and StepOne Plus R[illegible]l Time-PC[illegible] System. The following primers[illegible]igned fo[illegible] qRT-PCR reaction: HOXB7[illegible] 5'-A[illegible]ACCC-CTGGATGCGAAGCT-3'[illegible] 5'-C[illegible]TCAG-GTAGCGATTGTAGTG[illegible] β-c[illegible] 5'-AACGTCTTCTCC[illegible]TC[illegible]R: 5'- CCA-CAGCAGCAGAA[illegible]'; β-a[illegible] 5'-AAG-GTGAAGGTCGG[illegible]TCAA-3', R[illegible]ATGA-AGGGGTCAT[illegible]3'. Each sa[illegible]e was subjected to a[illegible]e-he[illegible]ated experiment and repeated tw[illegible]e. The Bio-R[illegible]R instrument was used to a[illegible]ze and process[illegible]ata (Bio-Rad, Hercul[illegible]A, USA). The β-acti[illegible] and U6 genes were[illegible]d as i[illegible]al parameters, and the gene exp[illegible]n wa[illegible]culated[illegible] the $2^{-\Delta\Delta Ct}$ method.

Wester[illegible]t

[illegible] tissue[illegible] to be analyzed were collect[illegible] The lysa[illegible]e-cooled on the ice was added[illegible]he cell or tissue samples, shaken, mixed vi[illegible] then placed on the ice for clea[illegible] for 30 min. The protein concentration was [illegible]rmined by the bicinchoninic acid (BCA) [illegible] (Pierce, Waltham, MA, USA). The protein [illegible]mple was denatured in a water bath at 100°C for 5 min, and an appropriate amount of the loading buffer was applied. Afte[illegible] parated through sodium dodecyl s[illegible]te (SD[illegible] gel electrophoresis, the protein [illegible] transferred to polyvinylidene difluoride ([illegible] Millipore, Billerica, MA, USA) *via* wet t[illegible] method, and 5% skim milk was u[illegible]d to blo[illegible] pro[illegible] tein for 1 hour. After t[illegible] the corres[illegible] primary antibodies w[illegible] added[illegible]nd incu[illegible] in the refrigerator a[illegible] over[illegible]. After being washed with Tris-[illegible]ed[illegible]ne an[illegible]ween (TBST; Sigma-[illegible]ric[illegible]ouis, [illegible] USA) 3 times the n[illegible]ay, the [illegible]po[illegible]g secondary antibo[illegible](1:1000) wer[illegible], and after 2 hours o[illegible]n at room te[illegible]perature, the protein [illegible] dev[illegible]d with enhanced chemiluminescence (ECL, [illegible]mo Fisher Scientific, Wa[illegible]MA, USA).

[illegible]tistical Anal[illegible]is

[illegible]tatistical ana[illegible]s was performed using Stati[illegible]l Product an[illegible]ervice Solutions (SPSS) 22.0 s[illegible]al softw[illegible] (IBM, Armonk, NY, USA). The [illegible]ed to compare the measurement data, and the categorical variables were analyzed [illegible]ing χ^2-test or Fisher's exact probability method. [illegible] analysis was performed using the [illegible]plan-Meier method and survival curves were plotted. Data were expressed as mean ± standard deviation ($\bar{x}\pm s$), and p-values < 0.05 were considered statistically significant.

Results

HOXB7 Had a High Level in Glioma Tissues and Cell Lines

HOXB7 level was conspicuously elevated in glioma tissues compared to paracancerous tissues, and the difference was statistically significant (Figure 1A). In addition, compared with HEB, HOXB7 was conspicuously expressed in glioma cell lines, especially in U251 and U87 cells, so we chose these two cells for subsequent experiments (Figure 1B).

HOXB7 Expression was Correlated with Lymph Node and Distant Metastasis in Glioma

According to 32 pairs of glioma tumor tissues and paracancerous tissues, the relationship between HOXB7 expression and age, sex, pathological stage, lymph node metastasis and distant metastasis of glioma patients was analyzed. As

