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Transcription factor YY1-activated GNG5 facilitates glioblastoma cell growth, invasion, stemness and glycolysis through Wnt/β-catenin pathway

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G protein subunit Gamma 5 (GNG5) has been found to be involved in regulating glioma progression. However, its function and mechanism in glioblastoma (GBM) progression need to be further elucidated. GBM cell proliferation, apoptosis, invasion and stemness were assessed by cell counting kit 8 assay, EdU assay, flow cytometry, transwell assay and sphere formation assay. The mRNA and protein levels of GNG5 and Yin Yang 1 (YY1) were determined by quantitative real-time PCR and western blot (WB). Detection of the glucose consumption, lactate production and ATP/ADP ratios were used to assess cell glycolysis. Besides, Wnt/β-catenin pathway-related protein levels were examined by WB. Mice xenograft model was also constructed to explore GNG5 roles in vivo. GNG5 was highly expressed in GBM, and its silencing inhibited GBM cell proliferation, invasion, stemness and glycolysis, while promoted apoptosis. Transcription factor YY1 could bind to the GNG5 promoter region and induce its expression. GNG5 overexpression reversed the inhibitory effects of YY1 silencing on GBM cell growth, invasion, stemness and glycolysis. YY1/GNG5 axis could activate the Wnt/β-catenin pathway, and Wnt/β-catenin pathway agonists SKL2001 could revert the effects of GNG5 silencing on GBM cell progression. Furthermore, GNG5 facilitated GBM tumor growth by mediating the Wnt/β-catenin pathway. YY1-mediated GNG5 promoted GBM progression through the Wnt/β-catenin pathway.

Keywords Glioblastoma, GNG5, YY1, Wnt/β-catenin

Glioma is a common tumor originating from glial cells[1,2], in which glioblastoma (GBM) is the most malignant and aggressive type[3]. There are no known causative factors for GBM other than genetics and radiation exposure[4]. The heterogeneity of GBM cell makes the development of therapeutic strategies difficult[5]. Therefore, it is of great meaning to explore effective molecular targets for GBM treatment.

G protein family is considered to be a vital regulator in cancer progression, which can serve as potential targets for cancer treatment[6,7]. G protein subunit Gamma 5 (GNG5) is a member of the G protein family, which plays important role in regulating cell biological function[8]. It had been revealed that GNG5 was overexpressed in glioma tissues and could promote tumor growth, suggesting that GNG5 might become a potential independent prognostic indicator for GBM[9,10]. However, its function and mechanism in GBM have not been fully elucidated.

Yin Yang 1 (YY1), a zinc finger-containing transcription factor, is ubiquitously expressed in mammalian cells and regulates many important biological processes[11]. YY1 selectively activates or represses gene transcription and thus functions a key role in human disease[12]. Studies had indicated that YY1 promoted DLEU1 transcription and facilitated cholangiocarcinoma progression by regulating the miR-149-5p/YAP1/TEAD2/SOX2 axis[13]. YY1 directly bound to the LINC01089 promoter region and suppressed LINC01089 transcription, thereby exacerbating tumor growth in lung cancer[14]. In regulating cell processes, YY1 activated insulin transcription and contributed to beta cell maturation and function[15]. Also, YY1 induced aerobic glycolysis and proliferation in neuroblastoma cells[16,17]. It was found that YY1 had high expression in GBM tissues, which could promote the

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tumorigenicity of GBM stem cells[18]. Here, jaspar software predicted the existence of binding sites between the transcription factor YY1 and GNG5 promotor region. However, the transcriptional regulation of GNG5 by YY1 in GBM has not been reported.

Wnt/β-catenin pathway can modulate embryonic development and cell processes[19]. β-catenin is key factor to Wnt signaling and its aberrant regulation leads to early events in various diseases, including colorectal cancer[20] and hepatocellular carcinoma[21]. Studies had revealed that GNG5 promoted breast cancer cell proliferation and glycolysis by Wnt/β-catenin pathway[22]. However, whether GNG5 regulates Wnt/β-catenin pathway in GBM progression remains unclear.

This study was to explore GNG5 roles and mechanisms in GBM malignant progression. Through our analyses, we verified that YY1 promoted GNG5 transcription to facilitate the malignant progression of GBM by Wnt/β-catenin pathway.

Methods

Samples collection

Tissue samples were collected from 33 GBM patients at Cangzhou Central Hospital. The Ethics Committee of Cangzhou Central Hospital approved this study [No. 2021-113-02(z)]. All experimental procedures in this study were performed in accordance with the Declaration of Helsinki. Meanwhile, normal brain tissues were collected from 33 volunteers with traumatic brain injury from this hospital. All samples were preserved at −80 °C. Informed consent was obtained from each participant. The relationship between GNG5 expression and clinicopathologic features of GBM patients was shown in Table 1.

Cell culture and transfection

Normal human astrocytes (NHA) and GBM cells (LN229, A172 and U251) (Procell, Wuhan, China) were included in our study to measure GNG5 protein expression, which were cultured in DMEM (Gibco, Grand Island, NJ, USA) containing 10% FBS and 1% penicillin/streptomycin. A172 and U251 cells were transfected with GNG5 lentiviral shRNA (sh-GNG5), pcDNA GNG5 overexpression vector, YY1 siRNA (si-YY1), and negative control (sh-NC, pcDNA and si-NC) (RiboBio, Guangzhou, China) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). After transfection for 24 h, A172 and U251 cells transfected with sh-NC/sh-GNG5 were treated with DMSO or SKL2001 (Yeasen, Shanghai, China) for 24 h.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from GBM tumor tissues and normal brain tissues using TRIzol reagent (Invitrogen), and cDNA was synthesized by Reverse Transcription Kit (Takara, Tokyo, Japan). PCR amplification was performed using SYBR Green (Takara) with specific primers (Table 2). GAPDH as an internal reference. Relative mRNA expression of GNG5 and YY1 was analyzed using the $2^{-\Delta\Delta Ct}$ method.

Cell counting kit 8 (CCK8) assay

A172 and U251 cells were harvested using trypsin and then re-inoculated into 96-well plates (2×10^3 cells/well) and then cells were cultured for 48 h. Each well was added with 10 μL of CCK8 reagent (Beyotime, Shanghai, China) and then cultured for 2 h. Cell viability was analyzed at 450 nm using a microplate reader (BioTek, Winooski, Vermont, USA).

