Biologics: Targets and Therapy

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ORIGINAL RESEARCH

LncRNA GAS5 Modulates the Progression of Glioma Through Repressing miR-135b-5p and Upregulating APC

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Purpose: The main purpose of this paper is to explore the interaction between GAS5 and miR-135b-5p to understand their function in the metastasis, invasion, and proliferation of glioma. This may provide new ideas for the pathogenesis and treatment of glioma.

Patients and Methods: Western blotting assays and RT-qPCR were employed to investigate the expression of related genes in glioma tissues or cell lines. CCK-8 was used to examine the impact of GAS5 on cell viability. Motile activities were adopted by the transwell and wound healing experiments. A double luciferase experiment was performed to elucidate transcriptional regulation.

Results: GAS5 showed low expression in glioma cells and tissues, and up-regulation of GAS5 could depress the invasion, proliferation, and metastasis of glioma. GAS5 negatively regulates miR-135b-5p, which can counteract the cellular effects caused by GAS5. APC was the target of miR-135b-5p, and GAS5 can regulate the expression of APC by sponging miR-135b-5p. APC overexpression reversed the effects of miR-135b-5p promotion on glioma cells, while miR-135b-5p has the opposite function. As a downstream target gene of GAS5, miR-135b-5p was negatively regulated by GAS5. The restoration of miR-135b-5p can remarkably reverse the impact of GAS5 on glioma cells. In addition, GAS5 increased the expression of APC in glioma cells by inhibiting miR-135b-5p.

Conclusion: GAS5 increased APC expression by restraining miR-135b-5p and partially blocked the progression of glioma, suggesting that it could be an advantageous therapeutic target for glioma intervention.

Keywords: glioma, lncRNA GAS5, miR-135b-5p, APC

Introduction

Gliomas are responsible for the majority of deaths from primary brain tumours.[1] Gliomas originate from glial cells or stem cells and are the most common primary tumors of the brain and spinal cord,[2] accounting for 80% of primary malignant tumors of the brain.[3] The main glial tumor groups included oligodendroglial tumors, astrocytic tumors, oligoastrocytic tumors, neuronal tumors, ependymal tumors, and mixed neuronal-glial tumors. Among adults with primary intracranial tumors, gliomas account for the majority.[4] For children, the most common glioma types are pilocytic astrocytomas and diffuse midline gliomas including diffuse intrinsic pontine gliomas of various grades.[1] The incidence of gliomas in general increases with age, with the most pronounced increase in glioblastoma. Many environmental factors have been studied in relation to glioma, but ionizing radiation (exposure to therapeutic doses) is the only established factor that has been identified as a causative agentitive factor.[5–7] Genome-wide association studies have shown that glioma risk is associated with single-nucleotide polymorphisms (SNPs).[8,9] In addition, telomerase RNA component (TERC) and telomerase reverse transcriptase (TERT), which are involved in telomere length regulation, were identified as candidate genes for increased glioma risk.[10,11]

The World Health Organization (WHO) classifies gliomas into four different grades based on tumor mitotic rate, necrosis, histology, angiogenesis, and nuclear atypia.[12] The malignant degree of glioma is strongly linked to prognosis.[13] To date, the conventional therapy for glioma consists of surgical resection, temozolomide (TMZ), and radiation, which is far from sufficient in combating cancer development.[14] The recurrence rate of patients is high due to the depth of glioma

Received: 16 January 2024
Accepted: 23 April 2024
Published: 23 May 2024

malignancy, and the 5-year survival rate is no more than 5%. Early identification and treatment of these tumors will greatly improve the treatment effect. There is an urgent need for new diagnostic and prognostic markers to identify the disease at an early stage and to differentiate subtypes of these tumors, thereby improving the current treatment modalities.[15]

LncRNAs are more than 200 bases in length and are noncoding RNAs. LncRNAs can widely participate in the regulation and expression of genes in eukaryotic cells. Additionally, abnormal expression of lncRNAs is strongly linked to the appearance and progression of tumors and is regarded as an emerging biomarker and potential therapeutic target in cancer epigenetics.[16] Long noncoding RNA growth arrest-specific 5 (GAS5) is known to act as a tumor suppressor and apoptosis promoter, and its downregulation is involved in a variety of tumors, such as lymphoma, cervical carcinoma, and osteosarcoma.[17–19] GAS5 exerts biological functions mainly through its intron encoding multiple snoRNAs.[20] In MA's study,[17] GAS5 inhibited the expression of miR-221-3p and upregulated the expression of IRF2 in non-small cell lung cancer. GAS5 also acted as a microRNA-23a sponge to promote autophagy and enhance autophagosome formation after GAS5 overexpression in breast cancer.[21] In addition, GAS5 slowed glioma progression by eradicating Sirtuin 1 and microRNA-10b in A172 and U251 cells.[22] As a competitive endogenous RNA, GAS5 restrained the proliferation and invasion of osteosarcoma cells through a combination of miR-23a-3p and promoted PTEN expression by mediating the PI3K/AKT pathway.[22] Moreover, GAS5 targets miR-106b to regulate the expression of PTEN, thus affecting the progression of EMT, as well as the invasion, migration, and proliferation of glioma cells.

MicroRNAs are noncoding small RNAs containing 20 ~ 40 nucleotides. Most of them have important functions.[23,24] In particular, diverse functions of miRNA have been reported in many diseases, including apoptosis, migration, differentiation, proliferation, and invasion.[25,26] Due to their essential role in eukaryotic cells, dysregulation of miRNAs can lead to the activation of oncogenes or suppressors in tumor diseases.[27–29] MiR-135b-5p is a conserved transcript among mammalians and is located in the gene locus of 1q32.1 in humans.[30] In gastric cancer, miR-135b-5p upregulates and maintains the expression of Kruppel-like factor 4 (KLF4), thereby promoting the proliferation, viability, migration, and invasion of cancer cells.[31] The expression of miR-135b-5p was significantly up-regulated in feces of CRC patients, and miR-135-5p can directly target the mRNA of ZNRF3, thereby activating the Wnt pathway. MiR-135b-5p is expected to be a non-invasive biomarker for the diagnosis of colorectal cancer patients at stage TNM III/IV and a potential candidate for colorectal cancer intervention strategies.[32]