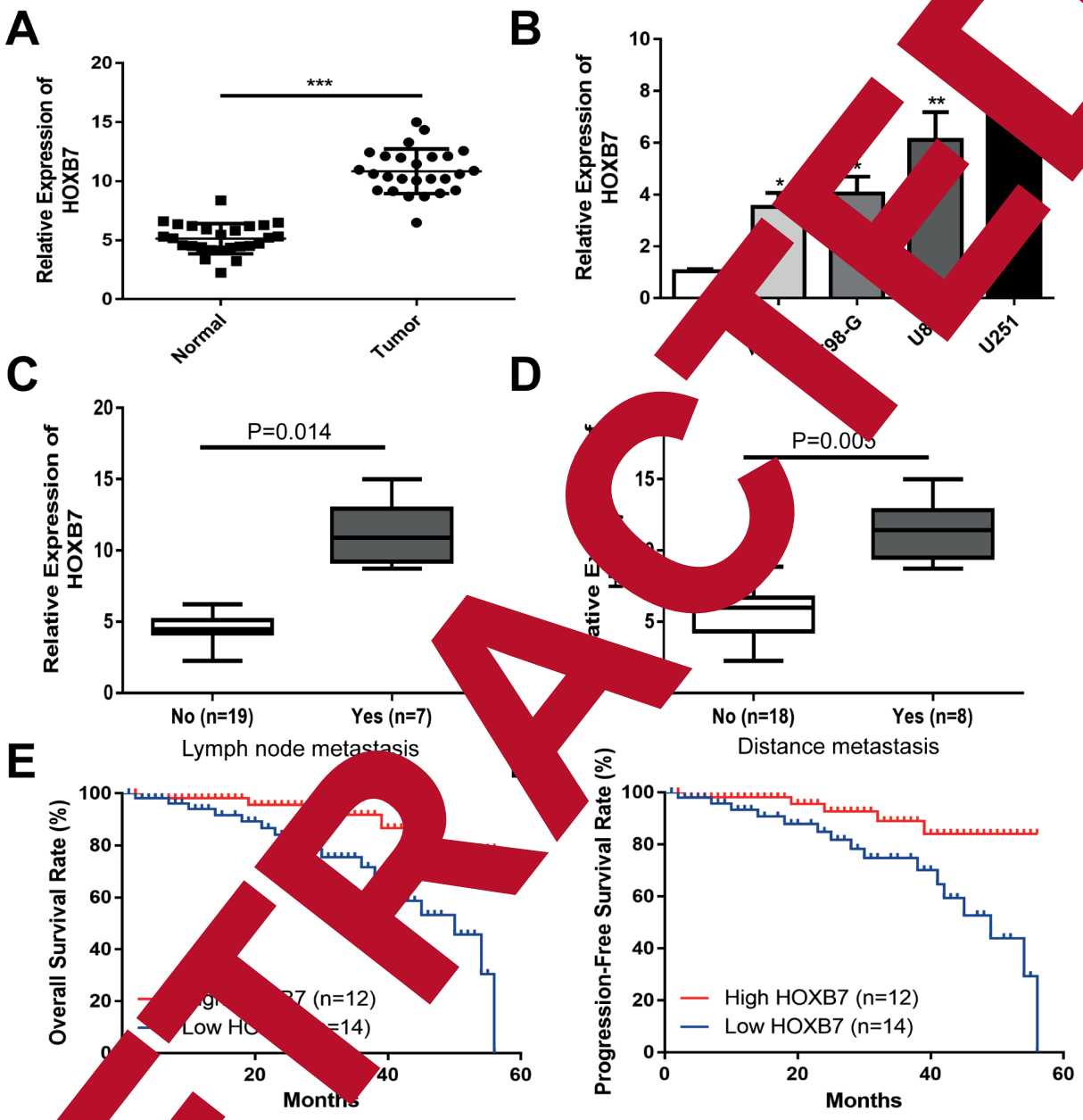

Fig HOX highly ssed in glioma tissues and cell lines. ***A,*** qRT-PCR was used to detect the HOXB7 expression in glio sues and cent tissues. ***B,*** qRT-PCR was used to detect HOXB7 expression levels in glioma cell lines. ***C,*** qRT-PCR to de fferential expression of HOXB7 in glioma tumor tissues with or without lymph node metastasis. PCR ect differential expression of HOXB7 in glioma tumor tissues with or without distant metastasis. ***E,*** erall su ve of the Kaplan-Meier in patients with glioma was shown based on the HOXB7 expression. ***F,*** The prog n-free surv l curve of the Kaplan-Meier was analyzed in patients with glioma based on HOXB7 expression and the patients with high expression was significantly worse than that of the low expression group. Data are mean ± SD 01, ***$p<0.001$.

shown in Table I, low expression of HOXB7 was positively correlated with glioma lymph node metastasis and distant metastasis, but not with age, gender and pathological stage (Figure 1C and 1D). In addition, to explore the interplay between the expression of HOXB7 and the prognosis of patients with glioma, we collected relevant follow-up data. The Kaplan-Meier survival curves showed that low expression of HOXB7 was conspicuously associated with overall survival rate and disease-free survival time ($p<0.05$; Figure 1E and 1F).