EdU assay

EdU assay was performed using EdU kit (Ribobio). A172 and U251 cells were harvested using trypsin and then re-inoculated into 96-well plates (4×10^4 cells/well). Then, cells were incubated with 50 μM EdU solution for 2 h, fixed with 4% polyformaldehyde for 30 min, decolored with 2 mg/mL glycine for 5 min, and treated with 0.5%

	Characteristics $n=33$	GNG5 expression		*P* value
		Low ($n=16$)	High ($n=17$)	
Gender				0.7238
Female	14	6	8	
Male	19	10	9	
Age (years)				0.7319
≤ 50	15	8	7	
> 50	18	8	10	
WHO grade				0.0134*
I + II	13	10	3	
III + IV	20	6	14	
Tumor size				0.0053*
≤ 3 cm	16	12	4	
> 3 cm	17	4	13	

Table 1. Relationship between GNG5 expression and clinicopathologic features of GBM patients *$P<0.05$

Name		Primers for PCR (5′–3′)
GNG5	Forward	CCCTTCCCACAAACTGGGAG
	Reverse	CGCGAAGAACTAAGAGGGGG
YY1	Forward	AAGCTGCACTTTCTTGGGGT
	Reverse	ACCATCTTCAGGCAACCAGG
β-actin	Forward	CTTCGCGGGCGACGAT
	Reverse	CCACATAGGAATCCTTCTGACC

Table 2. Primer sequences used for qRT-PCR

TritonX-100 for 10 min. After stained with Apollo solution and DAPI solution, EdU-positive cells were observed under a fluorescence microscope and counted using ImageJ software.

Flow cytometry

A172 and U251 cells were collected using trypsin and then suspended with 100 μL of Binding Buffer. Then, cells were stained with 5 μL Annexin V-FITC and 10 μL PI Staining Solution (Yeasen) for 15 min in the dark. Then, apoptotic cells were evaluated by flow cytometry (BD Biosciences, San Diego, CA, USA).

Transwell assay

Transwell assays were performed using transwell chambers-precoated with Matrigel (Corning Inc., Corning, NY, USA). A172 and U251 cells suspended with serum-free medium were seeded in the upper chamber and then the lower chamber was filled with 600 μL of culture medium containing 10% FBS. After fixation in 4% paraformaldehyde and staining with 0.1% crystal violet, invading cells were counted under a microscope in multiple selected fields of view using ImageJ software.

Sphere formation assay

A172 and U251 cells were collected and cultured in flat-bottomed ultra-low attachment 6-well plates (Corning) containing B27, bFGF, and EGF (Invitrogen) in serum-free medium. After culturing the cells at 37 °C in 5% CO_2 for 14 days, cell images were captured using an inverted microscope.

Assessment of cell glycolysis

According to kit instructions, Glucose Assay Kit, L-Lactate Assay Kit, and ADP/ATP Ratio Assay Kit (Abcam) were used to detect glucose consumption, lactate production and ATP/ADP ratio in A172 and U251 cells, respectively. Basing on the instructions of Seahorse XF Cell Mito Stress Test Kit (Seahorse Bioscience, Chicopee, MA, USA), the oxygen consumption rate (OCR) of GBM cells was analyzed with XF96 Extracellular Flux analyzer (Seahorse Bioscience).

Western blot (WB) analysis

Protein were extracted from NHA cells and GBM cells (LN229, A172 and U251) by RIPA buffer (Beyotime). After quantified using BCA Kit (Beyotime), proteins were separated by 10% SDS-PAGE gel and transferred onto PVDF membranes, which were then blocked by the addition of 5% skimmed milk. Membrane was incubated with antibodies, including anti-GNG5 (1:2500, PA5-60452, Invitrogen), anti-YY1 (1:1000, ab109228, Abcam), anti-Wnt3A (1:1000, ab219412, Abcam), anti-β-catenin (1:5000, ab32572, Abcam), anti-LC3 (1:3000, ab51520, Abcam), anti-P62 (1:10000, ab109012, Abcam), anti-Nanog (1:2000, ab109250, Abcam), anti-Sox2 (1:1000, ab97959, Abcam), anti-Oct4 (1:1000, ab19857, Abcam), anti-p-GSK-3β (1:20000, ab75814, Abcam), anti-GSK-3β (1:5000, ab32391, Abcam), anti-β-actin (1:1,000, ab8227, Abcam), and secondary antibody (1:50000, ab205718, Abcam). Protein signals were observed by ECL reagent (Beyotime), and gray values were analyzed by ImageJ software with β-actin as loading control.

ChIP assay

According to EZ-ChIP Kit (Millipore, Billerica, MA, USA) instructions, A172 and U251 cells were incubated with formaldehyde, and the cross-linked chromatin complexes were sonicated. Fragments were immunoprecipitated with anti-IgG or anti-YY1. The precipitated DNA was purified and the GNG5 enrichment in binding site 1/2 was detected by qRT-PCR.

Dual-luciferase reporter assay

The GNG5 promoter regions containing wild-type and mutant YY1 binding sites were cloned into pGL3-basic vectors to generate WT-GNG5 and MUT-GNG5 vectors. A172 and U251 cells were co-transfected with si-NC/si-YY1 and WT/MUT-GNG5 vectors using Lipofectamine 3000, and then luciferase activity was tested with corresponding assay kit (Beyotime).

Mice xenograft models

BALB/c nude mice (Vital River, Beijing, China) were subcutaneously injected with U251 cells transfected with sh-NC/sh-GNG5 in the right side of the back ($n=6$). Tumor volumes and weights were measured starting at 7 days, and then every 3 days thereafter. Mice were killed using 5% isoflurane after 22 days, and the tumor tissues were collected. Animal care and method procedure were authorized by Cangzhou Central Hospital Animal

Ethics Committee and Welfare Committee [No. 2021-113-02(z)]. Procedures also followed the guidelines for the care and use of animals provided by the National Institutes of Health Office of Laboratory Animal Welfare. The study is reported in accordance with ARRIVE guidelines.

Immunohistochemical (IHC) staining

Tumor tissues were prepared as paraffin sections to perform IHC staining using SP Kit (Solarbio, Beijing, China) with anti-GNG5 (1:100, ab238835, Abcam), anti-Ki67 (1:200, ab15580, Abcam) and anti-MMP9 (1:100, ab76003, Abcam) according to kit instructions.

Statistical analysis

Data are shown as mean ± SD. Statistical significance was evaluated by Student's *t* test or ANOVA followed by Tukey post-hoc test using GraphPad Prism 7.0 software. $P < 0.05$ was considered statistical significance.

Results

GNG5 was highly expressed in GBM

GEPIA and TCGA database analyses found the upregulation of GNG5 mRNA expression in GBM tissues (Fig. 1A, B). Besides, the HPA database (https://www.proteinatlas.org/) was used to analyze the IHC data of GNG5 in GBM patients and normal brain tissues, and the results showed that GNG5-positive cells were indeed increased in GBM patients (Fig. 1C). To confirm this, we then conducted QRT-PCR experiments, and the result showed that GNG5 mRNA expression was obviously upregulated in human GBM tissues (Fig. 1D). Moreover, Kaplan-Meier analysis revealed that GBM patients with high GNG5 expression had a significantly lower overall survival rate (Fig. 1E). Through analyzing the relationship between GNG5 expression and clinicopathologic features of GBM patients, we found that GNG5 expression was related to the WHO grade and tumor size of GBM patients (Table 1). In addition, GNG5 protein levels were detected to be elevated in GBM tissues and cells (Fig. 1F, G). Since A172 and U251 cells had superior GNG5 protein expression to LN229 cells, they were used for subsequent experiments.

