Neoplasma 2025; 72(1–2): 80–90

doi:10.4149/neo_2025_241227N539

KLHL7 enhances cell viability and cell cycle progression in glioma via glutamine metabolism by activating the β-catenin signaling pathway

Rui LIU[1,#], Xiaoju CHENG[2,3,#], Peirui WANG[1], Xiangping XIA[1], Gang LI[1], Fuan ZHANG[1], Chong HAN[1], Shengtao YAO[1,*]

[1]Department of Neurosurgery, Affiliated Hospital of Zunyi Medical University, Zunyi, China; [2]Department of Pathology, The First People's Hospital of Zunyi, Zunyi, China; [3]The Third Affiliated Hospital of Zunyi Medical University, Zunyi, China

**Correspondence: yst@zmu.edu.cn*
[#]Contributed equally to this work.

Received December 27, 2024 / Accepted April 10, 2025

Kelch-like family member 7 (KLHL7) is associated with cancer development and occurrence, but its role and mechanism in the malignant progression of gliomas remain poorly understood. This study aimed to investigate the regulatory effects and mechanisms of KLHL7 on cell cycle and glutamine metabolism in glioma. Glioma cell lines A172 and U87 and a xenograft mouse model were used to analyze the function of KLHL7 *in vitro* and *in vivo*, respectively. Gene expression levels and protein amounts were assessed by quantitative reverse-transcription polymerase chain reaction and western blotting, respectively. Cell viability was assessed using the CCK-8 assay, and the cell cycle was analyzed via flow cytometry. The glutamine content was measured using a biochemical assay. The level of KLHL7 was upregulated in patients with glioma. KLHL7 knockdown reduced cell viability, inhibited cell cycle progression, and decreased the glutamine content in A172 cells. KLHL7 silencing inhibited tumor growth *in vivo*. Furthermore, KLHL7 overexpression enhanced cell viability, cell cycle progression, and glutamine metabolism and activated the β-catenin signaling pathway in U87 cells. These findings indicate that KLHL7 promotes the malignant progression of glioma via the β-catenin signaling pathway and may serve as a biomolecule for the clinical prediction and treatment of the disease.

Key words: glioma; KLHL7; glutamine metabolism; β-catenin

Glioma is a common primary brain tumor, mainly derived from glial cells in brain tissue [1]. Nearly 40–50% of intracranial tumors occur within the skull (inside the brain and skull) [2]. In clinical practice, the treatment methods for glioma usually include surgery, radiotherapy, and chemotherapy (mainly temozolomide), and the choice of these methods depends on factors such as the location, histology, and genotype of the tumor, and the overall health status of the patient [3–5]. Presently, the treatment of glioma poses challenges and has poor therapeutic effects, leading to increased research on innovative therapies, such as immunotherapy and targeted molecular therapy [6, 7].

Cancer cells must accelerate the synthesis rate of biomolecules such as ATP, nucleotides, and lipids to support their rapid growth and proliferation [8, 9]. Several malignant tumors consume and utilize glutamine at a much faster rate than other amino acids; they have a high dependence on glutamine, also known as glutamine addiction [10–12]. As an amino acid abundant in plasma, glutamine is transported into the cytoplasm via a carrier (such as SLC1A5) on the cell membrane, following which it enters the mitochondria where it is deaminated by glutaminase to produce glutamate [13, 14]. Glutamate is further converted into alpha-ketoglutarate by glutamate dehydrogenase, which takes part in the tricarboxylic acid cycle (glutamine fermentation) [15, 16]. Glutamine metabolism is an important therapeutic target in cancer treatment [17]. Notably, Wnt/β-catenin-mediated glutamine metabolism plays an important role in the development and progression of cancer [18]. Myc, a key downstream target of Wnt/β-catenin signaling, transcriptionally controls the glutamine transporter SLC1A5 and regulates glutamine metabolism [19]. However, the specific mechanisms underlying glutamine metabolism in glioma remain poorly elucidated. Exploring the regulatory mechanisms governing glutamine metabolism in glioma may provide novel insights and targets for preventing and treating the disease.

KLHL7 is one of the Kelch-like (KLHL) proteins that modulate a variety of cellular processes, such as the cell

cycle, apoptosis, autophagy, and DNA damage repair, by connecting the E3-ubiquitin ligase Cullin 3 (CUL3) and various protein substrates [20]. However, recent investigations have unveiled KLHL7's emerging role in metabolic regulation, a novel function that has garnered considerable attention in cancer research. KLHL7-mediated TUT1 ubiquitination may contribute to the pathogenic mechanism of retinitis pigmentosa [21]. The increased expression of KLHL7 in hepatocellular carcinoma (HCC) and breast cancer may be linked to poor prognosis [22, 23] and increased HCC cell viability, colony formation, and drug resistance via the degradation of RASA2 [22]. Wnt/β-catenin pathway dysregulation is also associated with glioma pathogenesis [24] and can be regulated by KLHL12 via the ubiquitin-mediated degradation of DVLs [25]. Nevertheless, further exploration is warranted to elucidate the precise contribution of KLHL7 and its underlying regulatory mechanisms in glioma.

This study aimed to investigate the mechanism by which KLHL7 influences the progression of glioma. KLHL7 expression was increased in glioma patients, and KLHL7 silencing inhibited tumor growth *in vivo*. Moreover, KLHL7 enhanced cell viability and cell cycle progression in the glioma cells through glutamine metabolism by activating the β-catenin signaling pathway. These findings indicated that KLHL7 may serve as a new target for glioma treatment.

Patients and methods

Bioinformatics analysis. The Gene Expression Profiling Interactive Analysis (GEPIA) database was used to determine KLHL7 expression in glioma. Gene Set Enrichment Analysis (GSEA) was conducted to identify the altered molecular pathways associated with high and low KLHL7 scores.

Clinical sample collection. All clinical specimens were collected with informed consent from the participants using a protocol approved by the ethics committee of the Affiliated Hospital of Zunyi Medical University (Approval No. KLL-2023-589). Glioma (n=20) and non-neoplastic (n=20) brain tissues were harvested at the Affiliated Hospital of Zunyi Medical University and stored at –80 °C until use for measurements.

Immunohistochemistry (IHC) staining. IHC staining was done on human tissue microarrays (Shanghai Outdo Biotech, China), including glioma (n=52) and non-neoplastic (n=52) brain tissues, to detect KLHL7 expression. Glioma tissue sections were stained with anti-KLHL7 (#ab254785; Abcam) or anti-catenin (#ab16051; Abcam). The H-score system was assessed based on the staining intensity of protein and the percentage of positively stained cells. The glioma patients were divided into the high and low groups based on the H-scores (>6).