Knockdown of HOXB7 Inhibited Cell Proliferation, Migration and Invasiveness

To explore the impact of HOXB7 on the function of glioma cells, we first successfully constructed the sh-HOXB7 expression model and verified it by qRT-PCR (Figure 2A). We then performed cell proliferation, invasion and migration experiments in the U251 and U87 cell lines, respectively. It was found that the proliferation of cells in the sh-HOXB7 group was conspicuously decreased according to the CCK-8 assay, and the difference was statistically significant (Figure 2B). In addition, we used the transwell assay to explore the role of HOXB7 in the migration and invasiveness of glioma cells. The results showed that the number of transmembrane glioma cells in the transwell chamber of the sh-HOXB7 group was conspicuously smaller than that of the NC group, suggesting that sh-HOXB7 inhibited the cell invasive ability (Figure 2C).

Knockdown of EHMT2 Changed the Activation of the Wnt/β-Catenin

To further explore how HOXB7 promotes malignant progression of glioma, we examined the key proteins in the Wnt/β-catenin pathway and found by Western blot that the expression of β-catenin, CTNNB1, SOX9, CCND1, CCND2, C-MYC decreased conspicuously after knockdown of HOXB7 (Figure 3A).

β-Catenin was Lowly Expressed in Glioma Tissues and Cells

Subsequently, we found through bioinformatics research that HOXB7 and β-catenin may have some relation with glioma. The expression level of β-catenin was found markedly increased in glioma tumor tissue specimens compared to the adjacent tissues (Figure 3B). In addition, β-catenin was conspicuously higher in glioma cell lines than in NHA, and the difference was statistically significant (Figure 3C). Therefore, we examined HOXB7 and β-catenin levels in 32 glioma tumor tissues and in cell lines by qRT-PCR, and found that these two showed positive correlation (Figure 3D).

HOXB7 Modulated β-Catenin Expression in Glioma Cells

To figure out the interplay between HOXB7 and β-catenin, we constructed and transfected a β-catenin knockdown vector into a tumor cell line based on sh-HOXB7 transfection and qRT-PCR was performed to verify the expression efficiency (Figure 4A). Subsequently, the β-catenin protein

Table I. Association of HOXB7 expression with clinicopathologic characteristics of glioma.

Parameter	Number of cases	HOXB7 expression		*p*-value
		Low (%)	High (%)	
Age (y				0.716
<60	14	8	6	
≥6	12	6	6	
G				0.716
Ma	12	6	6	
Female	14	8	6	
e				0.793
	18	10	8	
T	8	4	4	
Ly metastasis				0.014
	19	13	6	
s	7	1	6	
stance metastasis				0.005
	18	13	5	
	8	1	7	

