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Original Article

LncRNA SNHG6 inhibits autophagy of gastric carcinoma cells via PI3K/AKT/mTOR signaling pathway

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Abstract: Objective: To investigate the role of lncRNA SNHG6 (SNHG6) in gastric carcinoma (GC) and its relationship with the PI3K/AKT/mTOR signaling pathway in order to provide more comprehensive and reliable reference for the diagnosis and treatment of GC. Methods: GC patients admitted to our hospital from May 2017 to August 2018 as well as healthy individuals who underwent physical examinations during the same time period were enrolled in this study. The serum SNHG6 level was quantified. Patients were followed up for 3 years to analyze the significance of SNHG6 in the diagnosis and treatment of GC. Finally, *in vitro* assays were performed to determine the influences of SNHG6 and PI3K/AKT/mTOR signaling pathway on biological behaviors and autophagy ability of GC cells. Results: SNHG6 showed high expression in patients with GC and its expression decreased after therapy. SNHG6 also demonstrated a favorable predictive value for the development of GC and the death of patients. The survival curve suggested that increased SNHG6 indicated a higher risk of death. Additionally, mRNA of PI3K/AKT/mTOR pathway related molecules was highly expressed in GC patients. In *in vitro* assays, GC cells showed stronger viability and invasion activity and weaker apoptosis and autophagy ability after targeted up-regulation of SNHG6. According to the rescue assay, the effect of up-regulating SNHG6 on GC cells could be completely reversed by suppressing the PI3K/AKT/mTOR pathway. Conclusion: With high expression in patients with GC, SNHG6 can promote the development of GC by activating the PI3K/AKT/mTOR signaling pathway and suppressing the autophagy of cells. Therefore, it is a potential breakthrough in the diagnosis and treatment of GC in the future.

Keywords: Gastric carcinoma, lncRNA SNHG6, PI3K/AKT/mTOR, autophagy

Introduction

Gastric carcinoma (GC) is a malignant tumour with a terribly high incidence worldwide. There are 10-20 million new GC cases each year, mostly among the elderly [1]. According to the study [2], GC is strongly correlated with factors including eating habits, bacterial infection, inflammation, nutritional status, and even inhalation of air, which is one primary cause of the increasing incidence of GC. Moreover, as GC is hereditary, the prevalence in individuals with a family history of GC was 3-5 times higher than that in healthy individuals without family history [3]. Patients with early GC have no special clinical manifestations, and may just suffer abdominal pain and anorexia. Accordingly, early GC is usually ignored or mishandled by patients due to the lack of medical and health knowledge, and most cases may have already entered in the middle or advanced stage at the time of diagnosis [4]. The clinical treatment effect on early GC is relatively ideal, and patients have a survival rate of 90-100% after therapy [5]. However, for advanced GC, effective treatment measures are still under exploration, so patients at advance stage still face a mortality of over 70% after active therapy [6]. Accordingly, GC has long been a crucial disease under research, and domestic and foreign scholars are constantly striving to explore new diagnosis and treatment methods for it.

In modern medicine, molecular pathogenicity is a focus of research on tumour diseases, and lncRNA is a typical one [7] which plays a crucial

part in life activities such as heredity and cell viability [8]. LncRNA SNHG6 (SNHG6) is a crucial lncRNA in colon cancer and diabetic microangiopathy, and it is also confirmed to have abnormal expression in cases with GC [9-11]. Yan et al. [12] have found the involvement of SNHG6 in biological behaviors of GC cells and it is probably a key breakthrough in the diagnosis and treatment of GC in the future. As we all know, autophagy is one of the most crucial links in the biological behavior of tumour cells. Through autophagy, cells have a faster life cycle, and the mechanism of currently used chemotherapy drugs is to kill tumour cells by activating their autophagy [13]. Accordingly, autophagy is of great significance in tumor molecular research. In previous studies, SNHG6 has been verified to be strongly linked to the autophagy of colorectal cancer cells [14], but the relationship with GC is not clear. In addition, these data also lay a reliable foundation for the follow-up study of SNHG6 in GC, but no other study has further confirmed their relationship. The PI3K/AKT/mTOR signaling pathway is a crucial part in the action pathway of SNHG6. For example, Meng et al. [15] have revealed that SNHG6 affects the proliferation and metastasis of colorectal cancer (CRC) cells via the PI3K/AKT/mTOR pathway, and Wang et al. [16] have found that SNHG6 promotes the epithelial-mesenchymal transition of breast cancer cells via this pathway. As a classical signaling pathway, PI3K/AKT/mTOR has been verified to affect various tumours, and is closely associated with the viability of GC cells [17, 18]. Accordingly, we inferred that the involvement of SNHG6 in GC was probably associated with PI3K/AKT/mTOR pathway.