Cell cultures. Human brain microvascular endothelial cells (HBMEC) and glioma cell lines were obtained from the BeNa Culture Collection. Different cell lines were cultured with high-glucose Dulbecco's Modified Eagle Medium and supplied with 10% fetal bovine serum at 37 °C.

Gene overexpression and knockdown. For overexpression, the coding sequence (CDS) region of KLHL7 was inserted into the pLVX-Puro vector. Short hairpin RNAs (shRNAs) were designed and inserted into a pLKO.1 vector to suppress KLHL7 expression. The recombinant plasmids, psPAX2, and pMD2G vectors were co-transfected into 293T cells, resulting in the successful construction and secretion of virus particles from the cell medium.

Cell Counting Kit-8 (CCK-8) assay. The CCK-8 assay was used to quantify the cell viability [26, 27]. After treatment for 48 h, cells in 96-well plates were incubated with CCK-8 solution (10 μl) for 4 h, and the absorbance was measured using a microplate reader.

Cell cycle measurement. The cells were incubated with RNase in the dark and stained with propidium iodide. The cell cycle in the different phases was evaluated using flow cytometry (CytoFLEX cytometer; Beckman Coulter, CA, USA) and the FlowJo software.

Glutamine measurement. The glutamine concentrations were measured using a glutamine assay kit (#ab197011; Abcam) according to the manufacturer's instructions. The glutamine level was measured by assessing the amount of ammonia produced based on the principle of glutamine conversion into ammonia.

Quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was reverse-transcribed into cDNA, and qRT-PCR was conducted based on the Fast SYBR Green PCR protocol. The related expression levels of the genes were normalized to those of ACTB (beta-actin) and analyzed using the $2^{-\Delta\Delta Ct}$ method. The primers used in the study were as follows: KLHL7-F 5'-AAGAAACTTGCTGCTCGGGA-3', KLHL7-R 5'-GGCTGCAGCAAGAACAACAC-3'; ACTB-F 5'-AGGATTCCTATGTGGGCGAC-3', ACTB-R 5'-ATAGCACAGCCTGGATAGCAA-3'.

Western blot. Total lysates were extracted using the radio-immunoprecipitation assay buffer. The nuclear-plasmic fractionation was isolated using the Nuclear and Cytoplasmic Extraction Kit (#78835; Thermo Fisher Scientific). The proteins were separated and transferred onto a polyvinylidene fluoride membrane, which was incubated with antibodies against KLHL7 (#K110746P; Solarbio), β-catenin (#ab32572; Abcam), H3 (#ab1791; Abcam), proliferating cell nuclear antigen (PCNA; #ab29; Abcam), c-Myc (#ab32072; Abcam), and b-actin (#81115-1-RR; Proteintech). The protein signals were visualized using an enhanced chemiluminescence reagent (Beyotime) and analyzed for gray value using ImageJ software.

***In vivo* tumor xenograft model.** Male BALB/c nude mice (n=6) were subcutaneously injected with 5×10^6 U251 cells transduced with KLGL7 shRNA or shNC lentivirus vector in the armpits. The tumor volumes were measured every three days and calculated using the following formula: $\text{width}^2 \times \text{length} \times 0.5$. Thirty days after injection, the mice

were sacrificed, and tumor xenografts were collected, photographed, and weighed. All animal experiments were conducted in accordance with our institution's laboratory animal guidelines and were approved by the Committee on the Ethics of Animal Experiments of the Affiliated Hospital of Zunyi Medical University (Approval No. zyfy-an-2023-0245).

Immunofluorescence staining. The tumor tissue sections were fixed, blocked with 1% fetal bovine serum, and stained with anti-PCNA and secondary antibodies. The stained cells were examined and photographed using a confocal microscope.

Statistical analysis. All data were analyzed using GraphPad Prism 8.4.2 software and are shown as the mean values ± standard deviations. Differences between and among groups were calculated using the Student's t-test and analysis of variance methods, respectively. A p-value of <0.05 was considered statistically significant.

Results

KLHL7 expression was increased in patients with glioma. The GEPIA dataset was analyzed to explore the clinical significance of KLHL7. KLHL7 was highly expressed in glioma tissues compared to normal healthy brain tissue (Figure 1A). Similar findings were observed for the mRNA levels (Figure 1B) and the IHC analysis (Figures 1C, 1D) in glioma and normal brain tissues from the hospital cohort and human tissue microarrays, respectively. Moreover, human tissue microarrays were further used to analyze the relationship between KLHL7 and clinical characteristics. KLHL7 expression was significantly related to the histological type and World Health Organization grade (Table 1). Further analysis of the impact of KLHL7 expression on key signaling pathways via GSEA indicated that the upregulation of KLHL7 was closely related to the Benporath proliferation (Figure 1E), Reactome cell cycle, (Figure 1F), Reactome signaling by Wnt (Figure 1G), and Hallmark Myc targets v1 (Figure 1H) signaling pathways.

KLHL7 silencing reduced cell viability, inhibited cell cycle progression, and decreased glutamine levels in A172 cells. Evaluation of the levels of KLHL7 in different glioma cell lines (A172, T98G, U87, U251) and HBMEC using RT-PCR and western blot revealed that KLHL7 expression was greater in the glioma cells (Figures 2A, 2B). We constructed a KLHL7 interference lentivirus and transduced it into the A172 cells; the interference efficacy of shRNAs was ideal (Figures 2C, 2D). In addition, KLHL7 knockdown significantly reduced cell viability and induced cell cycle arrest in the G0–G1 phase (Figures 2E–2G). Glutamine plays an important role in tumor metabolism, which affects tumor growth [28]. In the current study, the glutamine content was significantly decreased following KLHL7 knockdown (Figure 2H). Wnt/β-catenin pathway dysregulation is associated with glioma pathogenesis [24] and can be regulated by KLHLs, such as KLHL12 [25]; therefore, the expression levels of β-catenin and downstream targets (Myc and PCNA) [29] were measured by western blotting. KLHL7 knockdown inhibited β-catenin entry into the nucleus and reduced the expression levels of PCNA and c-Myc (Figures 2I, 2J).