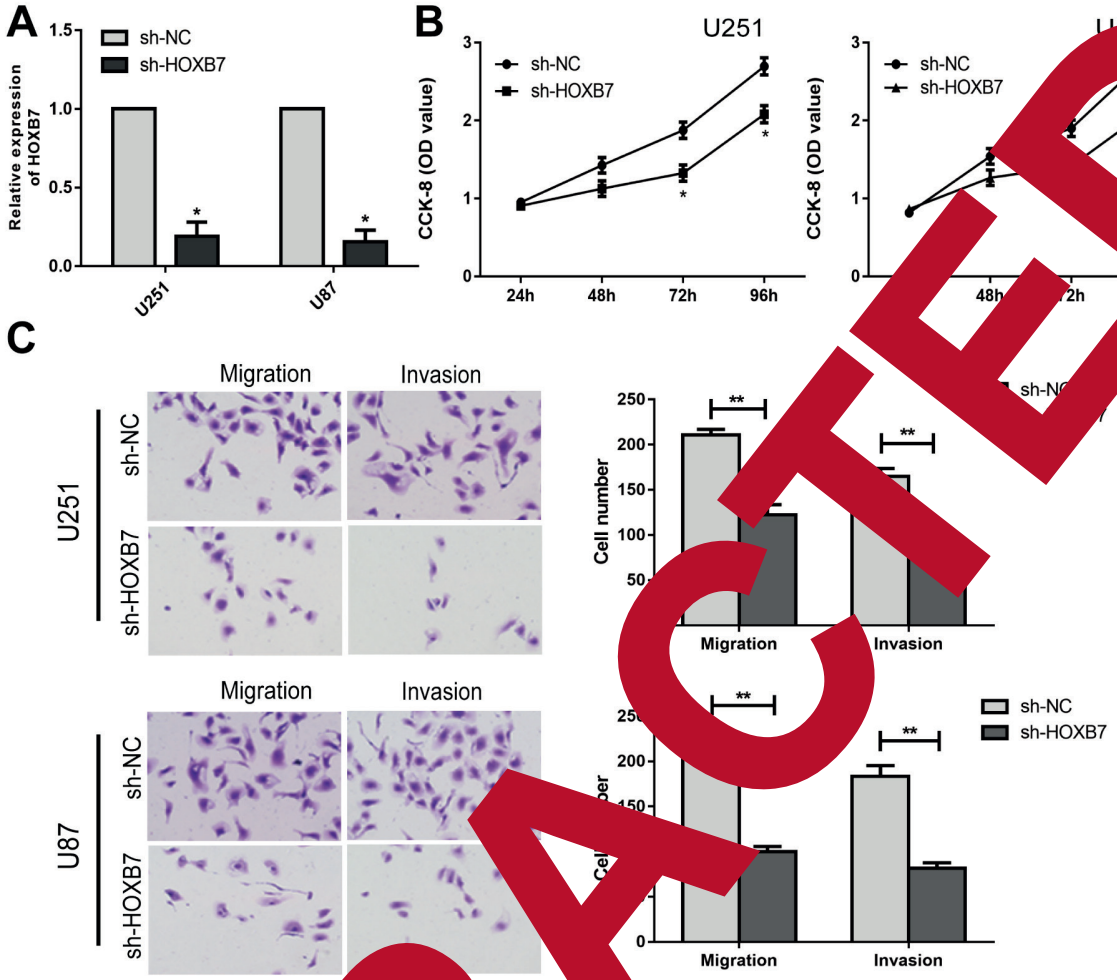

Figure 2. Silencing HOXB7 in[illegible] glioma[illegible] proliferation as well as invasion and migration. ***A,*** qRT-PCR verified the interference efficiency of HOX[illegible]fter trans[illegible]HOXB7 in U251 and U87 cell lines. ***B,*** The CCK-8 assay revealed that the proliferation of cells in[illegible]HOX[illegible]nspicuously decreased. ***C,*** The transwell migration invasion assay detected that sh-HOXB7 i[illegible]bited[illegible]n and migration ability of glioma cells in U251 and U87 cell lines (Magnification: 40 ×). Data are me[illegible]SD, *p<[illegible]

expression [illegible]as examined[illegible]stern blotting (Figure 4B)[illegible] proliferation an[illegible]ation were detected [illegible]CK-8 and transwell migration assays. The [illegible]ts sho[illegible]that the invasiveness and migra[illegible]bility[illegible]he sh-H[illegible]XB7 and the sh-β-catenin g[illegible]lower [illegible]that of the sh-HOXB7 [illegible]and the si[illegible]roup[illegible]ure 4C).

Discussion

[illegible]ioma is the most common primary intracra-[illegible]tumor, with multiple invasive growth and un-c[illegible]rders. Tumor cells usually grow in brain tissue [illegible]in 2 cm of the tumor, and the pathological grade is progressively worse[3]. Molecular genetics and epigenetics have always been the research hotspots of tumors. The establishment of new disciplines such as tumor molecular epidemiology and molecular pathology has further revealed the incidence, development, proliferation and invasiveness of central nervous system tumors. The molecular mechanism of neovascularization also opens the prelude to molecularly targeted therapy for glioma[4-7]. Among them, molecular targeted therapy is developing from the initial single target inhibition to multi-target therapy, and the interaction between multi-target cell signaling pathways and bypass activation has become a research hotspot in the field of targeted therapy[7,8].