GNG5 knockdown inhibited GBM cell progression

To investigate the role of GNG5 in GBM progression, A172 and U251 cells were transfected with sh-GNG5. Decreased GNG5 protein expression indicated that sh-GNG5 transfection was successful (Fig. 2A). Moreover, viability and apoptosis were detected in sh-GNG5-transfected GBM cells treated with apoptosis inhibitor Z-VAD-FMK. The results showed that Z-VAD-FMK significantly inhibited apoptosis but did not affect viability

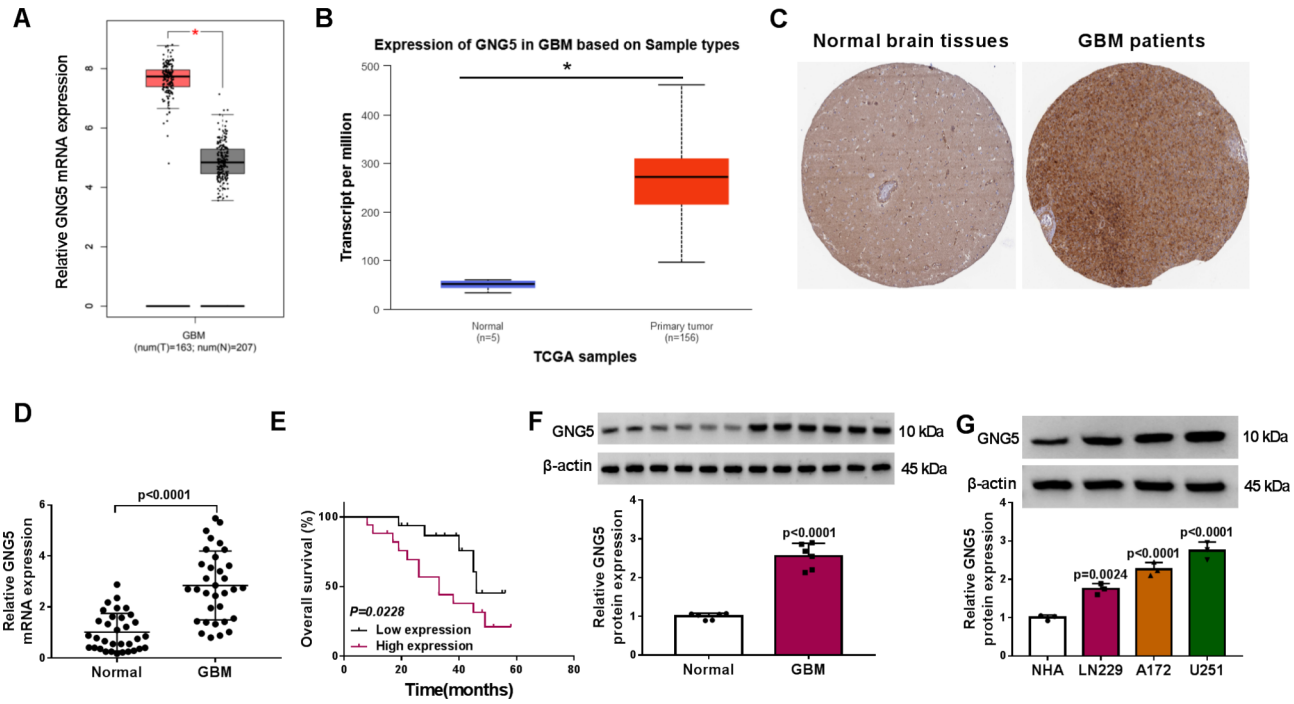

Fig. 1. GNG5 expression in GBM. **A**, **B** GEPIA and TCGA databases analyzed the GNG5 mRNA expression in GBM tissues and normal tissues. **C** HPA database analyzed the IHC data of GNG5 in GBM patients and normal brain tissues. **D** GNG5 expression was detected using qRT-PCR in human normal tissues ($n = 33$) and GBM tissues ($n = 33$). **E** Kaplan-Meier analysis based on follow-up results of clinical samples. **F** GNG5 protein expression was detected by WB in human normal tissues ($n = 6$) and GBM tissues ($n = 6$). **G** WB analysis for detection of GNG5 protein expression in NHA and GBM cells ($n = 3$). **D** and **F**, Unpaired *t* test; **G** two-way ANOVA