The adenomatous polyposis coli (APC) gene is a tumor suppressor gene located in the human chromosome region 5q21–22,[33,34] which plays a vital role in cellular proliferation, migration, DNA repair, and chromosomal segregation.[35] One of the best-known mechanisms of APC is the regulation of the Wnt/b-catenin signaling pathway.[36] A mutation in APC in mouse intestinal epithelial cells can decrease the level of E-cadherin at the cell membrane and association between catenin and E-cadherin,[37] thus affecting cell adhesion. Mutations in APC are usually deletion, insertion, or frameshift, which introduce premature stop codons and lead to the production of truncated APC proteins that lack normal function and have tumorigenic properties.[38] APC is lost in a variety of cancers, including breast, colorectal, and prostate cancers, and its loss leads to decreased overall survival in patients with non-small cell lung cancer and breast cancer.[39] This implies that APC- deficient patients have a worse prognosis than APC-competent patients. In recent years, it has been found that APC gene mutation status can be used as a predictor of poor prognosis in patients with stage III colorectal cancer.[40] However, the regulatory mechanism of APC in glioma remains unclear.

Although the role and mechanics of GAS5 in glioma have been reported, whether miR-135b-5p is involved in the development and progression of glioma and whether GAS5 can target and regulate miR-135b-5p to meditate the progression of glioma have not been studied. In our study, we researched the biological functions and expression characteristics of miR-135b-5p and GAS5 in glioma cells and tissues, and also explored the regulatory function of GAS5 on miR-135b-5p, further proving that GAS5 can inhibit the progression of glioma by reducing miR-135b-5p and facilitating the expression of APC.

Materials and Methods

Cell Culture and Tissue Collection

Twenty-two glioma tissues and paired corresponding normal tissues were collected from the Third Affiliated Hospital of Chongqing Medical University. Tissue samples were conserved in 2 mL cryotubes in liquid nitrogen for 1 hour and then

transferred to −80°C. The human glioma cell lines T98, LN229, A172, and U251 and the normal human astrocyte cell line (NHA) were acquired from the China Center for Type Culture Collection (Wuhan, China). Glioma cell lines were cultivated in high glucose DMEM containing 10% FBS (Moregate, Australia), and NHAs were grown in AM medium (Astrocyte Medium, ScienCell, USA) at 37°C and 5% CO_2.

Cell Transfection

GAS5 overexpression plasmid, sh-GAS5, miR-135b-5p inhibitor, miR-135b-5p mimic, APC overexpression plasmid, and their controls were purchased from Gene Pharma (Shanghai, China). The above plasmids and controls were transfected into T98 or A172 cells using Lipofectamine 3000 (Thermo, USA).

Reverse Transcription-Quantitative (RT-qPCR)

Total RNA from cell lines and tissues was extracted using a TRIzol reagent kit (Invitrogen, USA) according to the manufacturer's instructions. The primers were designed and provided by Sangon Biotech, Shanghai. qRT-PCR was performed using SYBR Green I (Takara, Japan). The miRNA and mRNA expression levels were normalized against U6 and GAPDH, respectively.[31,41–44] Reaction conditions for qRT-PCR were as follows: 95°C 30s, 55°C 30s, and 72°C 90s, for a total of 40 cycles. All samples were repeated three times, and the primers used in this study are listed in Table 1.

Cell Counting Kit-8 Assay

Cells in the logarithmic phase of growth (2000 cells/well) were cultured on 96-well plates filled with 150 μL complete medium for 1 day. After different treatments, CCK-8 solution (10 μL, Sigma, Germany) was added and cultured for 4 h at 37°C. After that, the optical density of each well at 450 nm was detected.

Wound Healing Assay

First, cells were seeded into 6-well plates. A line was drawn in the cell surface with a pipette tip (200 μL) when the cells reached 70–85% confluence. After 24 h, the scratch width was measured to assess wound healing, photographs were taken, and ImageJ was used to evaluate the gap distance.

Transwell Assay

The polycarbonate membrane bourdon chamber in the transwell apparatus was used to detect cell infiltration. A total of 5 × 10^4 cells were plated in the upper chambers, while 100 μL of DMEM with 10% FBS was added to the lower chambers. After 24 h of incubation at 37°C, a cotton swab was used to remove the cells on the top of the membrane, and then the cells were fixed with 4% paraformaldehyde followed by staining in crystal violet. Finally, the cells were observed and photographed under an inverted microscope (×100).

Table 1 The Primer Sequences Used in Study

Gene	Primer sequence
GAS5	F: 5′-CTTGCCTGGACCAGCTTAAT-3′
	R: 5′- CAAGCCGACTCTCCATACCT-3′
APC	F: 5′-AGGGTGTCACTGGAGACAGA-3′
	R: 5′-TCTTCAGTGCCTCAACTTGCT-3′
U6	F: 5′-CTCGCTTCGGCAGCACA-3′
	R: 5′-AACGCTTCACGAATTTGCGT-3′
GAPDH	F: 5'-GTTCGTCATGGGTGTGAACC-3'
	R: 5'-CATCCACAGTCTTCTGGGTG-3'
miR-135b-5p	F: 5'-TGCGATATGGCTTTTCATTCCTA-3'
	R: 5'-CGCAGGGTCCGAGGTATTC-3'

Dual-Luciferase Reporter Gene Experiment

The mutant (MUT) or wild-type (WT) binding sequence between GAS5 and miR-135b-3p or APC 3'-UTR and miR-135b-3p was linearized with restriction enzymes and cloned and inserted into the pmirGLO dual luciferase vector. These reporters were cotransfected with miR-135b-5p mimics or mimics-NC using Lipofectamine 3000 reagent. Then, after 48 h, a Dual luciferase Reporter Assay System kit (Promega) was used to measure luciferase activity. The experiment was conducted three times in total.