The HOXB7 gene is a class of evolutionarily highly conserved DNA sequences with a conserved sequence of 183 nucleotides in length encoding a homeodomain (HD) consisting of 61 amino acids[16,17]. The region is folded into three helical structures containing an amino-terminal arm and passed through the helix between the second and third helices. The turn-helix module binds specifically to the DNA sequence of the target gene, and recognizes a 10-12 bp DNA sequence centered at 5'-TAAI, -3'[17]. In this study, we focused on the effects of HOXB7 on the biological function of glioma cells. The results showed that HOXB7 was conspicuously up-regulated in gliomas, suggesting that HOXB7 has a potential cancer-promoting effect in gliomas. To further figure out the role of HOXB7 in the progression of glioma, we used RT-PCR to detect the expression of HOXB7 in 32 glioma tissues and its adjacent paracancerous t found that HOXB7 expression in was conspicuously higher than t cent tissues and was positive lymph node and distant metastasi believe that HOXB7 may p glioma. Tumor metastasi tumor cells are scatte distal target organ microenvironment. of HOXB7 on t we constructe virus. The r migration mote th and plays a pivotal r mo

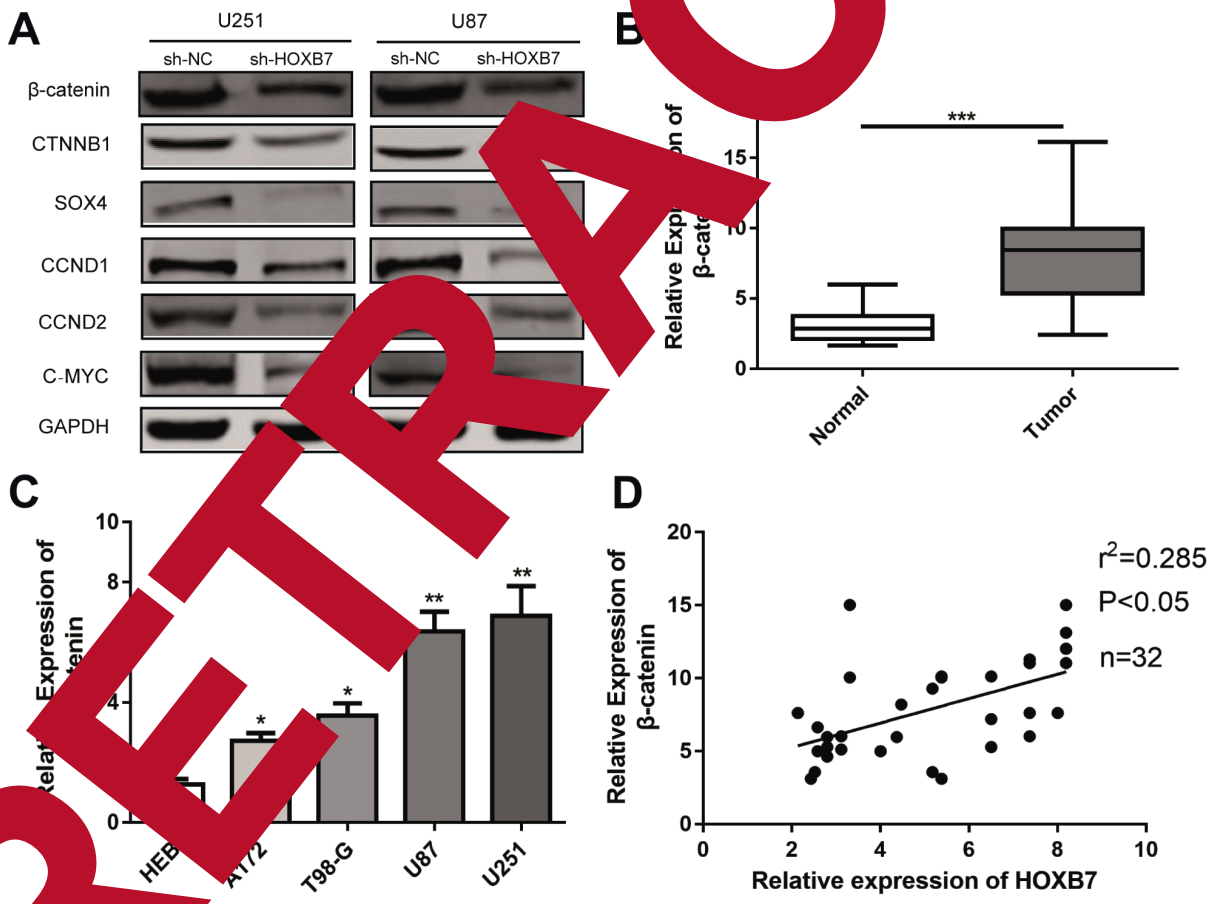

Figure 3. Silencing HOXB7 downregulates the expression of the Wnt/β-catenin signaling pathway in gliomas. ***A,*** Western blot verified the expression levels of β-catenin, CTNNB1, SOX4, CCND1, CCND2, C-MYC after transfection of sh-HOXB7 in U251 and U87 cell lines. ***B,*** qRT-PCR detected differential expression of β-catenin in glioma tumor tissues and adjacent tissues. ***C,*** qRT-PCR detected β-catenin expression in glioma cell lines. ***D,*** The expression of HOXB7 was significantly positively correlated with β-catenin in glioma tissues. Data are mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