Accordingly, this study explored the detailed influence of SNHG6 on the autophagy of GC cells and the related pathway to provide more comprehensive and reliable reference for the diagnosis and treatment of GC in the future.

Materials and methods

Data about patients

A total of 54 GC patients admitted to the Second Affiliated Hospital of Shanxi Medical University from May 2017 to June 2018 and 60 healthy individuals who underwent physical examination during the same period were enrolled and retrospectively analyzed. The experiment was approved by the Ethics Committee of the Second Affiliated Hospital of Shanxi Medical University ((2019) KY NO. (294)) and all participants signed informed consent. There was no significant difference between GC patients and healthy individuals in clinical baseline data, such as age and gender (all $P>0.05$).

Inclusion and exclusion criteria

The inclusion criteria of GC patients: 1. Patients confirmed with GC by pathologic biopsy; 2. Patients at pathological stage of 0-I (namely T2, N0, and M0); 3. Patients with detailed case data; 4. Patients with an age of over 18 years. The exclusion criteria of GC patients: 1. Patients with comorbid multiple tumors; 2. Patients with other cardiovascular or cerebrovascular diseases, autoimmune diseases, infectious diseases or organ dysfunction; 3. Pregnant and lactating patients; 4. Referred patients; 5. Patients who had received antibiotics, surgery, radiotherapy or chemotherapy within half a year before admission. The inclusion criteria of healthy individuals: 1. Individuals with an age of over 18 years; 2. Individuals with detailed case data and without a major medical history; 3. Individuals with normal physical examination results.

Sample collection

Patients were given radical surgery after admission by the same senior surgical team from the digestive surgery department in our hospital. Fasting peripheral venous blood (4 mL) was extracted from patients before therapy (at admission) and after therapy (7 days after operation) and from healthy individuals at admission. The serum was centrifugated and stored in a refrigerator (-80°C) for subsequent analyses.

PCR assay

The expression of SNHG6 and PI3K/AKT/mTOR pathway related molecules in serum was detected by PCR. Total RNA was extracted from serum by Trizol, and transcribed into cDNA with a reverse transcription kit, and then amplified according to the kit guidelines under reaction conditions: 95°C/30 s, followed by 40 cycles of 95°C/5 s, 95°C/30 s, and 60°C/30 s. $2^{-\Delta\Delta Ct}$ was adopted to calculate the relative expres-

Table 1. Primer sequences

	F (5'-3')	R (5'-3')
SNHG6	ATACTTCTGCTTCGTTACCT	CTCATTTTCATCATTTGCT
GAPDH-1	AATCCCATCACCATCTTCC	CATCACGCCACAGTTTCC
PI3K	AGTTTGCCCCTCCTGATGTT	GTAAGTCGGCGAGATAGCGT
AKT	TGTCTCGTGAGCGCGTGTTTT	CCGTTATCTTGATGTGCCCGTC
mTOR	ACAGTGAAAGTGAAGCCGAGAG	CAAGGAGATAGAACGGAAGAAGC
GAPDH-2	CGCTAACATCAAATGGGGTG	TTGCTGACAATCTTGAGGGAG

sion of SNHG6 (internal reference: GAPDH-1) and PI3K/AKT/mTOR pathway related molecules (internal reference: GAPDH-2). The primer sequences were constructed by Shanghai Yihe Applied Biotechnology Co., Ltd. (**Table 1**).