Western Blot

Cells were lysed with RIPA containing protease inhibitor PMSF (MCE, USA) to lyse cells. Total protein lysates were quantified according to the BCA kit (MCE, USA). Equal amounts of proteins mixed with SDS–PAGE loading buffer were transferred onto PVDF membranes. The membrane was then blocked with 5% nonfat milk and incubated with the primary antibody (1:1000) overnight at 4°C. Then, the secondary antibody (1:10,000) was added after washing, and another 1 h was needed for incubation. The experiment was conducted three times in total.

Statistical Analysis

Data were expressed as the mean ± standard deviation of three repeated experiments. All data were analyzed using GraphPad Prism 8.0. Student's *t*-test was performed to evaluate the differences between the two groups. Multiple comparisons were conducted with 1-way ANOVA, followed by Tukey post hoc test. $P<0.05$ was considered statistically significant.

Results

GAS5 Was Abnormally Decreased in Glioma Tissues and Cells and Related to a Low Survival Rate

Data analysis using CGGA (http://www.cgga.org.cn/) database indicated that patients with high expression of GAS5 had a significantly higher survival rate (Figure 1A). To examine the level of GAS5 in glioma tissues and cells, quantitative PCR was used. The data indicated that GAS5 expression in glioma tissues and cells was markedly decreased (Figure 1B) indicating that GAS5 may be a potential factor promoting glioma deterioration.

Up-Regulation of GAS5 Suppressed the Metastasis, Invasiveness, and Proliferation of Glioma Cells

Firstly, GAS5 overexpression vector and shRNA vector were constructed to investigate the biological role of GAS5 in glioma. After transfection, Western blot and RT-qPCR verified that the overexpression and knockdown effects of the vectors were satisfactory in T98 and A172 cells, respectively (Figure 2A). Subsequently, CCK-8 and transwell experiment showed that the overexpression of GAS5 in T98 cells reduced cell proliferation, migration, and invasion, while the

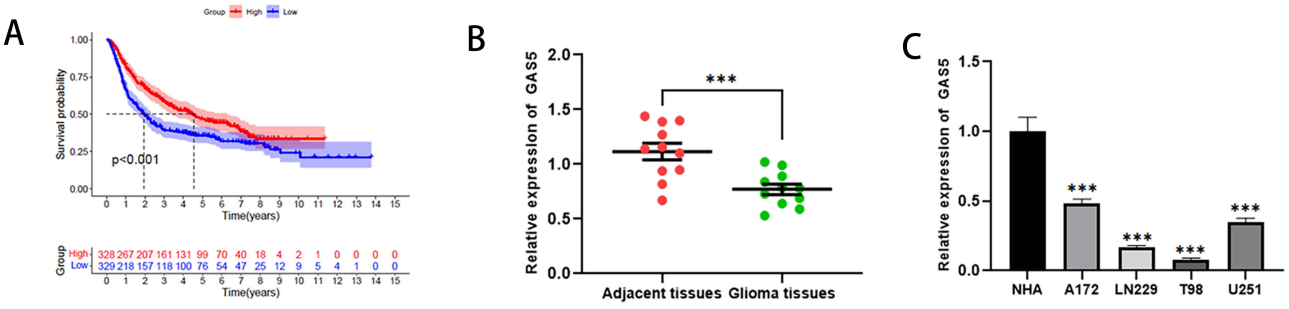

Figure 1 Reduced GAS5 in glioma tissues is related to low survival rates. (**A**) Correlation between GAS5 expression and overall survival was significantly higher in patients with high expression of GAS5. (**B**) The mRNA expression level of GAS5 in glioma tissues and adjacent tissues. (**C**) The mRNA expression level of GAS5 in normal glial and glioma cells. *** means $P < 0.001$.