Table 1. Relationship between KLHL7 expression and their clinicopathological characteristics of patients with glioma

Characteristics	Cases	KLHL7		p-value
		Low (n=21)	High (n=31)	
Age (years)				0.2357
≥50	27	13	14	
<50	25	8	17	
Sex				0.3039
Male	30	12	22	
Female	15	9	9	
Predominant side				0.2812
Left	24	10	14	
Middle	8	5	3	
Right	20	6	14	
Predominant site				0.2227
Frontal lobe	23	6	17	
Temporal lobe	15	9	6	
Parietal lobe	7	3	4	
Others	7	3	4	
Histological type				0.0025
Astrocytoma	12	10	2	
Oligodendroglioma	3	1	2	
Glioblastoma	37	10	27	
WHO grade				0.0006
II	11	10	1	
III	4	1	3	
IV	37	10	27	

Note: statistical analyses were performed by the Chi-square test
Abbreviation: WHO - World Health Organization

KLHL7 silencing inhibited tumor growth *in vivo*. The tumor mouse model was constructed using U251 cells transduced with KLHL7 interference lentivirus, and the tumor volume and weight were recorded. As shown in Figures 3A–3C, KLHL7 knockdown significantly inhibited tumor growth *in vivo*, including the volume and weight. PCNA staining was performed on tumor tissues to analyze cell proliferation. KLHL7 knockdown resulted in lower PCNA expression in the tumor tissues (Figure 3D). In addition, the expression levels of KLHL7, PCNA, c-Myc, and nuclear β-catenin were significantly reduced in the KLHL7 knockdown mice (Figures 3E, 3F).

KLHL7 overexpression enhanced cell viability and cell cycle progression in U87 cells via glutamine metabolism. We constructed KLHL7 overexpressing lentivirus and transduced it into U87 cells to further refine our conclusions. The overexpression of KLHL7 was measured after treatment with KLHL7 overexpressing lentivirus (Figures 4A, 4B). U87 cells were transduced with KLHL7 overexpression lentiviral vector and treated alone or with a glutaminase inhibitor (CB-839;

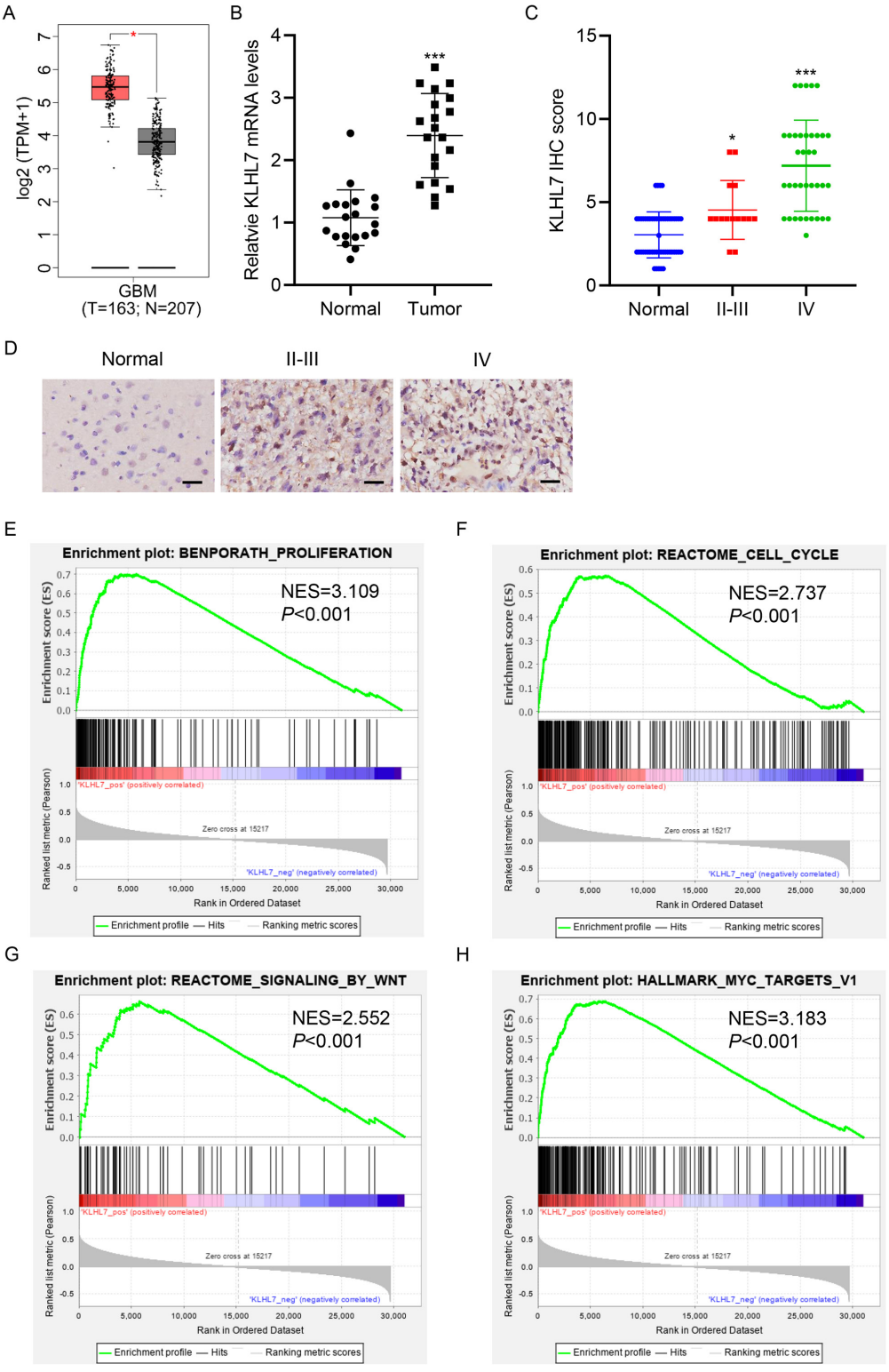

Figure 1. KLHL7 expression is increased in patients with glioma. A) The GEPIA database was used to analyze the expression of KLHL7 in glioma and normal brain tissues. B) qRT-PCR was used to detect the expression of KLHL7 in 20 cases of glioma and 20 cases of normal brain tissue. C, D) IHC staining of human tissue microarrays was performed to analyze the expression of KLHL7 in 52 cases of glioma and 52 cases of normal brain tissues (scale bar, 50 μm). GSEA analysis of the impact of KLHL7 expression on the E) Benporath proliferation, F) Reactome cell cycle, G) Reactome signaling by Wnt, and H) Hallmark Myc targets v1 signaling pathways. Data are presented as the mean ± standard deviation (SD). *p<0.05, ***p<0.001 vs. normal