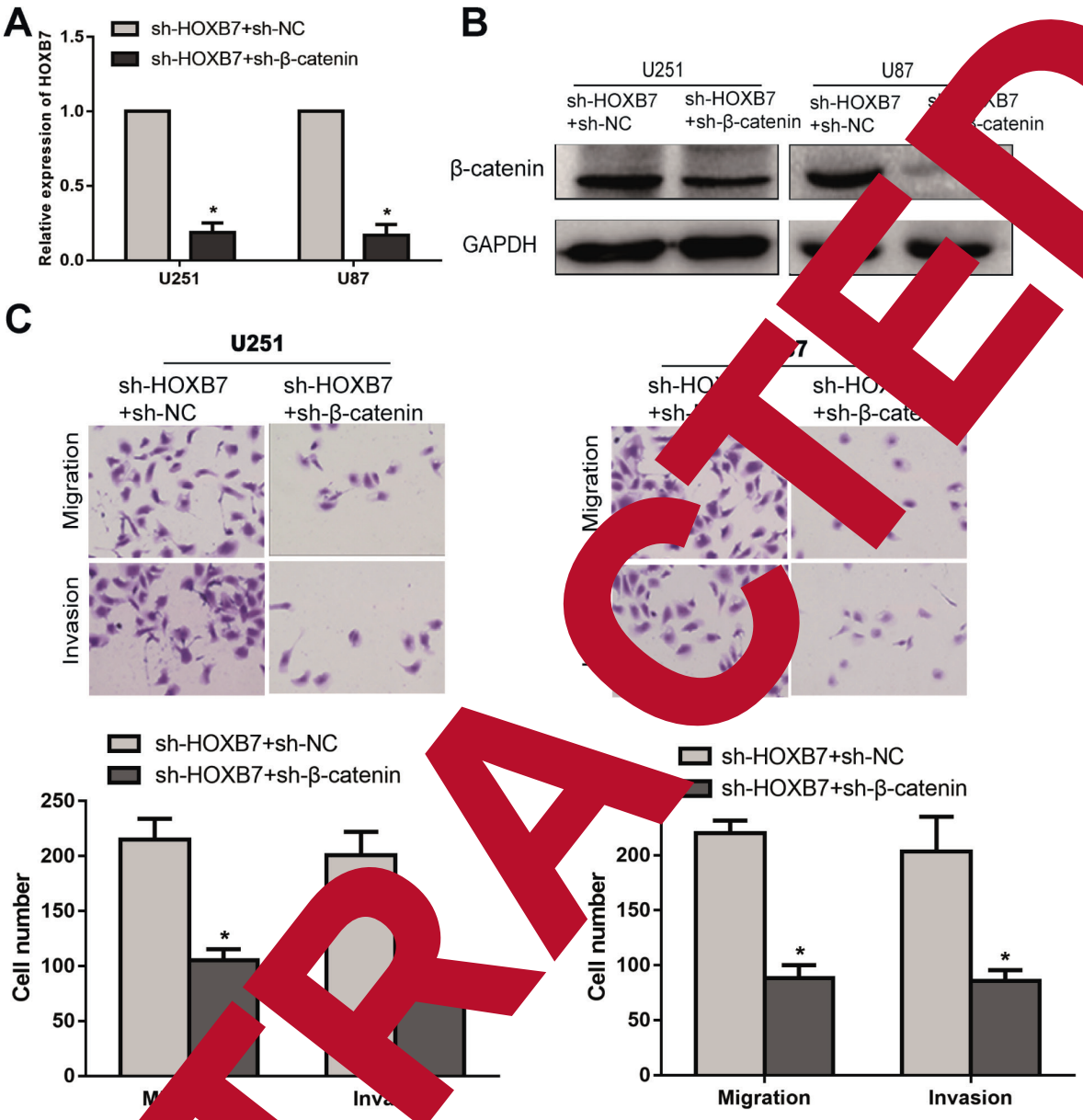

Figure 4. HOX[illegible] regulate[illegible] pression of β-catenin in glioma tissues and cell lines. ***A,*** qRT-PCR was used to detect HOXB7 exp[illegible]on levels in cel[illegible]-transfected with HOXB7 and β-catenin. ***B,*** Western blot was used to detect β-catenin expressio[illegible] in cell lines co-tra[illegible]d with HOXB7 and β-catenin. ***C,*** The transwell migration assay was used to analyze the role [illegible]OXB7 a[illegible]d β-catenin in the regulation of invasion and migration of glioma cells (Magnification: 40×). Data are mean[illegible], *#$p<0$

The W[illegible]nalin[illegible]thway includes at least [illegible]athwa[illegible]nonical pathway, the Wnt/β-[illegible]nin pati[illegible], and two non-canonical pathw[illegible] There is no Wnt signal in normal matur[illegible] the β-catenin in the cytoplasm [illegible]s to E-cadherin on the cell membrane, and a [illegible]ll part binds to APC, Gsk-3p and Axin in the c[illegible]m. After the complex forms, Gsk-36 can degr[illegible] β-catenin by phosphorylation, so the level of free β-catenin in the cytoplasm is extremely low, and does not enter the nucleus to regulate the expression of the corresponding genes[10-12]. The Wnt signal inhibits the activity of Gsk-3p binding to Axin by transmitting a signal to the intracellular scattered protein Dsh (disheveled Dsh/Dvl) by binding to the specific membrane receptor Fz, so that β-catenin cannot be phosphorylated and degraded and accumulates in the cytoplasm

and enters the nucleus. In the nucleus, β-catenin binds to T cell transcription factor TCF/NGer factor LEF, and activates Cyclin D1 and C-myc gene expression to promote cell proliferation[13-15]. The uncertainty of the Wnt activation pathway and the specificity of its receptor protein increase the difficulty of studying the Wnt signaling pathways[15]. Abnormalities in the Wnt/β-catenin signaling pathway are closely related to the development of