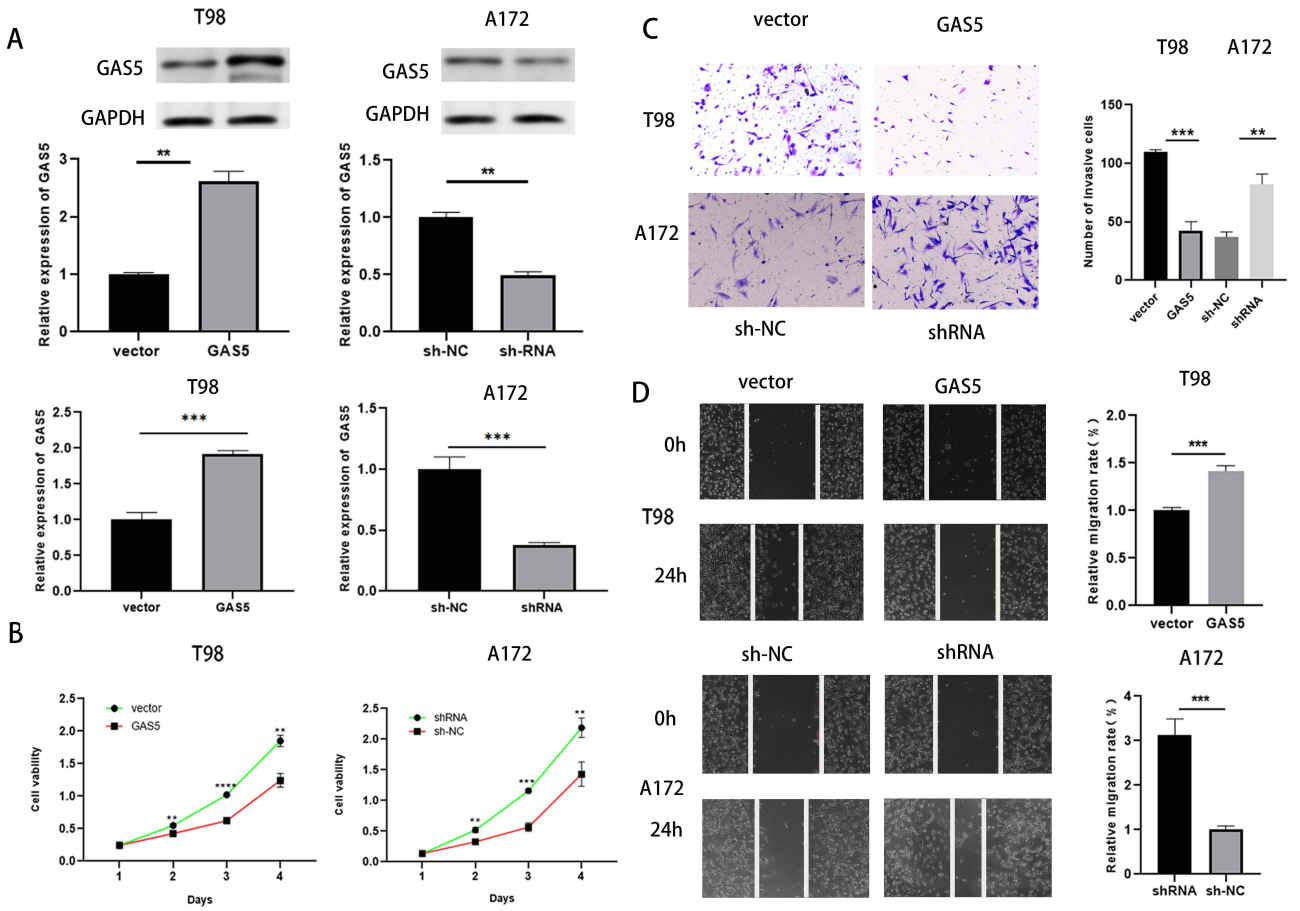

Figure 2 GAS5 suppressed the invasion, propagation, and migration of glioma cells. (**A**) Western blot and qRT-PCR were implied to detect the overexpression and knockdown efficiency of GAS5. (**B, C, D**)CCK-8, scratch test (magnification, x40) and transwell assay (magnification, x100) were applied to detect cell proliferation, invasion and migration ability after overexpression or knockdown of GAS5. ** means $P < 0.01$, *** means $P < 0.001$.

knockdown of GAS5 in A172 cells showed the opposite phenomenon (Figure 2B-D). In conclusion, up-regulation of GAS5 inhibited the proliferation, migration, and invasion of glioma cells.

GAS5 Acted as a Molecular Sponge for miR-135b-5p

The miRNA related to lncRNA GAS5 was searched in StarBase v2.0, and referring to literature reports,[32] we found that GAS5 had a complementary sequence to miR-135b-5p (Figure 3A). We subsequently performed a dual luciferase validation experiment. Compared with the control, the miR-135b-5p mimic obviously weakened the luciferase activity of WT-GAS5 but not that of GAS5-MUT in A172 cells (Figure 3B). RT-qPCR results showed that compared with the adjacent tissues and NHA cells, miR-135b-5p in glioma tissues and cells increased significantly (Figure 3C). Additionally, the overexpression and knockdown of GAS5 triggered the up-regulation and declined of miR-135b-5p and there was a negative correlation between them (Figure 3D). These results illustrated that miR-135b-5p was a target of GAS5, which is a natural sponge of miR-135b-5p.

GAS5 Regulated Cell Proliferation, Migration, and Invasion by Sponging miR-135b-5p

To further investigate the role of GAS5 and miR-135b-5p in glioma progression, we constructed a functional gain model of miR-135b-5p. By analyzing the RT-qPCR results, overexpression effects were significant (Figure 4A). We then performed reverted experiments to explore the regulation of GAS5 on miR-135b-5p. The result showed that the cell viability of GAS5+miR-135b-5p_mmic group was clearly higher contrasted to GAS5 group (Figure 4B). The