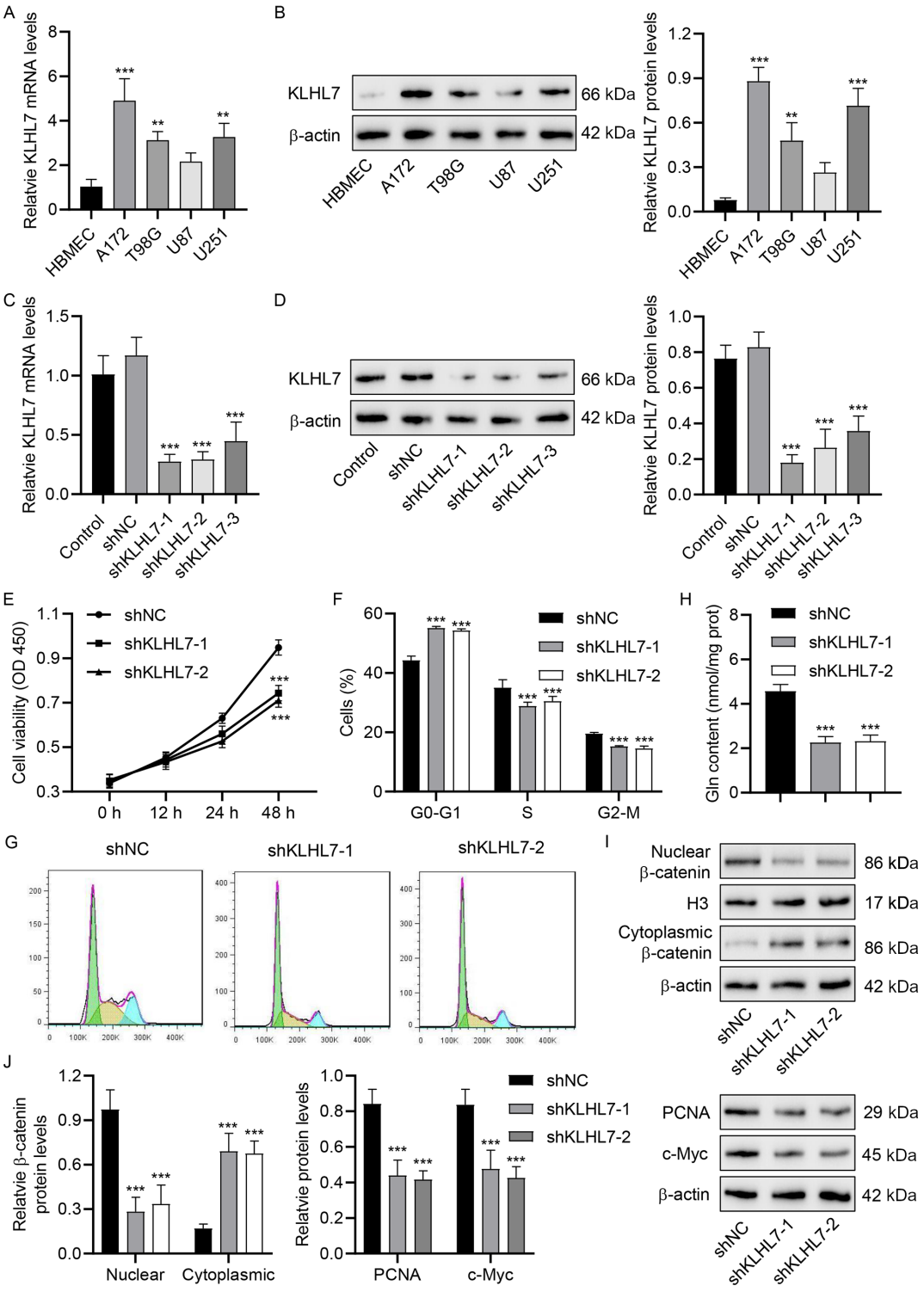

Figure 2. KLHL7 silencing reduces cell viability, inhibits cell cycle progression, and decreases glutamine levels in A172 cells. Multiple glioma cell lines (A172, T98G, U87, and U251) and brain microvascular endothelial cells (HBMEC) were used to determine the expression of KLHL7 via A) qRT-PCR and B) western blotting. KLHL7 interference lentivirus was constructed and transduced into the A172 cells; KLHL7 expression was evaluated via C) qRT-PCR and D) western blotting. After the transduction of KLHL7 interference lentivirus into the A172 cells, E) the CCK-8 assay was used to assess cell viability, F, G) flow cytometry was performed to analyze the cell cycle, H) a biochemical assay was performed to determine the glutamine levels, and I, J) western blot assay was used to detect the expression of β-catenin (nucleus/cytoplasm), PCNA, and c-Myc. Data are presented as the means ± SD from three independent experiments. **p<0.01, ***p<0.001 vs. HBMEC or shNC