Follow-up

All patients were followed up for 3 years through telephone and re-examination in the hospital at an interval of 2-3 months, and the medical records of each patient in this study were sorted by nurses in our hospital. Their survival was recorded, on which a survival curve was drawn.

Bioinformatics analyses

SNHG6 expression was analyzed according to Oncomine (www.oncomine.org), ENCORI (https://starbase.sysu.edu.cn/), and GEPIA databases (http://gepia.cancer-pku.cn/index.html), and the association of SNHG6 and PI3K/AKT/mTOR pathway with the prognosis of GC was also analyzed according to the Plotter database (http://kmplot.com/analysis/index.php?p=background).

Data about cells

Human GC cells (SGC-7901 and MGC-803) and normal gastric mucosa cells (GES-1) purchased from ATCC were subjected to incubation (37°C, 5% CO_2) with corresponding medium after cell thawing, and then passaged after cell confluency reached 80%.

Cell transfection

Under the guidelines of a Lipofectamine 2000 kit, 50 μL virus suspension of lentiviral vectors with targeted up-regulation of SNHG6 (pcDNA3.1-SNHG6) [1.0×10^8 TU/mL, multiplicity of infection (MOI)=50], lentiviral vectors with targeted silencing of SNHG6 (sh-SNHG6), and corresponding empty vector controls (SNHG6-NC) were transfected into SGC-7901 and MGC-803, for 10 hours, followed by transfection effect verification through PCR.

Cell viability determination

After transfection, GC cells at the logarithmic phase were seeded in a 96-well plate (2×10^4 cells/mL), and then added with 10 μL CCK-8 at 0, 24, 48, and 72 h, followed by 2 h incubation each time. Finally, the optical density of cells (450 nm) was measured with a microplate reader, and a cell growth curve was drawn.

Cell apoptosis determination

GC cells at the logarithmic phase were washed with PBS three times, and then added with 5 μL FITC and 5 μL PI. Afterwards, the cells were subjected to 15 min incubation (indoor temperature) with dark surroundings. Finally, a flow cytometer was adopted for apoptosis determination of the cells. Normal cells were adjusted by fluorescence compensation as controls, and the apoptotic cells were early apoptotic cells (Q3 quadrant in the lower right corner), and the number of apoptotic cells was expressed as a percentage.

Cell invasion determination

The upper and lower compartments of the Transwell chamber were added with GC cells at the logarithmic phase (1×10^6/mL) and FBS, respectively, and then treated with 24 h incubation. Cells in the upper compartment were wiped off, and the rest were immobilized and dyed. Lastly, transmembrane cells were counted under a microscope.

Protein quantification

Protein lysate (200 μL) was adopted to lyse cells, and the total protein was acquired and mixed with loading buffer. Subsequently, the mixture was treated in a water bath for denaturation, and then subjected to SDS-PAGE and transferred to a PVDF membrane, followed by immersion in 5% fetal bovine serum (FBS) and addition with primary antibodies PI3K (1:1000), AKT (1:1000), mTOR (1:1000), Beclin1 (1:1000), LC3 (1:1000), and GAPDH