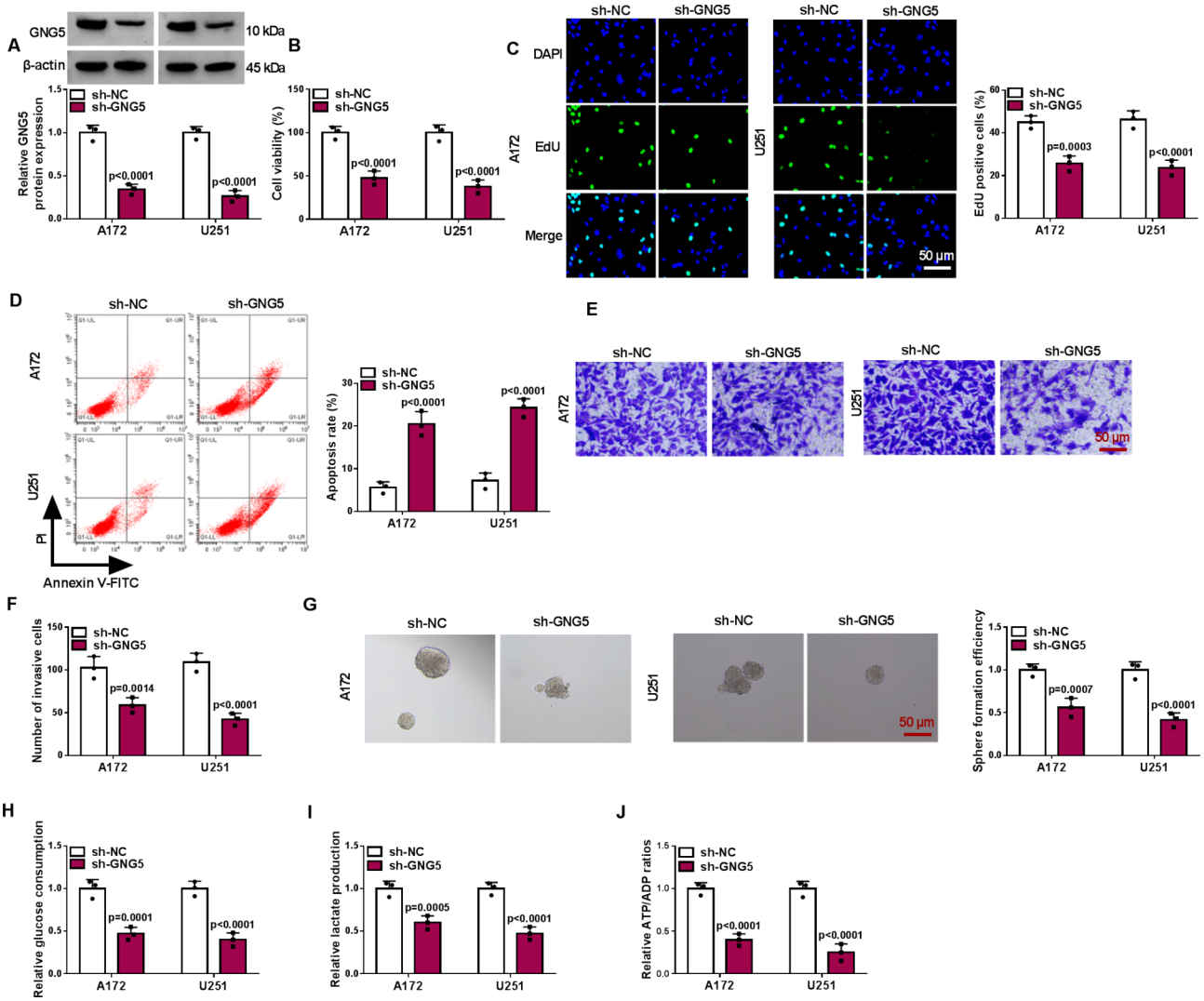

Fig. 2. Effects of GNG5 knockdown on GBM cell progression. A172 and U251 cells were transfected with sh-NC/sh-GNG5 ($n = 3$). **A** The sh-GNG5 transfection efficiency was assessed using WB. Cell proliferation, apoptosis, invasion and stemness were determined by CCK8 assay (**B**), EdU assay (**C**), flow cytometry (**D**), transwell assay (**E**, **F**) and sphere formation assay (**G**). **H**–**J** Glucose consumption, lactate production, and ATP/ADP ratio were detected to evaluate glycolysis. **A**–**J** two-way ANOVA

in sh-GNG5-transfected GBM cells (Supplementary Fig. 1A-B), which ruled out the effect of apoptosis on proliferation inhibition. Further analyses demonstrated that GNG5 knockdown suppressed cell viability, EdU positive cell rate, invasive cell number and sphere formation efficiency, while increased apoptosis (Fig. 2B–G). To further confirm our results, we examined the expression of stem cell marker proteins (Nanog, Sox2 and Oct4), and determined that GNG5 knockdown reduced the stemness of GBM cells (Supplementary Fig. 2A–B). Meanwhile, GNG5 knockdown repressed OCR, glucose consumption, lactate production and ATP/ADP ratios in GBM cells (Fig. 2H–J and Supplementary Fig. 3A–C). Besides, we found that GNG5 knockdown did not affect the protein expression of autophagy-related marker LC3II/I and P62 (Supplementary Fig. 4), suggesting that GNG5 had no effect on GBM cell autophagy. These demonstrations showed that GNG5 might contribute to GBM cell growth, invasion, stemness and glycolysis.

YY1 could induce GNG5 transcription

The jaspar software predicted that there existed 2 binding sites between YY1 and the GNG5 promoter region (Fig. 3A). ChIP assay showed that GNG5 binding site 1 was enriched in the YY1 antibody (Fig. 3B, C). The decreased expression of YY1 protein confirmed the successful transfection of si-YY1 (Fig. 3D). YY1 knockdown inhibited the luciferase activity of the WT-GNG5 vector in GBM cells, while had no effect on that of the MUT-GNG5 vector (Fig. 3E, F). These data confirmed that YY1 could bind to GNG5 promoter. GEPIA and TCGA database analysis revealed that YY1 was highly expressed in GBM tissues at the mRNA level (Fig. 3G, H). Besides, GEPIA database analyzed that YY1 expression was positively correlated with GNG5 expression in GBM tissues (Fig. 3I). Then, we detected that YY1 indeed had elevated expression in GBM tissues and was positively