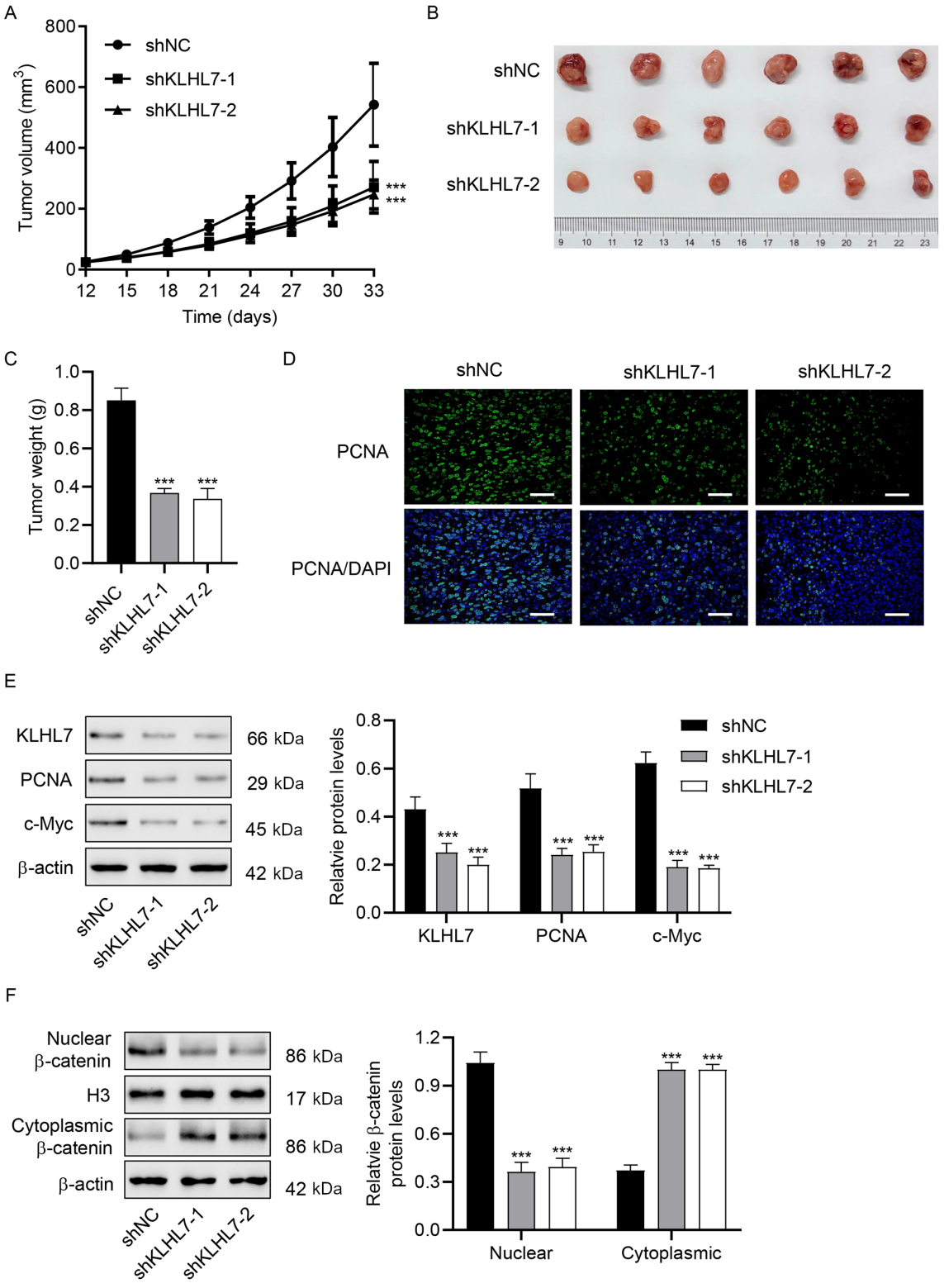

Figure 3. KLHL7 silencing inhibits tumor growth *in vivo*. Construction of a nude mouse subcutaneous tumor model using U251 cells transduced with KLHL7 interference with lentivirus. A) Tumor volume, B) tumor size, C) tumor weight, and D) PCNA staining of tumor tissues to analyze tumor cell proliferation (n=6). Scale bar, 50 μm. Western blot was used to measure the expression of E) KLHL7, PCNA, c-Myc, and F) β-catenin (nucleus/cytoplasm) in the tumor tissues (n=3). Data are presented as the means ± SD. ***p<0.001 vs. shNC

10 μM). KLHL7 overexpression significantly enhanced cell viability, accelerated cell cycle progression, and increased glutamine synthesis in the cells (Figures 4C–4F). However, further treatment with CB-839 reversed the promoting effect of KLHL7 on cell viability and cell cycle progression and increased glutamine levels. These results indicate that KLHL7 enhanced cell viability and cell cycle progression in glioma U87 cells by regulating glutamine metabolism.

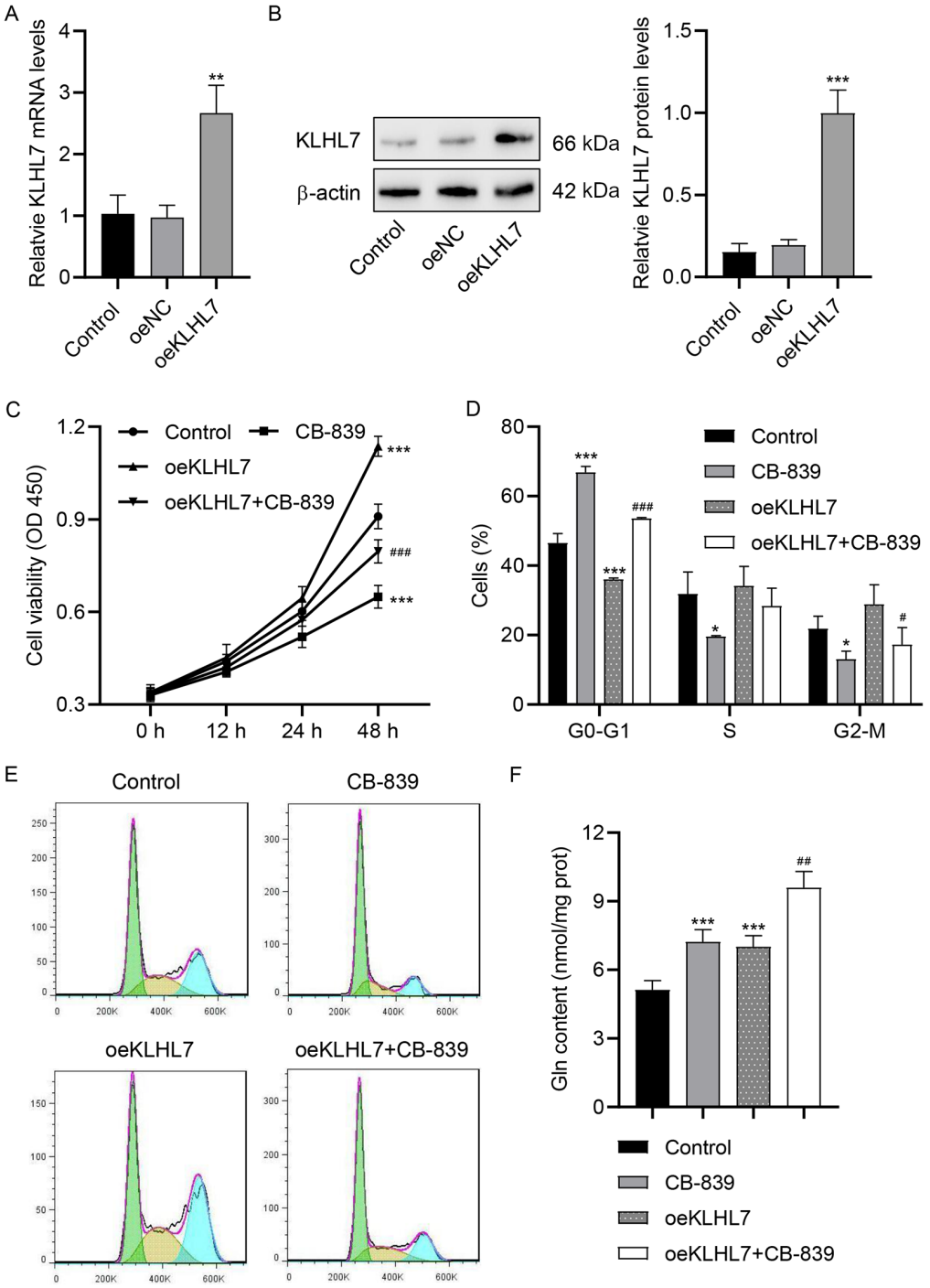

Figure 4. KLHL7 overexpression enhances cell viability and cell cycle progression in glioma U87 cells via glutamine metabolism. The KLHL7 overexpression lentiviral vector was constructed and transduced into U87 cells. A) qRT-PCR and B) western blot were performed to detect the expression of KLHL7. U87 cells transduced with KLHL7 overexpression lentiviral vector were treated alone or in combination with CB-839 (10 μM). C) Cell viability was detected using the CCK-8 assay. D, E) The cell cycle was analyzed using flow cytometry. F) A biochemical assay was used to detect the glutamine levels. Data are presented as the means ± SD from three independent experiments. *p<0.05, **p<0.01, ***p<0.001 vs. control; #p<0.05, ##p<0.01, ###p<0.001 vs. oeKLHL7