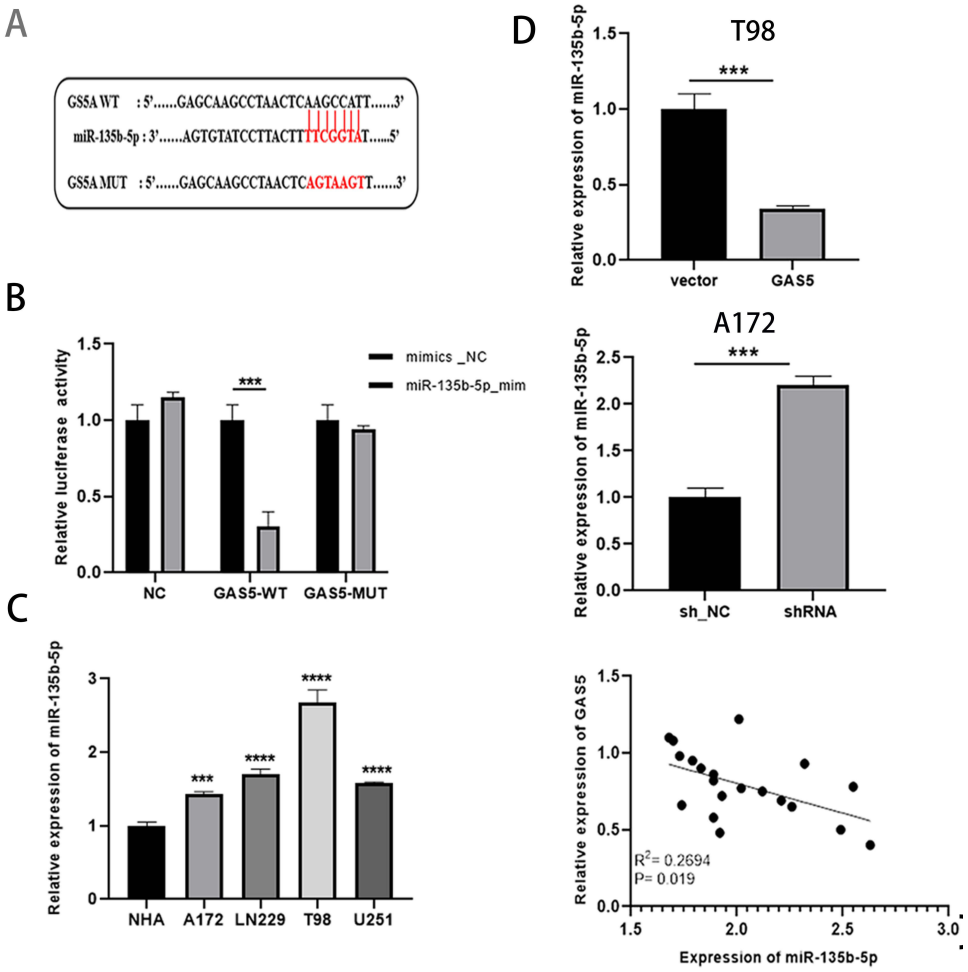

Figure 3 GAS5 acted as a molecular sponge for miR-135b-5p. (**A**) Starbase was used to predict the target sites between GAS5 and miR-135b-5p. (**B**) Dual luciferase reporter assay was used to verify the binding of GAS5 to miR-135b-5p. (**C**) qRT-PCR was taken to detect the expression of miR-135b-5p in glioma cells and NHA. (**D**) The expression of miR-135b-5p was determined by qRT-PCR after GAS5 overexpression and knockdown, and there was a negative correlation between them. *** means $P < 0.001$, **** means $P < 0.0001$.

cotransfection of GAS5 and miR-135b-5p_mmic rescued GAS5-induced invasion and migration inhibition of T98 cells (Figure 4C). Taken together, GAS5 regulated the evolution of glioma cells dependent on sponging miR-135b-5p.

MiR-135b-5p Targeted APC and GAS5 Regulated APC Expression by Targeting miR-135b-5p

Through a literature review and database prediction,[19] we found that 3′UTR of APC has a binding target for miR-135b-5p in sequence (Figure 5A). Moreover, there was no correlation between APC expression level and the overall survival rate of glioma patients (Figure 5B). We performed a dual luciferase assay to verify whether APC could combine with miR-135b-5p. Compared with mmic_NC, transfection of miR-135b-5p_mmic significantly inhibited the luciferase activity of APC-WT group, but not APC-MUT group (Figure 5C), suggesting the interaction between miR-135b-5p and APC. Whereafter, APC expression was clearly decreased in glioma tissues and cells compared with adjacent tissues and NHA cells, respectively (Figure 5D). Data analysis obtained from TCGA database showed that there was no significant correlation between GAS5 and APC expression levels in glioma samples (Figure 5E). Western blot result showed that APC was down-regulated after overexpression of miR-135b-5p and increased after the knockdown of miR-135b-5p (Figure 5F). Furthermore, the overexpression of GAS5 elevated APC, while co-transfection of GAS5 and miR-135b-5p_mmic

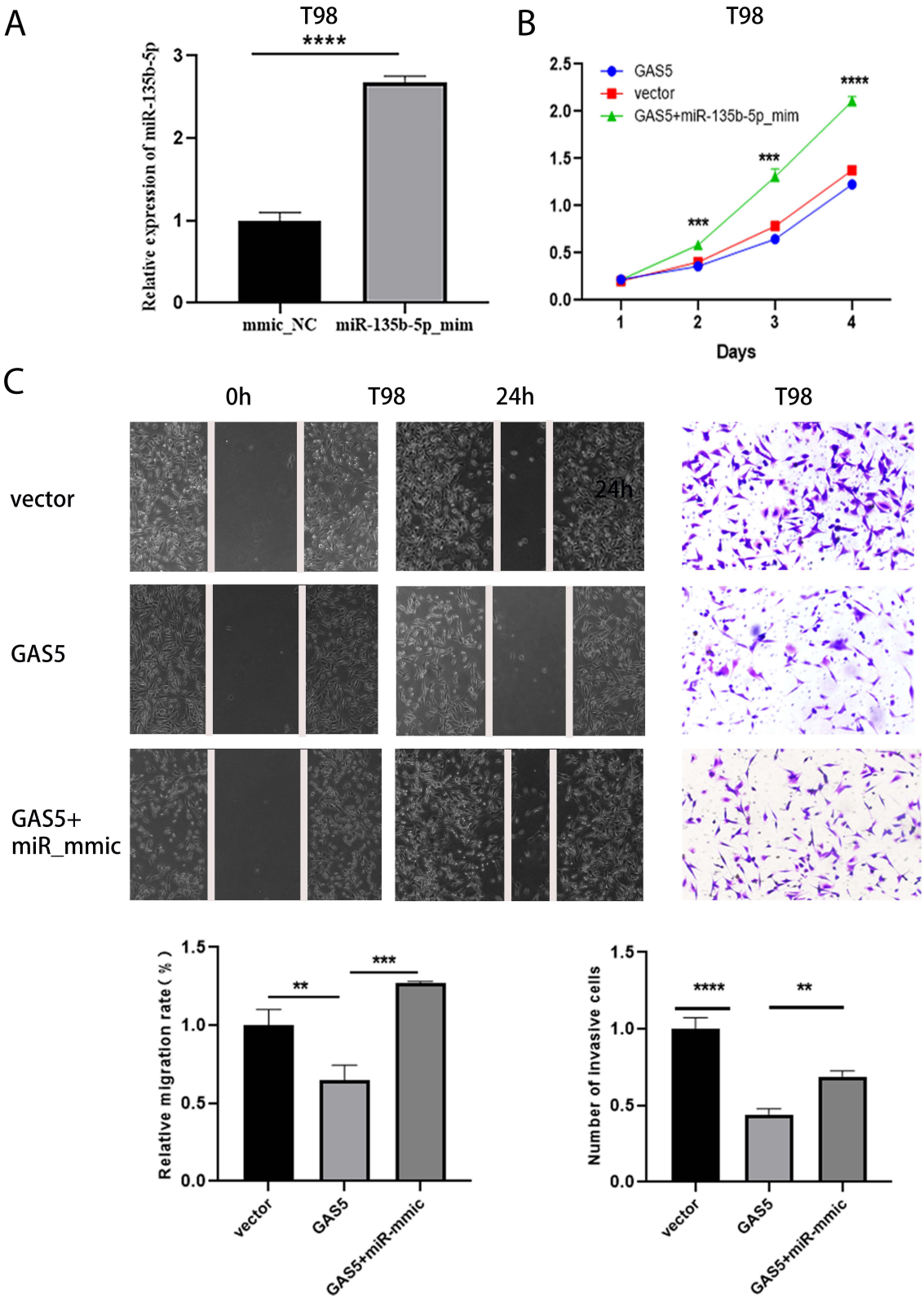

Figure 4 GAS5 regulated cell proliferation, migration and invasion by sponging miR-135b-5p. (**A**) qRT-PCR was used to detect the transfection efficiency of miR-135b-5p. (**B, C**) CCK-8, scratch test (magnification, x40) and transwell assay (magnification, x100) were applied to detect cell proliferation, invasion and migration ability after transfection with vector, GAS5, GAS5+ miR-135b-5p_mmic. ** means $P < 0.01$, *** means $P < 0.001$, **** means $P < 0.0001$.