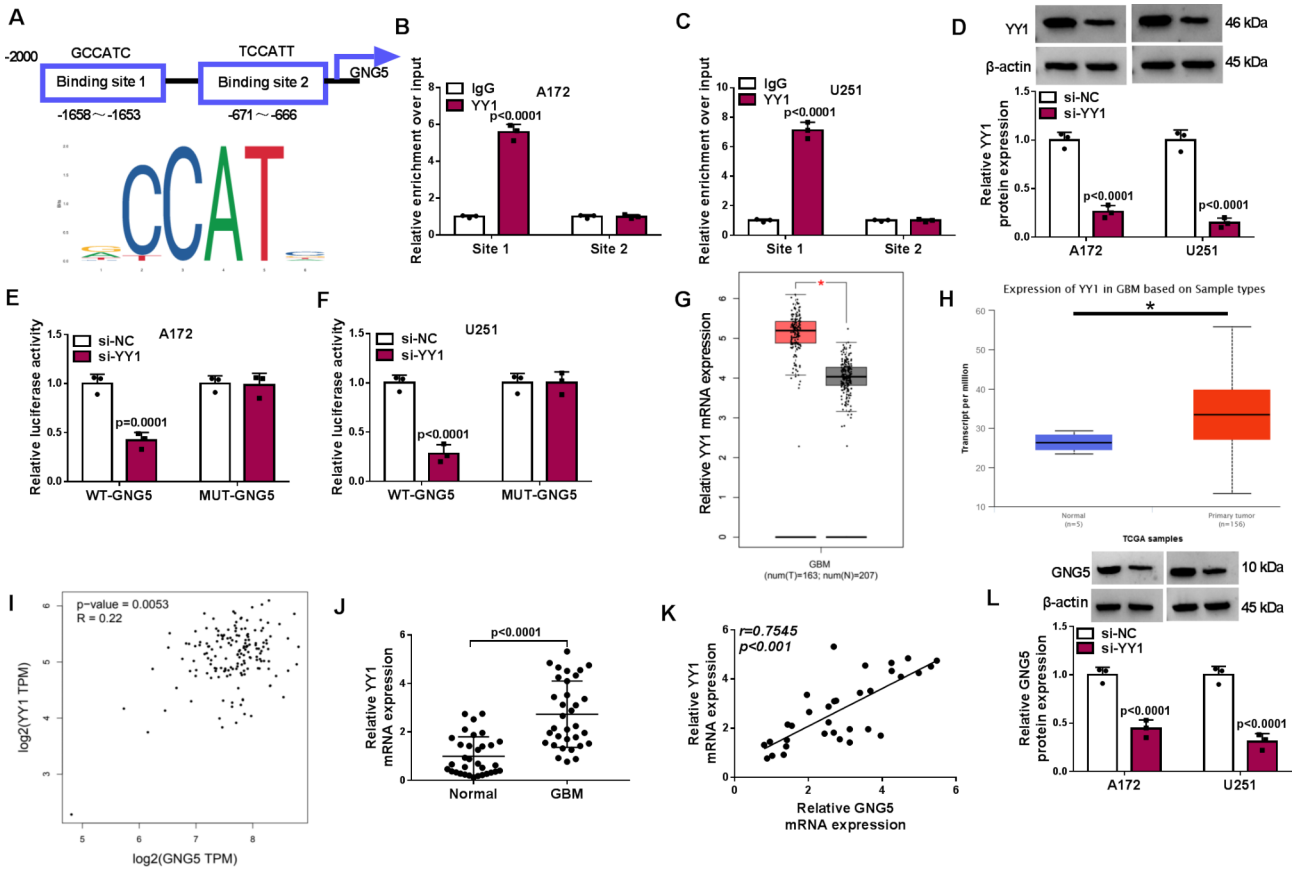

Fig. 3. Effect of transcription factor YY1 on GNG5. **A** Jaspar was used to predict the binding sites of YY1 and GNG5. **B**, **C** The interaction between YY1 and GNG5 was detected using ChIP assay ($n=3$). **D** The si-YY1 transfection efficiency was confirmed by WB analysis ($n=3$). **E**, **F** Dual-luciferase reporter assay was used to test the interaction between YY1 and GNG5 ($n=3$). **G**, **H** GEPIA and TCGA databases analyzed YY1 mRNA expression in GBM tissues and normal tissues. **I** GEPIA database analyzed the relationship between YY1 and GNG5 expression in GBM tissues. **J** YY1 mRNA expression was tested in human normal tissues ($n=33$) and GBM tissues ($n=33$) by qRT-PCR. **K** Pearson correlation coefficient for detecting correlation between YY1 and GNG5 expression in GBM tissues ($n=33$). **L** GNG5 protein expression was detected by WB in cells transfected with si-YY1/si-NC ($n=3$). **B**–**F** and **L**, two-way ANOVA; **J** Unpaired *t* test

correlated with GNG5 mRNA expression (Fig. 3J, K). Furthermore, YY1 knockdown could repress GNG5 protein expression in GBM cells (Fig. 3L). The above indicated that YY1 might promote GNG5 expression in GBM cells.

YY1 affected GBM cell progression by modulating GNG5 expression

To study YY1 roles in GBM progression and explore whether YY1 mediated GNG5 to play a regulator function, A172 and U251 cells were co-transfected with si-YY1 and GNG5 overexpression vector. GNG5 overexpression vector markedly enhanced GNG5 protein level suppressed by si-YY1 (Fig. 4A). YY1 knockdown inhibited GBM cell viability, EdU positive cell rate, and promoted apoptosis rate, while these effects were reversed by GNG5 overexpression (Fig. 4B–D). Moreover, GNG5 overexpression also abolished the suppressive effect of YY1 knockdown on invasive cell number, sphere formation efficiency, glucose consumption, lactate production and ATP/ADP ratios (Fig. 4E–I). The above demonstrated that YY1 could promote GBM progression by enhancing GNG5 expression.

YY1/GNG5 axis mediated the Wnt/β-catenin pathway

To investigate the effect of YY1 and GNG5 on the activity of Wnt/β-catenin pathway, we measured the levels of Wnt/β-catenin pathway-related proteins in A172 and U251 cells co-transfected with si-YY1 and GNG5 overexpression vectors. The results revealed that YY1 knockdown decreased the protein expression of Wnt3A and β-catenin, but this effect could be reversed by GNG5 overexpression (Fig. 5A, B). To further confirmed this, A172 and U251 cells were transfected with sh-GNG5 and then treated by Wnt/β-catenin pathway agonists SKL2001. As shown in Fig. 5C, D and Supplementary Fig. 5, GNG5 knockdown reduced Wnt3A and β-catenin levels, as well as promoted p-GSK-3β/GSK-3β levels, while SKL2001 could abolish above results. Above data confirmed that YY1/GNG5 axis activated the Wnt/β-catenin pathway.