KLHL7 overexpression enhanced cell viability and cell cycle progression and increased glutamine levels by activating the β-catenin signaling pathway. U87 cells were transduced with a KLHL7 overexpressing vector and treated with the Wnt/β-catenin inhibitor XAV939. The inhibitor significantly reduced cell viability, induced cell cycle arrest in the G0–G1 phase, and decreased glutamine levels (Figures 5A–5D). XAV939 also reversed the promoting effect of KLHL7 overexpression on cell viability, cell cycle progression, and glutamine levels. Western blotting showed

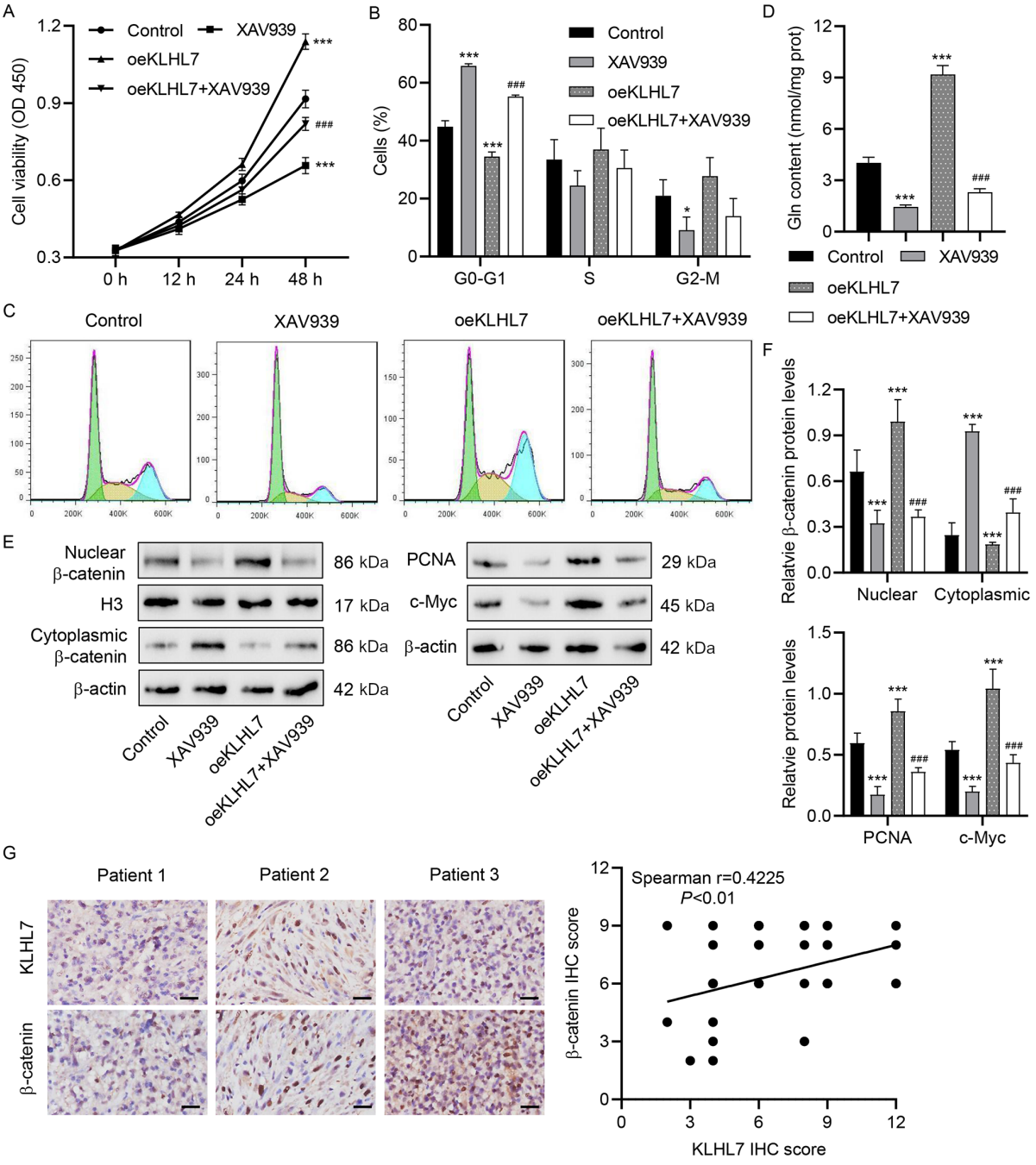

Figure 5. KLHL7 overexpression enhances cell viability and cell cycle progression and increases glutamine levels in U87 cells by activating the β-catenin signaling pathway. U87 cells were transduced with a KLHL7 overexpressing lentivirus vector and treated with XAV939. A) Cell viability was assessed using the CCK-8 assay. B, C) The cell cycle was analyzed using flow cytometry. D) The glutamine level was detected using a biochemical analysis, and E, F) western blotting was performed to determine the expression of β-catenin (nucleus/cytoplasm), PCNA, and c-Myc. G) IHC staining of human tissue microarrays was conducted to analyze the correlation between KLHL7 and β-catenin protein expression (scale bar, 100 μm). Data are presented as the means ± SD from three independent experiments. *p<0.05, ***p<0.001 vs. control; ###p<0.001 vs. oeKLHL7

that the expression levels of nuclear β-catenin, PCNA, and c-Myc were significantly increased in the KLHL7 overexpression group; this trend was reversed in the XAV939 group (Figures 5E, 5F). IHC staining of human tissue microarrays showed a significant positive correlation between KLHL7 and β-catenin protein expression (Figure 5G). These findings indicate that KLHL7 enhanced cell viability and cell cycle progression and increased glutamine levels by activating the β-catenin signaling pathway in glioma U87 cells.

Discussion

KLHL7 has been associated with various biological processes, including cell cycle, growth, and differentiation [22]. Prior studies on KLHL family-mediated ubiquitin degradation involved several types of tumors. KLHL dysregulation may be associated with cell cycle deregulation in cancers, especially in small-cell lung cancer, benign teratomas, and ovarian cancer [30]. However, the function of KLHL7 in cancer is poorly investigated, and the molecular mechanism involved remains unclear. In this study, we investigated the role of KLHL7 in human glioma. KLHL7 silencing reduced glioma cell viability, induced cell cycle arrest in the G0–G1 phase, decreased glutamine levels, delayed tumor growth in mice, and downregulated expression of PCNA and c-Myc. Our findings indicate that KLHL7 functions as an oncogene and supports glioma cell growth via glutamine metabolism by activating the Wnt/β-catenin pathway.