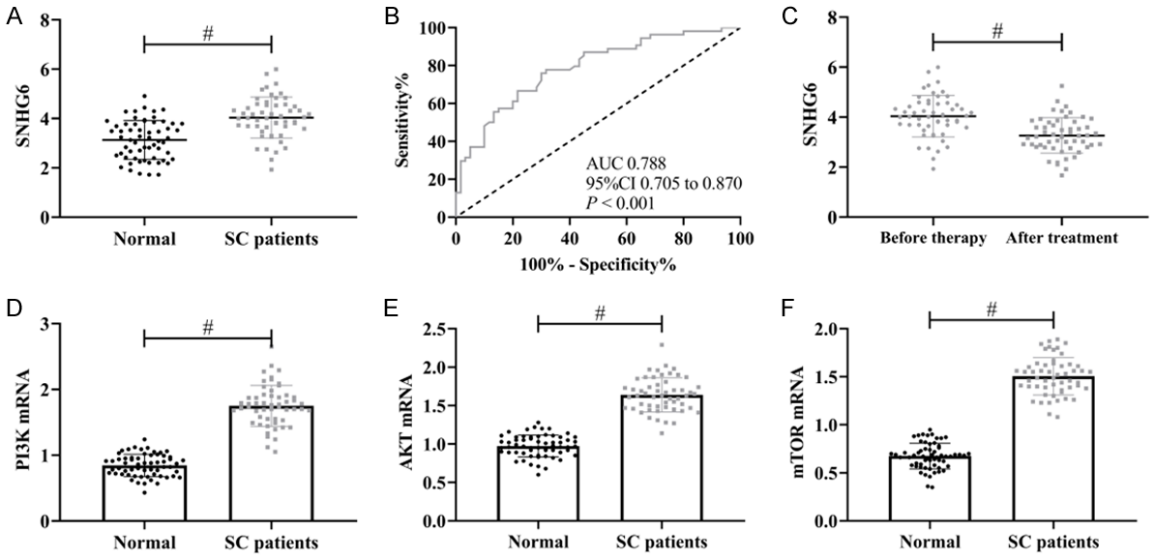

Figure 1. Expression of SNHG6 and PI3K/AKT/mTOR pathway related molecules. A: Comparison of SNHG6 level between GC patients and healthy individuals. B: ROC curve of SNHG6 in forecasting the development of GC. C: SNHG6 in GC patients before and after therapy. D: Comparison of PI3K mRNA level between GC patients and healthy individuals. E: Comparison of AKT mRNA level between GC patients and healthy individuals. F: Comparison of mTOR mRNA level between GC patients and healthy individuals. #P<0.05 in terms of inter-group comparison.

(1:10000). The membrane was subjected to overnight incubation (4°C), followed by addition of secondary antibody (1:6000) the next day. Lastly, it was developed through ECL in the dark, and photographed to calculate the relative expression of the proteins (internal reference: GAPDH).

Signaling pathway intervention

Specific inhibitor NVP-BEZ235 for PI3K/AKT/mTOR signaling pathway (10 nmol/L) was adopted to treat SGC-7901 and MGC-803 cells for 24 h (NVP-BEZ235 group), and an equal amount of normal saline was used to intervene cells (blank group). These cells were quantified as above.

Rescue assay

SGC-7901 and MGC-803 cells intervened by NVP-BEZ235 were then transfected with pcDNA3.1-SNHG6 and classified as Group A. Cells transfected with pcDNA3.1-SNHG6 were classified as Group B, and cells in Group C were not treated. The biological behaviors of each group were determined.

Statistical analyses

SPSS22.0 was adopted for statistical analyses. Count data (n/%) were analyzed using the Chi-square test, and measurement data ($\bar{x}$±sd) obtained from three independent experiments were analyzed using the independent-samples t test, one-way ANOVA, paired t test, and LSD post-hoc test. The predictive value was explored using the ROC curves. Survival rate was calculated using the Kaplan-Meier method, and compared using the Log-rank. P<0.05 denoted a remarkable difference.

Results and discussion

Expression of SNHG6 and PI3K/AKT/mTOR pathway related molecules and their clinical value

The results revealed higher serum SNHG6 expression in GC patients than that in healthy individuals (P<0.05, **Figure 1A**). According to ROC curve-based analysis, serum SNHG6>3.56 had a sensitivity of 77.78% and a specificity of 68.33% in predicting the development of GC (both P<0.001, **Figure 1B**). Additionally, serum SNHG6 expression in GC patients decreased greatly after therapy (P<0.05, **Figure 1C**), and the serum mRNA levels of PI3K, AKT and mTOR in patients with GC were higher than those in healthy individuals (all P<0.05, **Figure 1D-F**).