multiple tumors[12,13]. However, the molecular mechanism by which β-catenin enters the nucleus to activate downstream target genes in the Wnt signaling pathway remains unclear. Some studies[11,12] have shown that β-catenin shuttles from the cytoplasm into the nucleus by direct reaction with nuclear complexes. There are also studies[15] showing that TCF/LEF and β-catenin act together to activate Pygopus/bcl9 and then mediate β-catenin entry into the nucleus. Therefore, we focused on the role of HOXB7 in activating the Wnt/β-catenin pathway to promote the development of glioma. To demonstrate whether HOXB7 promotes the development of glioma by regulating the Wnt/β-catenin pathway, we examined the expression changes of key proteins inc[illegible] β-catenin, CTNNB1, SOX4, CCND1, C[illegible] and C-MYC after knockdown of HOXB7 [illegible] western blot. The result suggested that HOXB[illegible] indeed promote the proliferation and metast[illegible] of glioma *via* activating this pathway.

Subsequently, to figure ou[illegible] HOXB7 promotes the development of [illegible]ma b[illegible]ulating β-catenin, we analyzed th[illegible]catenin [illegible]ression after silencing HOXB7 [illegible]cater[illegible] revealed that HOXB7 [illegible]an p[illegible]roliferation and metastasis of g[illegible]a throu[illegible] β-catenin. In addition, our [illegible]lts showed [illegible]ilencing β-catenin could [illegible]he biological [illegible]avior of glioma after [illegible]kdo[illegible] HOXB7, suggesting that HOXB7 may promote [illegible]alignant progression of gl[illegible]a by regulating p[illegible]in.

Conclusions

We fou[illegible]t HO[illegible] was greatly associated [illegible]mph [illegible]stasis and distant metastasis a[illegible]poor prog[illegible]s. In addition, it may inhibit the [illegible]sion and migration of glioma by regulati[illegible]atenin signaling pathway.

Conflict of Interest

The A[illegible]ors declare that they have no conflict of interest.