KLHL7 is an adapter protein for Cul3-containing ubiquitin ligase complexes and belongs to the E3 ubiquitin family. Cul3-based E3 ubiquitin ligases play a critical role in mediating targeted protein ubiquitination in eukaryotic cells; however, they have a dual role in cancer [31–33]. The abundance of KLHL7 is closely linked to tumor development and regulates both invasion and proliferation in cancer [22]. However, the roles of KLHL7 in tumor growth and its underlying molecular mechanisms in glioma remain unknown. In the present study, KLHL7 was highly expressed in glioma tissues compared to normal brain tissues. KLHL7 knockdown reduced glioma cell viability, induced cell cycle arrest, and inhibited tumor growth *in vivo*.

Glutamine is an indispensable nutrient for cell cycle progression through the G1 phase; the absence of glutamine drastically reduces cell proliferation [34–36]. Additionally, glutamine metabolism has become an important therapeutic target in cancer treatment [17]. In the current study, KLHL7 overexpression enhanced the viability and cell cycle progression of glioma cells, effects that were reversed by the glutaminase inhibitor CB-839, suggesting that KLHL7 functions as an oncogene and supports glioma cell growth via glutamine metabolism. KLHLs display different biological functions through their specific substrate recognition in multiple diseases.

Dysregulation of the Wnt/β-catenin pathway is associated with the pathogenesis of gliomas [24] and can be regulated by KLHL12 via the ubiquitin-mediated degradation of DVLs [25]. In the current study, KLHL7 overexpression enhanced glioma cell viability and cell cycle progression and increased the glutamine level, effects that were reversed by the Wnt/β-catenin inhibitor XAV939. Wnt/β-catenin-mediated glutamine metabolism plays an important role in the development and progression of cancer [18]. These data suggest that KLHL7 promotes tumor growth via glutamine metabolism by activating the β-catenin signaling pathway.

In conclusion, our data confirms that KLHL7 is overexpressed in glioma and promotes its progression by regulating the Wnt/β-catenin pathway. These findings provide insight into the mechanism of glioma progression and provide potential targets for glioma therapy.

Acknowledgments: This study was supported by the Science and Technology Bureau of Guizhou [ZK (2021) 467] and The Science and Technology Plan Project of Guizhou Health Commission (gzwkj2025-458).

References

[1] LUO Z, TIAN M, YANG G, TAN Q, CHEN Y et al. Hypoxia signaling in human health and diseases: implications and prospects for therapeutics. Signal Transduct Target Ther 2022; 7: 218. https://doi.org/10.1038/s41392-022-01080-1

[2] HUANG Y, ZHENG T, LIU Y, FANG R. Comparison of microscopic transsphenoidal surgery and neuroendoscopic transsphenoidal surgery in pituitary adenoma resection and the risk factors of postoperative cerebrospinal fluid leakage. Am J Transl Res 2023; 15: 4746–4754.

[3] CHEN B, XIA L, XU CS, XIAO F, WANG YF. Paxillin functions as an oncogene in human gliomas by promoting cell migration and invasion. Onco Targets Ther 2016; 9: 6935–6943. https://doi.org/10.2147/ott.S114229

[4] CHEON YJ, JUNG TY, JUNG S, KIM IY, MOON KS et al. Efficacy of Gamma Knife Radiosurgery for Recurrent High-Grade Gliomas with Limited Tumor Volume. J Korean Neurosurg Soc 2018; 61: 516–524. https://doi.org/10.3340/jkns.2017.0259

[5] JESUS JA, SOUSA IMO, DA SILVA TNF, FERREIRA AF, LAURENTI MD et al. Preclinical Assessment of Ursolic Acid Loaded into Nanostructured Lipid Carriers in Experimental Visceral Leishmaniasis. Pharmaceutics 2021; 13: 908. https://doi.org/10.3390/pharmaceutics13060908

[6] LI B, LI H, ZHANG L, REN T, MENG J. Expression analysis of human glioma susceptibility gene and P53 in human glioma and its clinical significance based on bioinformatics. Ann Transl Med 2023; 11: 53. https://doi.org/10.21037/atm-22-5646

[7] XU J, LYU S, ZHAO Y, ZHANG X, LIU Z et al. Ratio of CA19-9 Level to Total Tumor Volume as a Prognostic Predictor of Pancreatic Carcinoma After Curative Resection. Technol Cancer Res Treat 2022; 21: 15330338221078438. https://doi.org/10.1177/15330338221078438

[8] CONDON KJ, SABATINI DM. Nutrient regulation of mTORC1 at a glance. J Cell Sci 2019; 132: jcs222570. https://doi.org/10.1242/jcs.222570

[9] YOUNG MJ, SACHIDANANDAM R, HALES DB, BRARD L, ROBINSON K et al. Identification of Somatic Mitochondrial DNA Mutations, Heteroplasmy, and Increased Levels of Catenanes in Tumor Specimens Obtained from Three Endometrial Cancer Patients. Life (Basel) 2022; 12: 562. https://doi.org/10.3390/life12040562

[10] RAMIREZ-PEÑA E, ARNOLD J, SHIVAKUMAR V, JOSEPH R, VIDHYA VIJAY G et al. The Epithelial to Mesenchymal Transition Promotes Glutamine Independence by Suppressing GLS2 Expression. Cancers (Basel) 2019; 11: 1610. https://doi.org/10.3390/cancers11101610

[11] MORITA M, KUDO K, SHIMA H, TANUMA N. Dietary intervention as a therapeutic for cancer. Cancer Sci 2021; 112: 498–504. https://doi.org/10.1111/cas.14777

[12] HAN G, TAKAHASHI H, MURAO N, GHENI G, YOKOI N et al. Glutamate is an essential mediator in glutamine-amplified insulin secretion. J Diabetes Investig 2021; 12: 920–930. https://doi.org/10.1111/jdi.13497

[13] VERNIERI C, CASOLA S, FOIANI M, PIETRANTONIO F, DE BRAUD F et al. Targeting Cancer Metabolism: Dietary and Pharmacologic Interventions. Cancer Discov 2016; 6: 1315–1333. https://doi.org/10.1158/2159-8290.Cd-16-0615