https://doi.org/10.2147/BTT.S454058 DovePress

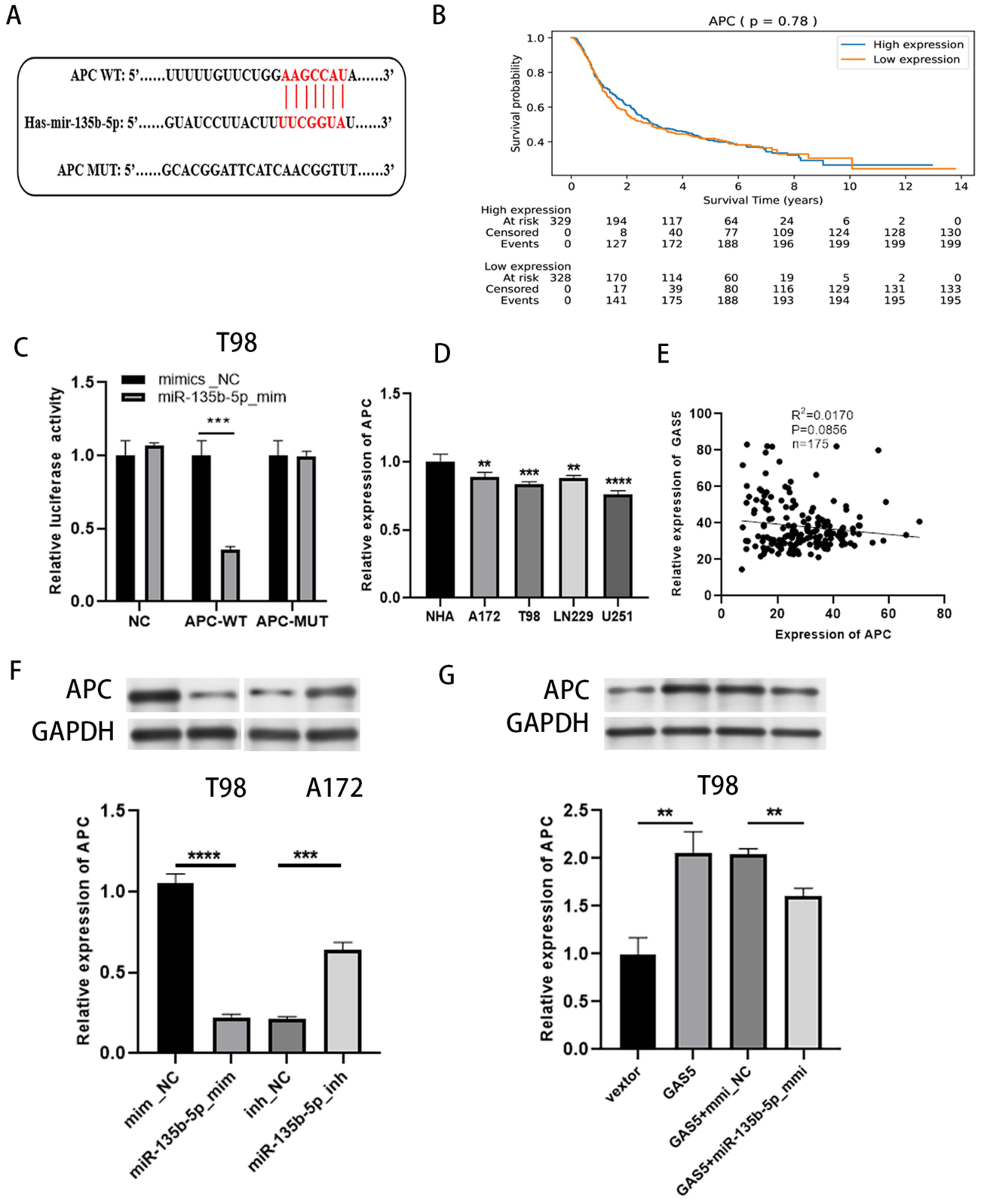

Figure 5 MiR-135b-5p targeted APC and GAS5 regulated APC expression by targeting miR-135b-5p. (**A**) Starbase was taken to predict the biding sites between APC and miR-135b-5p. (**B**) CCGA database analysis the relationship between APC expression level and survival rate of glioma patients. (**C**) Dual luciferase reporter gene assay was implied to exam the interaction between miR-135b-5p and APC. (**D**) The expression of APC in glioma cells and NHA was detected by qRT-PCR. (**E**) Relationship between GAS5 and APC expression levels in glioma samples from TCGA database. (**F**) Western blot was taken to detect the APC expression after increase or inhibition of miR-135b-5p. (**G**) The expression of APC was detected by western blot after transfected with GAS5, GAS5+ miR-135b-5p_mmic or controls in T98 cells. ** means P < 0.01, *** means P < 0.001, **** means P < 0.0001.

attenuated the effect (Figure 5G). In conclusion, APC was a target of miR-135b-5p and GAS5 indirectly promoted the expression of APC by sponging miR-135b-5p.

Increasement of APC Attenuated the Effects of miR-135b-5p Overexpression on Glioma Cells

The expression level of APC was detected after transfecting the overexpression plasmid of APC into T98 cells, and the RT-qPCR result was satisfactory (Figure 6A). Subsequently, CCK-8, scratch test and transwell assay showed that overexpression of miR-135b-5p could promote cell proliferation, migration, and invasion, but cotransfection of APC could attenuate this effect (Figure 6B-C).