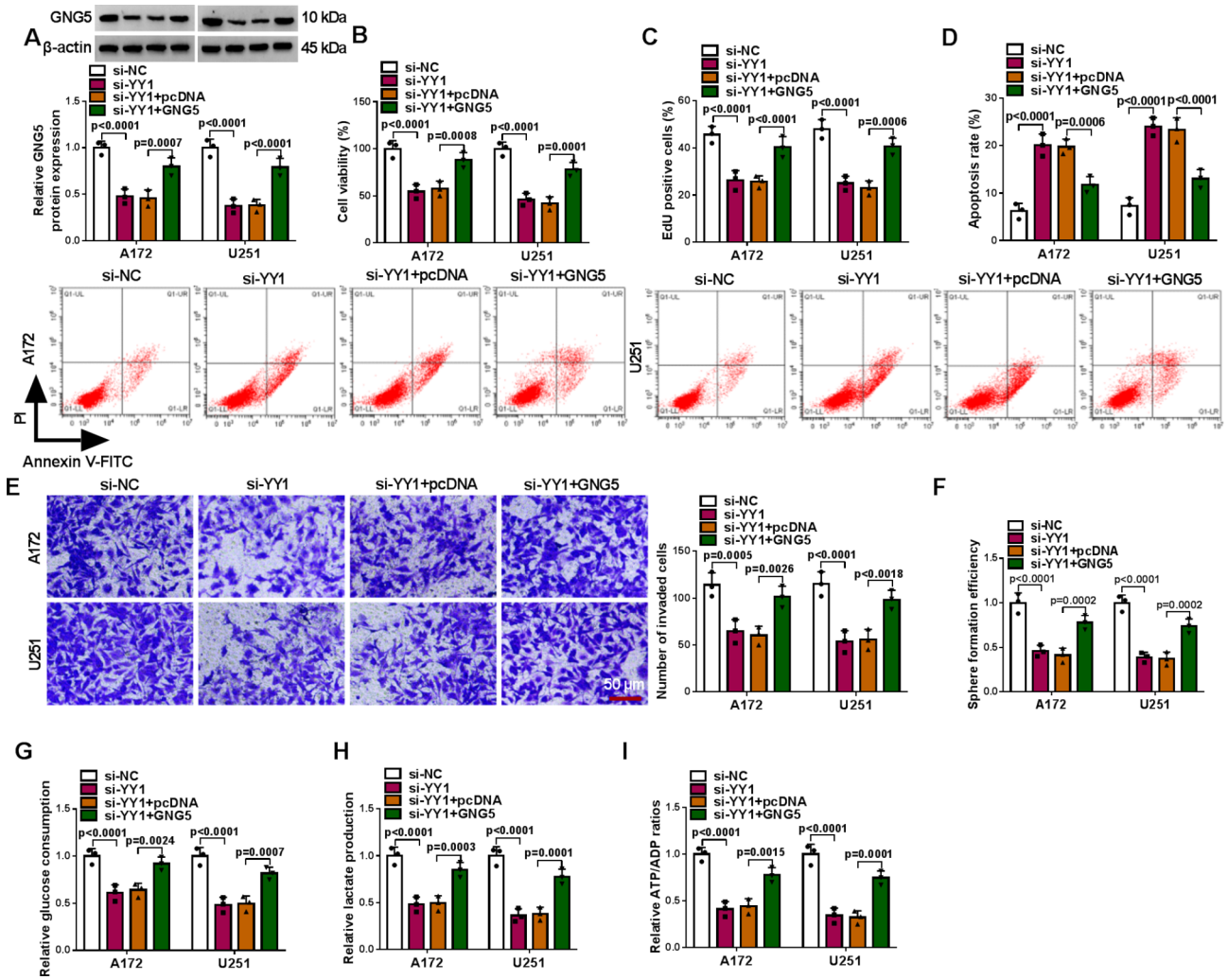

Fig. 4. Effect of si-YY1 and GNG5 on processes in GBM cells. A172 and U251 cells were transfected with si-NC/si-YY1/pcDNA/GNG5 overexpression vector ($n = 3$). **A** WB analysis for detecting GNG5 protein expression. CCK8 assay (**B**), EdU assay (**C**), flow cytometry (**D**), transwell assay (**E**) and sphere formation assay (**F**) were used to test cell proliferation, apoptosis, invasion and stemness. **G**–**I** Glucose consumption, lactate production and ATP/ADP ratios were tested to assess cell glycolysis. **A**–**I**, two-way ANOVA

SKL2001 reversed the effects of GNG5 knockdown on GBM cell progression

To examine whether GNG5 regulated GBM cell progression by Wnt/β-catenin pathway, we detected the functions of A172 and U251 cells transfected with sh-GNG5 and treated with SKL2001. SKL2001 treatment eliminated sh-GNG5-mediated the inhibition on cell viability, EdU positive cell rate, invasive cell number, sphere formation efficiency, glucose consumption, lactate production and ATP/ADP ratios, as well as the promotion on apoptosis rate (Fig. 6A–H). These data indicated that GNG5 activated Wnt/β-catenin pathway to accelerate GBM cell growth, invasion, stemness and glycolysis.

GNG5 knockdown suppressed GBM tumor growth in nude mice

To determine GNG5 roles in vivo, we constructed mouse xenograft model. GNG5 knockdown significantly suppressed tumor volume and weight (Fig. 7A, B). Besides, we observed the decreased expression of GNG5, Wnt3A and β-catenin in the tumor tissues of sh-GNG5 group (Fig. 7C, D). In addition, IHC staining showed that the numbers of Ki67, MMP9 and GNG5-positive cells were significantly reduced in the tumor tissues of sh-GNG5 group (Fig. 7E). Taken together, GNG5 might promote GBM tumor growth by Wnt/β-catenin pathway.

Discussion

With the advancement of medical technology, GBM patients' prognosis has not been greatly improved though its treatment options have been improving[23,24]. Currently, surgery and chemoradiotherapy are the standard treatment for GBM, but about 90% of GBM will recur locally within 2 years[4,25]. Neurological dysfunction seriously affects the health and life of GBM patients, so there is an urgent need to develop new approaches for GBM treatment.

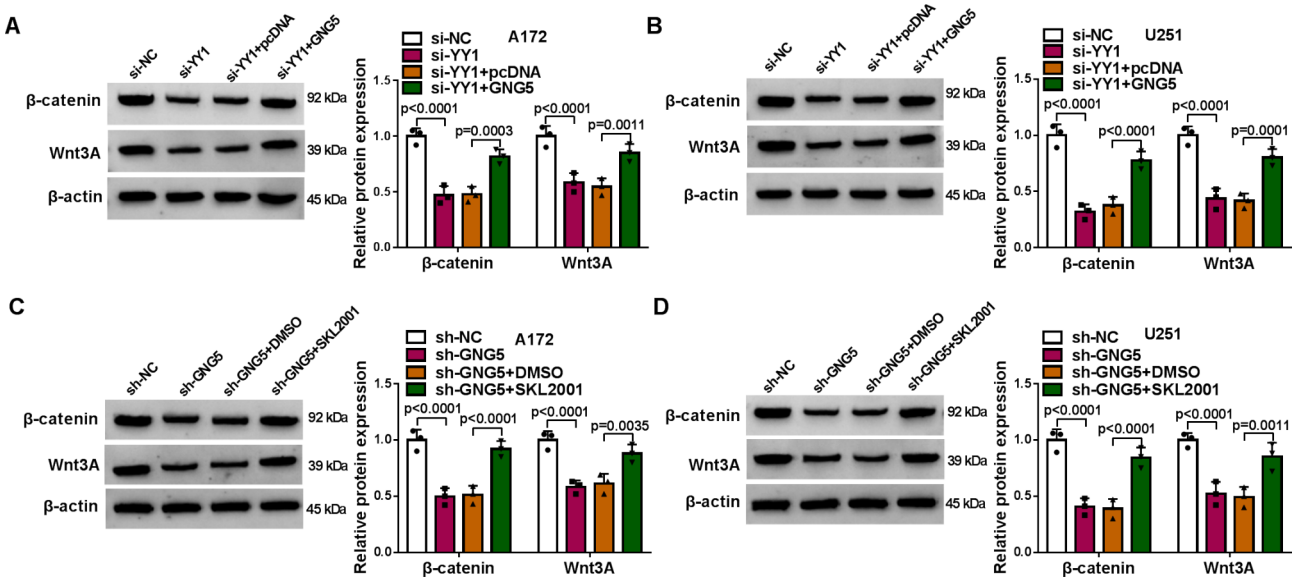

Fig. 5. Effect of YY1 and GNG5 on Wnt/β-catenin pathway. **A**, **B** WB analysis was used to test the protein expression of Wnt3A and β-catenin in A172 and U251 cells transfected with si-NC/si-YY1/pcDNA/GNG5 overexpression vector ($n = 3$). **C**, **D** The protein expression of Wnt/β-catenin pathway was detected by WB analysis in A172 and U251 cells transfected with sh-NC/sh-GNG5 and treated with DMSO/SKL2001 ($n = 3$). **A**–**D**, two-way ANOVA