[14] LENGAUER F, GEISSLINGER F, GABRIEL A, VON SCHWARZENBERG K, VOLLMAR AM et al. A metabolic shift toward glycolysis enables cancer cells to maintain survival upon concomitant glutamine deprivation and V-ATPase inhibition. Front Nutr 2023; 10: 1124678. https://doi.org/10.3389/fnut.2023.1124678

[15] XING J, LI J, FU L, GAI J, GUAN J et al. SIRT4 enhances the sensitivity of ER-positive breast cancer to tamoxifen by inhibiting the IL-6/STAT3 signal pathway. Cancer Med 2019; 8: 7086–7097. https://doi.org/10.1002/cam4.2557

[16] EKICI S, RISK BB, NEILL SG, SHU HK, FLEISCHER CC. Characterization of dysregulated glutamine metabolism in human glioma tissue with (1)H NMR. Sci Rep 2020; 10: 20435. https://doi.org/10.1038/s41598-020-76982-7

[17] KIM HM, JUNG WH, KOO JS. Site-specific metabolic phenotypes in metastatic breast cancer. J Transl Med 2014; 12: 354. https://doi.org/10.1186/s12967-014-0354-3

[18] LIU H, ZHANG H, LIU X, GUO W, LIU Q et al. Pancreatic stellate cells exploit Wnt/β-catenin/TCF7-mediated glutamine metabolism to promote pancreatic cancer cells growth. Cancer Lett 2023; 555: 216040. https://doi.org/10.1016/j.canlet.2022.216040

[19] WU WH, YANG YL, WANG T, SUN XM, WEI MG et al. Ginsenoside compound K restrains hepatic fibrotic response by dual-inhibition of GLS1 and LDHA. Phytomedicine 2024; 135: 156223. https://doi.org/10.1016/j.phymed.2024.156223

[20] YAO L, HAO Q, WANG M, CHEN Y, CAO H et al. KLHL29-mediated DDX3X degradation promotes chemosensitivity by abrogating cell cycle checkpoint in triple-negative breast cancer. Oncogene 2023; 42: 3514–3528. https://doi.org/10.1038/s41388-023-02858-5

[21] KIM J, TSURUTA F, OKAJIMA T, YANO S, SATO B et al. KLHL7 promotes TUT1 ubiquitination associated with nucleolar integrity: Implications for retinitis pigmentosa. Biochem Biophys Res Commun 2017; 494: 220–226. https://doi.org/10.1016/j.bbrc.2017.10.049

[22] CHEN L, LI Y, CHEN Y. KLHL7 promotes hepatocellular carcinoma progression and molecular therapy resistance by degrading RASA2. iScience 2023; 26: 106914. https://doi.org/10.1016/j.isci.2023.106914

[23] KUROZUMI S, JOSEPH C, SONBUL S, GORRINGE KL, PIGERA M et al. Clinical and biological roles of Kelch-like family member 7 in breast cancer: a marker of poor prognosis. Breast Cancer Res Treat 2018; 170: 525–533. https://doi.org/10.1007/s10549-018-4777-z

[24] TIAN Y, HU X, WANG X, HUANG Y, HAO J et al. Evaluating KLHDC8A as a biomarker for glioma: impact on survival and proliferation through the Wnt/β-catenin pathway. Discov Oncol 2024; 15: 732. https://doi.org/10.1007/s12672-024-01625-6

[25] YE G, WANG J, YANG W, LI J, YE M et al. The roles of KLHL family members in human cancers. Am J Cancer Res 2022; 12: 5105–5139.

[26] LI J, ZHANG B, FENG Z, AN D, ZHOU Z et al. Stabilization of KPNB1 by deubiquitinase USP7 promotes glioblastoma progression through the YBX1-NLGN3 axis. J Exp Clin Cancer Res 2024; 43: 28. https://doi.org/10.1186/s13046-024-02954-8

[27] ZHAO S, WANG X, WU R, WANG F, TANG X et al. KBTBD8/RRP15 as a potential novel therapeutic target associates with lenvatinib-inhibited progression in hepatocellular carcinoma both in vitro and in vivo. J Adv Res 2024; S2090-1232(24)00594-0. https://doi.org/10.1016/j.jare.2024.12.017

[28] ISAKOVA AA, DRUZHKOVA IN, MOZHEROV AM, MAZUR DV, ANTIPOVA NV et al. Glioblastoma Sensitization to Therapeutic Effects by Glutamine Deprivation Depends on Cellular Phenotype and Metabolism. Biochemistry (Mosc) 2024; 89: 1744–1758. https://doi.org/10.1134/s0006297924100079

[29] HAN S, JIN X, HU T, CHI F. ARHGAP25 suppresses the development of breast cancer by an ARHGAP25/Wnt/ASCL2 feedback loop. Carcinogenesis 2023; 44: 369–382. https://doi.org/10.1093/carcin/bgad042

[30] WEN Y, LOCKE KG, KLEIN M, BOWNE SJ, SULLIVAN LS et al. Phenotypic characterization of 3 families with autosomal dominant retinitis pigmentosa due to mutations in KLHL7. Arch Ophthalmol 2011; 129: 1475–1482. https://doi.org/10.1001/archophthalmol.2011.307

[31] SENFT D, QI J, RONAI ZA. Ubiquitin ligases in oncogenic transformation and cancer therapy. Nat Rev Cancer 2018; 18: 69–88. https://doi.org/10.1038/nrc.2017.105

[32] BULATOV E, CIULLI A. Targeting Cullin-RING E3 ubiquitin ligases for drug discovery: structure, assembly and small-molecule modulation. Biochem J 2015; 467: 365–386. https://doi.org/10.1042/bj20141450

[33] ABDALLA O, MASCARENHAS B, CHENG HM. Death of a Protein: The Role of E3 Ubiquitin Ligases in Circadian Rhythms of Mice and Flies. Int J Mol Sci 2022; 23: 10569. https://doi.org/10.3390/ijms231810569

[34] MA C, GURKAN-CAVUSOGLU E. A comprehensive review of computational cell cycle models in guiding cancer treatment strategies. NPJ Syst Biol Appl 2024; 10: 71. https://doi.org/10.1038/s41540-024-00397-7

[35] LI X, PENG X, LI Y, WEI S, HE G et al. Glutamine addiction in tumor cell: oncogene regulation and clinical treatment. Cell Commun Signal 2024; 22: 12. https://doi.org/10.1186/s12964-023-01449-x

[36] SOLOMON LA, PODDER S, HE J, JACKSON-CHORNENKI NL, GIBSON K et al. Coordination of Myeloid Differentiation with Reduced Cell Cycle Progression by PU.1 Induction of MicroRNAs Targeting Cell Cycle Regulators and Lipid Anabolism. Mol Cell Biol 2017; 37: e00013–17. https://doi.org/10.1128/mcb.00013-17