Discussion

As reported by the World Health Organization (WHO), glioma is one of the most common primary brain tumors in adults.[45] Epigenetic alterations, including DNA methylation and posttranscriptional modifications, play important functions in the etiology and biology of gliomas.[46] In recent years, molecular markers of glioma, such as O6-methylguanine-DNA methyltransferase (MGMT), phosphatase and tensin homolog (PTEN), and isocitrate dehydrogenase (IDH), have become increasingly important in predicting treatment outcome, prognosis, and diagnosis.[47] Tumor targeted therapy is a new treatment strategy for glioma that uses drugs or other substances to identify and destroy cancer cells without compromising the survival and overall survival of normal healthy cells.[48] Pathways involved in tumor growth, invasion, and angiogenesis processes represent major processes in glioma.[49] Multi-target kinase inhibitors or single-target kinase inhibitors combined with multiple signaling pathways can improve the therapeutic effect.[49] Additionally, the prognosis for high-grade gliomas is not satisfying. Uncovering the underlying mechanisms may help to explore specific therapeutic strategies.[50] GAS5, a well-known lncRNA that functions as a cancer suppressor, was inhibited in multiple tumors, including lung cancer, gliomas, gastric cancer, prostate cancer, etc.[20] Chen L et al demonstrated that GAS5 overexpression sensitizes A549 cells to radiotherapy through regulating miR-21/PTEN/Akt axis.[51] Another report proved that LncRNA GAS5 modulates the progression of non-small cell lung cancer through repressing miR-221-3p and up-regulating IRF2.[52] Currently, numerous studies generally agree that in glioma cells, GAS5 is expressed at low levels; additionally, downregulation of GAS5 can act as a predictive warning factor for poor prognosis when glioma is of low grade.[53–55] Consistent with these statements, the impacts of GAS5 on suppressing cell migration, migration, and invasion of glioma cells were exhibited in our current study. We also confirmed that GAS5 was associated with the survival rate of glioma patients.

Duan et al found that miR-498 promoted the proliferation, migration, and invasion of prostate cancer cells and reduced radiosensitivity by targeting PTEN.[56] Accumulated reports have shown that numerous abnormally expressed miRNAs were discovered in gliomas through RNA sequencing, proving that miRNAs may be associated with the formation and deterioration of gliomas.[37,38] It has been certified that lncRNA often rely on acting as sponges for miRNAs to play a role in cancers.[57,58] Accumulated reports have shown that numerous abnormally expressed miRNAs were discovered in gliomas through RNA sequencing, proving that miRNAs may be associated with the formation and deterioration of gliomas.[37,38] For example, GAS5 can regulate numerous pathological and physiological processes in cells by targeting numerous miRNA molecules.[59] In lung cancer, GAS5 can sponge miR-205 and regulate PTEN expression to affect cell proliferation and metastasis.[60] Chen et al showed that GAS5, through promoting the expression of miR-424, suppressed the progression of glioma cells and restrained xenograft growth in vivo.[61] In the present work, we verified that miR-135b-5p was the downstream target of GAS5, and there is a negative correlation. This finding has not been previously reported. It has been proven that up-regulation of miR-135b-5p promotes cancer metastasis in lung cancer, neck squamous, and head cell carcinoma.[62,63] We found that the expression level of miR-135b-5p in glioma cells was significantly higher than that in NHA cells. However, its expression data in clinical samples could not be found in multiple databases, which limited the validation of some of our findings. Besides, miR-135b can inhibit the development of tumors and reverse drug resistance.[64–66] Though miR-135b-5p has been found to modulate gene expression in various cancers, the role in glioma has not been reported. This work provided a certain basis for its cancer-promoting effect. We demonstrated that the expression of miR-135b-5p was increased in glioma tissues and cells, and it facilitated the malignant phenotypes of glioma cells. Moreover, overexpression of miR-135b-5p reversed the effect of GAS5 on glioma cells. Based on