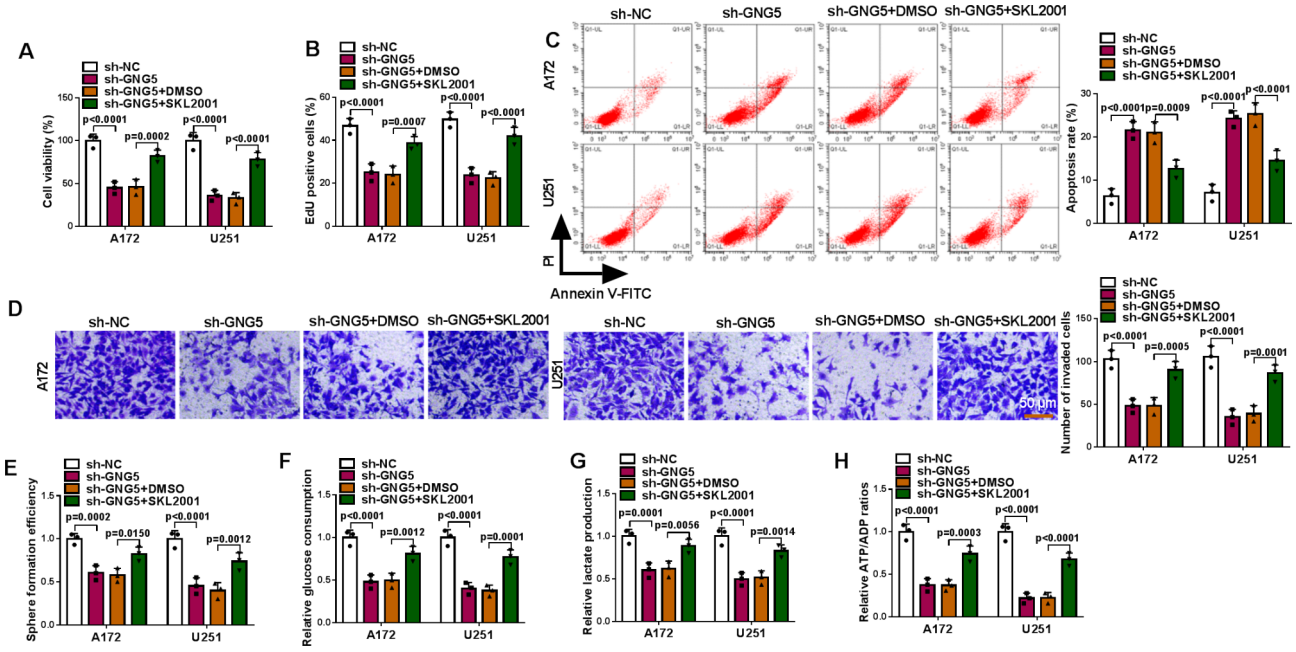

Fig. 6. Effect of SKL2001 and GNG5 knockdown on GBM cells. A172 and U251 cells were transfected with sh-NC/sh-GNG5 and then treated with DMSO/SKL2001 ($n = 3$). Cell proliferation, apoptosis, invasion and stemness were tested using CCK8 assay (**A**), EdU assay (**B**), flow cytometry (**C**), transwell assay (**D**) and sphere formation assay (**E**). **F**–**H** Glucose consumption, lactate production and ATP/ADP ratios were detected to analyze cell glycolysis. **A**–**H** two-way ANOVA

GNG5 plays an important role in a range of cell processes. It was found that miR-let-7b-5p modulated GNG5 protein levels to affect lung cancer cell proliferation and apoptosis[26]. Also, GNG5 promoted glioma cell functions, and its expression level was negatively correlated with patient's overall survival[9]. Previous studies confirmed that GNG5 had the highest expression in GBM, and it might act as potential GBM therapy target[10]. Based on database bioinformatics analysis and qRT-PCR, we confirmed the elevated GNG5 expression in GBM tissues