References

1) Hadjipanayis CG, Stummer W. 5-ALA [illegible] proval for glioma surgery. J Neur[illegible]ol 2019; 14[illegible] 479-486.
2) Ma Q, Long W, Xing C, Chu J, [illegible] Wang HY, Liu Q, Wang RF. Cancer stem cell[illegible]mmuno-suppressive microenvir[illegible]ent in gl[illegible]ro Immunol 2018; 9: 292[illegible]
3) Yuan X, Liu D, Wan[illegible] X. Sig[illegible]nce of nu[illegible]ar magnetic reso[illegible]e comb[illegible] with Ki-67 and VEGF detection in [illegible]n[illegible]nd proc[illegible]s evaluation of brai[illegible]oma[illegible] 2018; [illegible]0-415.
4) Uhm JH, P[illegible]AB. Treatm[illegible]li[illegible]n the 21st century: [illegible]xciting decade[illegible]ur-gical treatment[illegible] in the molecu[illegible]ra. Mayo Clin Proc[illegible]; 92[illegible]004.
5) Chen KQ, Liu F, C[illegible] Chen XQ. The prognostic [illegible]rapeutic valu[illegible]-L1 in gli-oma. Front [illegible]acol 2018; 9: 150[illegible]
6) Ameratunga M, Pavlakis N, Wheeler H, Grant R, Simes J, Khasra[illegible] Anti-angiogenic therapy for high-grade glio[illegible] Cochrane Database Syst Rev [illegible]018; 11: CD[illegible]18.
7) [illegible] Antonakis DG. Molecular pathoge[illegible]grade glioma. Neurosurg Clin N Am 2019; 30: 17-25.
8) Mao F, Wang B, Xiao Q, Cheng F, Lei T, Guo D. LRIG[illegible]s in glioma: functional roles, molecular me[illegible]chanisms, and potential clinical implications. J Neurol Sci 2017; 383: 56-60.
9) Lu HW, Liu XD. UCA1 promotes papillary thyroid carcinoma development by stimulating cell proliferation via Wnt pathway. Eur Rev Med Pharmacol Sci 2018; 22: 5576-5582.
10) Dunach M, Del Valle-Perez B, Garcia de Herreros A. p120-catenin in canonical Wnt signal-ing. Crit Rev Biochem Mol Biol 2017; 52: 327-339.
11) Duan P, Bonewald LF. The role of the wnt/beta-catenin signaling pathway in formation and maintenance of bone and teeth. Int J Biochem Cell Biol 2016; 77: 23-29.
12) Ma S, Lei Y, Zhang L, Wang J. Research on the inhibiting effect of tanshinone IIA on colon cancer cell growth via COX-2-Wnt/beta-catenin signaling pathway. J BUON 2018; 23: 1337-1342.
13) Hu XY, Hou PF, Li TT, Quan HY, Li ML, Lin T, Liu JJ, Bai J, Zheng JN. The roles of Wnt/beta-catenin signaling pathway related lncRNAs in cancer. Int J Biol Sci 2018; 14: 2003-2011.
14) Cheng X, Xu X, Chen D, Zhao F, Wang W. Therapeutic potential of targeting the Wnt/beta-catenin signaling pathway in colorectal cancer. Biomed Pharmacother 2019; 110: 473-481.
15) Fan X, Huang X, Li Z, Ma X. MicroRNA-372-3p promotes the epithelial-mesenchymal transition in breast carcinoma by activating the Wnt pathway. J BUON 2018; 23: 1309-1315.

16) Errico MC, Jin K, Sukumar S, Care A. The widening sphere of influence of HOXB7 in sol-id tumors. Cancer Res 2016; 76: 2857-2862.

17) Tsuboi M, Taniuchi K, Shimizu T, Saito M, Saibara T. The transcription factor HOXB7 regulates ERK ki-nase activity and thereby stimulates the motility and invasiveness of pancre-atic cancer cells. J Biol Chem 2017; 292: 17681-17702.

18) Stiegelbauer V, Vychytilova-Faltejskova P, Karbiener M, Pehserl AM, Reicher A, Resel M, Heitzer E, Ivan C, Bullock M, Ling H, Deutsch A, Wulf-Goldenberg A, Adiprasito JB, Stoeger H, Haybaeck J, Stotz M, Hoefler G, Slaby O, Calin [illegible] Pichler M. miR-196b-5p regulates colorectal cancer cell migration and metastasis through in-teraction with HOXB7 and GA[illegible] Clin Cancer Res 2017; 23: 5255-5266.

19) Joo MK, Park JJ, Chun [illegible]mpact of [illegible]bo genes in gastrointestinal cancer. World J Gastroenterol 2016; 22: 82[illegible]56.