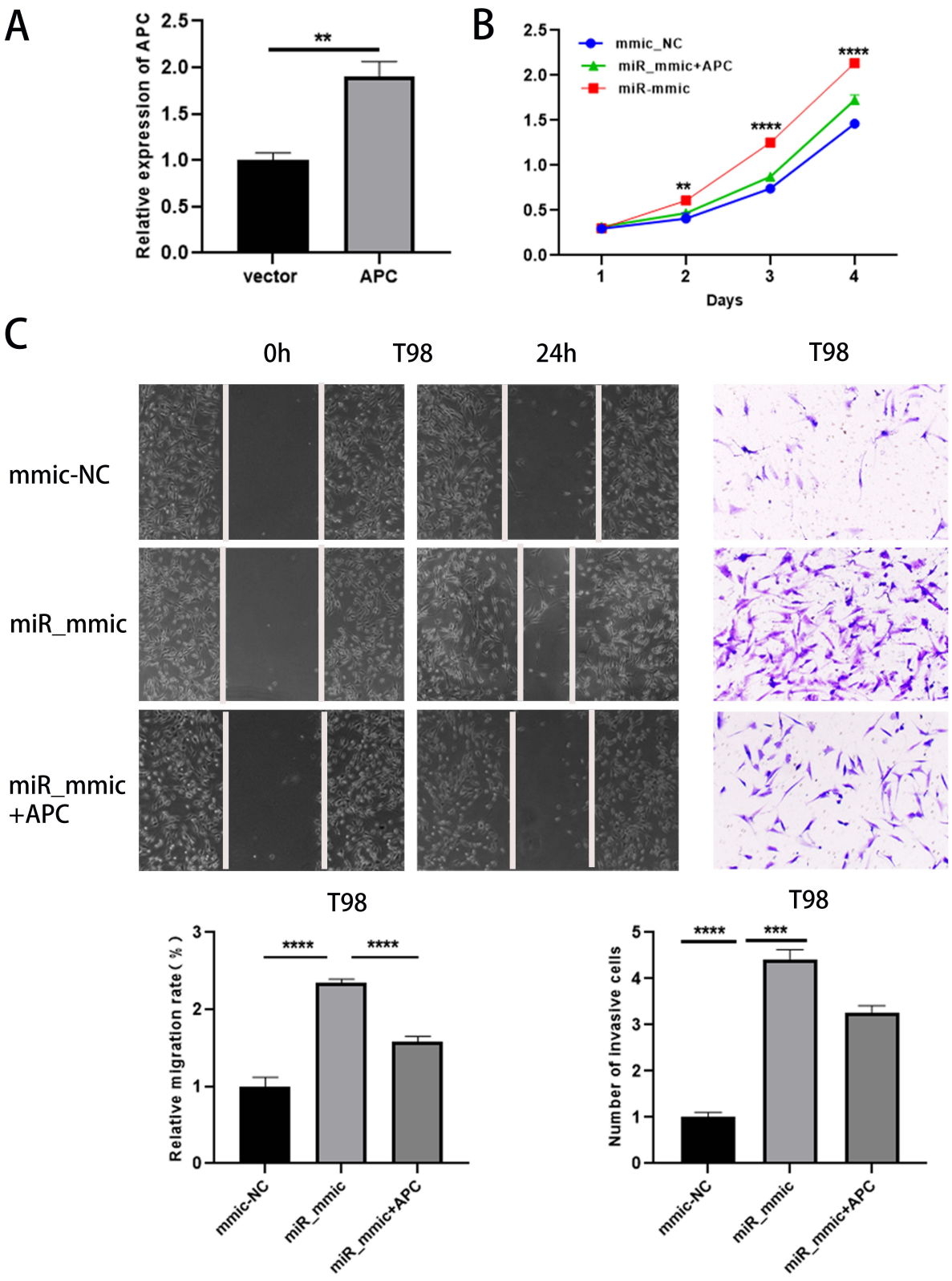

Figure 6 Increasement of APC attenuated the effects of miR-135b-5p overexpression on glioma cells. (**A**) The efficiency of APC overexpression was detected by qRT-PCR. (**B, C**) CCK-8, scratch test (magnification, x40) and transwell assay (magnification, x100) were performed to detect the proliferation, migration and invasion ability after transfection with mmic_NC, miR_mmic, miR_mmic+APC in T98 cells. ** means $P < 0.01$, *** means $P < 0.001$, **** means $P < 0.0001$.

these results, we proposed that miR-135b-5p was an oncomiR in glioma, and the effect of GAS5 were obtained by sponging miR-135b-5p.

MiRNAs play an important regulatory role in the occurrence and development of tumors by targeting the 3'-untranslated region (UTR) of messenger RNA (mRNA). Wang et al demonstrated that miR-135b-5p overexpression reduces chemoresistance via directly degrading the mRNA of upstream regulator of integrin subunit alpha 2 (ITGA2) in gastric cancer.[67] Duan et al found that miR-498 promoted the proliferation, migration, and invasion of prostate cancer cells and reduced radiosensitivity by targeting PTEN.[56] Li et al proposed that miR-135b-5p expression downregulates PPM1E to activate AMPK signaling, which inhibits LPS-induced TNF-α production via suppression of ROS production and NF-κB activation.[68] Jin et al have confirmed that miR-135b can affect tumor metastasis through the Wnt pathway.[69] β-catenin is a positive regulator of Wnt pathway, while APC is an important negative regulator, which has been confirmed by some studies.[70–72] APC lost by upregulation of miR-135 contributes to the development of colorectal, breast, and gastric cancers.[30,73,74] In glioma cells, we found that APC was the target gene of miR-135b-5p, and APC decreased with the overexpression of miR-135b-5p. This regulatory mechanism has also been validated in diffuse large B cell lymphoma.[44] In addition, we certified that overexpression of GAS5 led to an increase in APC, but this effect was reversed by miR-135b-5p_mmic. Meanwhile, TCGA data analysis showed that there was no significant correlation between the expression levels of GAS5 and APC in glioma clinical samples, and there was no significant correlation between APC and glioma patient survival after analyzing the CGGA database. These results are not what we expected. It is possible that the expression of APC is related to other factors, which needs further study. In conclusion, GAS5 indirectly regulates APC through sponging miR-135b-5p.

This study has some limitations. Firstly, although APC is generally considered a negative regulator of the Wnt pathway, we did not explore Wnt/β-contenin pathway changes. Because APC is a multi-domain protein, it contains binding sites for many proteins, including the Wnt/Wg pathway components β-catenin and axin, microtubules, the cytoskeletal regulators IQGAP1 and EB1, and the Rac guanine-nucleotide-exchange factor (GEF) Asef1. It has been proposed that mutations in APC contribute to cancer development through processes other than Wnt signaling. We were discussing and designing the validation of regulatory mechanisms independent of the WNT pathway, which would be a huge job, so we decided to do it separately. Secondly, there is a lack of in vivo experimental validation, which is also an important work that we intend to study separately at a later stage. Last but not least, the regulatory functions of GAS5 in glioma cells, such as chemoresistance, apoptosis, cell cycle progression, and immune evasion, need to be further studied.

Conclusion

In summary, we unraveled the molecular mechanism of GAS5 affecting glioma progression by sponging miR-135b-5p and regulating APC through a suite of in vitro experiments, which is of great significance for elucidating the mechanism of glioma development. In addition, this study provided a reference for the prognosis evaluation of glioma patients and new ideas and directions for clinical multi-target treatment of glioma.

Abbreviations

GAS5, Long noncoding RNA growth arrest-specific 5; APC, Adenomatous polyposis coli; GAPDH, Glycine-3-proline dehydrogenase; CGGA, Chinese Glioma Genome Atlas.

Ethics Approval and Consent for Participation and Publication

The research was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of The Third Affiliated Hospital of Chongqing Medical University (ethics number: KLYS (2021) 80). All 22 patients signed informed consent for participation and publication.

Acknowledgments

We would like to thank the authors for their contributions and the support of The Third Affiliated Hospital of Chongqing Medical University.

Funding

The research was sponsored by Science and Technology project of Yubei District, Chongqing (2022-NS-24).

Disclosure

The authors report no conflicts of interest in this work.

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