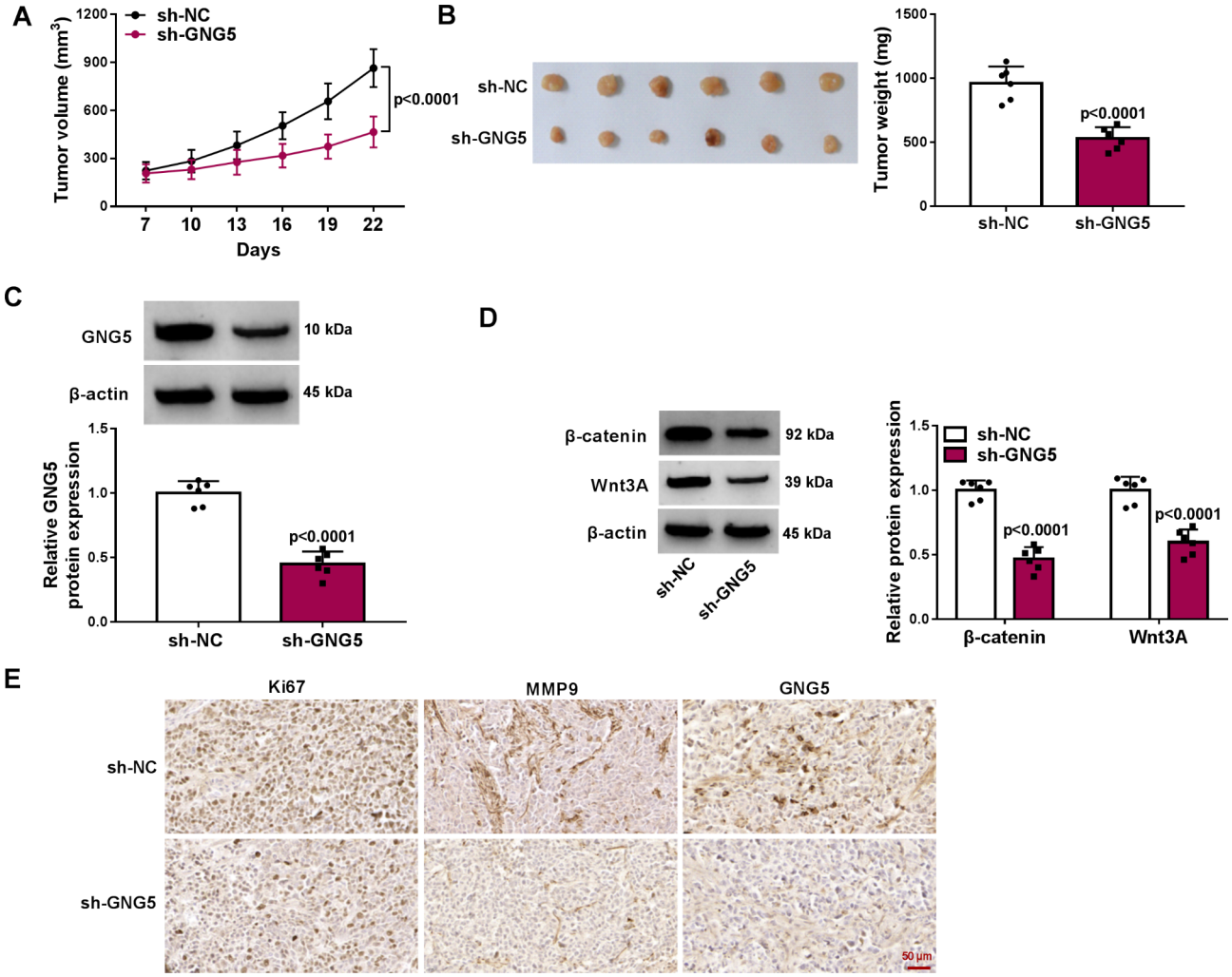

Fig. 7. Effect of GNG5 on GBM tumor growthin vivo. Tumor volume (**A**) and weight (**B**) were measured ($n=6$). **C**, **D** The protein expression of GNG5, Wnt3A and β-catenin in mice tumor tissues was tested WB analysis ($n=6$). **E** Ki67, MMP9 and GNG5 positive cells in mice tumor tissues were detected by IHC staining. **A** and **D** two-way ANOVA; **B** and **C** Unpaired *t*-test

and cells. Besides, follow-up results showed that high GNG5 expression was associated with poor prognosis in GBM patients. Importantly, function analysis revealed that GNG5 silencing suppressed GBM cell growth, invasion, stemness and glycolysis, as well as GBM tumorigenesis in vivo, verifying that targeted reduction of GNG5 might be an effective strategy to restrain GBM progression.

YY1 activates gene transcription to be involved in cell biological processes[27]. For example, YY1 positively regulated LINC01608 transcription to accelerate hepatocellular carcinoma progression[28]. YY1 promoted LINC00673 transcription, which in turn regulated breast cancer cell cycle and apoptosis[29]. In this, we found that YY1 could bind to the GNG5 promoter and enhance GNG5 transcription. Consistent with previous study[18], we detected high YY1 expression in GBM tissues. Furthermore, YY1 knockdown inhibited GBM cell growth, invasion, stemness, and glycolysis, while GNG5 overexpression reversed these effects. All data further confirmed that YY1 contributed to GBM progression via upregulating GNG5.

Wnt/β-catenin is an extremely conserved pathway in biological evolution and serves vital role in cell growth and differentiation[30]. Research has found that the Wnt/β-catenin pathway promotes progenitor cell injury in a fibrotic environment[31], and regulates cell inflammatory tumor environment[32]. Wnt/β-catenin pathway was verified to be activated in GBM process[33,34]. Here, we found that YY1/GNG5 could activate Wnt/β-catenin pathway. In addition, Wnt/β-catenin pathway agonist SKL2001 abolished the regulation of GNG5 knockdown on GBM cell progression, confirming that GNG5 exerted a regulatory effect in GBM dependent on the activity of Wnt/β-catenin pathway.

Of course, there are some limitations to this study. For in vivo experiments, the construction of intracranial orthotopic transplantation tumor models will further enrich our findings. However, due to technical limitations, we cannot carry out relevant research on intracranial orthotopic transplantation tumor models at present, which will be the direction of our future research. Currently, we have only explored the regulation of the YY1/GNG5 axis on the activity of Wnt/β-catenin pathway at the cellular level, and analyzed the reversal effect of Wnt/β-

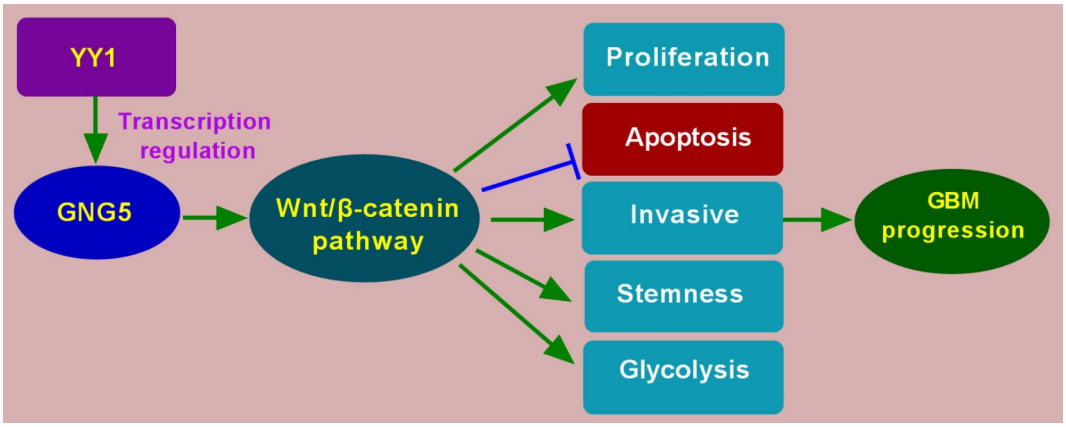

Fig. 8. Mechanistic diagram of the present study. YY1 promoted GNG5 transcription to activate Wnt/β-catenin pathway, thus facilitating GBM cell proliferation, invasion, stemness, glycolysis, and suppressing apoptosis

catenin pathway inhibitors on sh-GNG5-mediated GBM progression. Further in vivo studies are necessary to determine whether the YY1/GNG5 axis mediates GBM tumorigenesis through regulating Wnt/β-catenin pathway, which is a direction for future studies.

Conclusions

In conclusion, our findings provide a new approach for targeted therapy of GBM. Our data confirmed that YY1-activated GNG5 facilitated GBM cell growth, invasion, stemness, and glycolysis by mediating the Wnt/β-catenin pathway (Fig. 8).

Data availability

The datasets generated and/or analyzed during the current study are not publicly available due research design but are available from the corresponding author on reasonable request.

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SL: study concepts, study design, literature research, experimental studies, manuscript preparation and editing; LLZ, FY and HJD: definition of intellectual content, literature research experimental studies, manuscript preparation and editing; all authors read and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Cangzhou Central Hospital [No. 2021-113-02(z)], and carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Informed consent was signed by all patients and controls.

Consent for publication
Not applicable.
Animal care and method procedure were authorized by Cangzhou Central Hospital Animal Ethics Committee and Welfare Committee [No. 2021-113-02(z)]. Procedures also followed the guidelines for the care and use of animals provided by the National Institutes of Health Office of Laboratory Animal Welfare. The study is reported in accordance with ARRIVE guidelines.

Additional information
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