Prognostic value of SNHG6

A total of 50 GC patients were followed up successfully, and the other 4 patients failed to

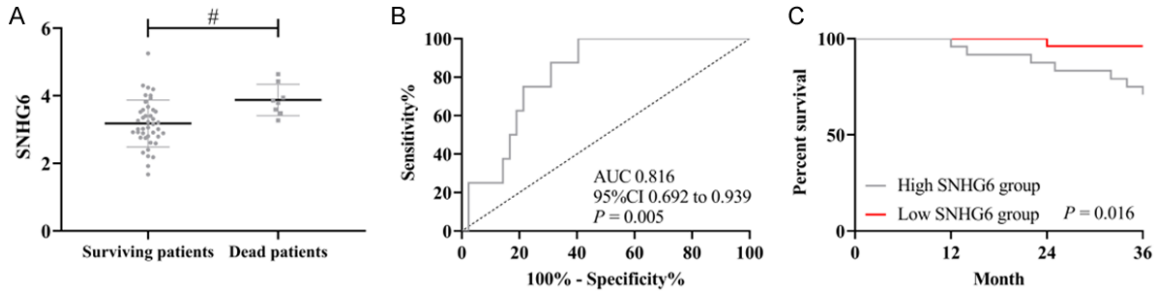

Figure 2. Prognostic value of SNHG6. A: SNHG6 expression in dead patients and survivors. B: ROC curves of post-therapy SNHG6 level in forecasting the death of patients. C: Impact of SNHG6 on GC prognosis. #P<0.05 in terms of inter-group comparison.

Table 2. COX analysis of prognostic death in GC patients

	Univariate analysis			Multi-factor analysis		
	HR	95% CI	P	HR	95% CI	P
SNHG6	6.142	2.542-12.363	<0.05	3.097	1.184-7.866	<0.05

be tracked because their phone could not be connected. The results revealed a total mortality of 16.0% (8 deaths). Patients who didn't survive presented notably higher SNHG6 expression than survivors after therapy (P< 0.05, **Figure 2A**). According to ROC curve-based analysis, serum SNHG6>3.27 had a sensitivity of 100.0% and a specificity of 59.52% in predicting the death of patients within 3 years (P<0.05, **Figure 2B**). GC patients were assigned to the high SNHG6 group (SNHG6> 3.27 after therapy, n=24) and low SNHG6 group (SNHG6≤3.27 after therapy, n=26) in the light of cut-off value. According to comparison of the low and high SNHG6 groups, the former presented a greatly lower mortality than the latter (P<0.05, **Figure 2C**). In addition, through the COX model, SNHG6 was found to be an independent risk factor affecting the survival of GC patients (P<0.05, **Table 2**).

SNHG6 expression in online databases

In the Oncomine database, cases with CRC presented the most abnormal expression of SNHG6, followed by cases with lymphoma, and cases with GC also presented such expression (**Figure 3A**). Six cases out of 9 studied cases presented high SNHG6 expression (**Figure 3B**). In the ENCORI database, tumors such as bladder cancer, bile duct cancer and esophageal cancer also presented high SNHG6 expression (**Figure 3C**). In the GEPIA database, ovarian serous cystadenocarcinoma and acute myeloid leukemia showed a low SNHG6 level, but other tumors generally showed high expression of SNHG6 (**Figure 3D**).

Association of SNHG6 with the survival of GC patients

The association of SNHG6 with the survival of GC patients was analyzed based on the Plotter database, and no correlations were found between SNHG6 expression and overall survival (OS) and first progression survival (FPS) (**Figure 4A** and **4B**), but a strong correlation was found between it and post-progression survival (PPS) (**Figure 4C**).

Association of PI3K/AKT/mTOR pathway with the survival of GC patients

According to the Plotter database, PI3K, AKT and mTOR expression was closely related to the survival of GC patients (**Figure 5**).

Impact of SNHG6 on biological behaviors of GC cells

First of all, PCR was performed to verify the success rate of transfection. The results showed notably higher expression of SNHG6 in pcDNA3.1-SNHG6 group than that in the other two groups, and lower expression in sh-SNHG6 group than that of NC-SNHG6 group (P<0.05), which verified the success in transfection. According to the results of biological behavior test, cells of pcDNA3.1-SNHG6 group presented stronger viability and invasion activity and weaker apoptosis than the cells of sh-

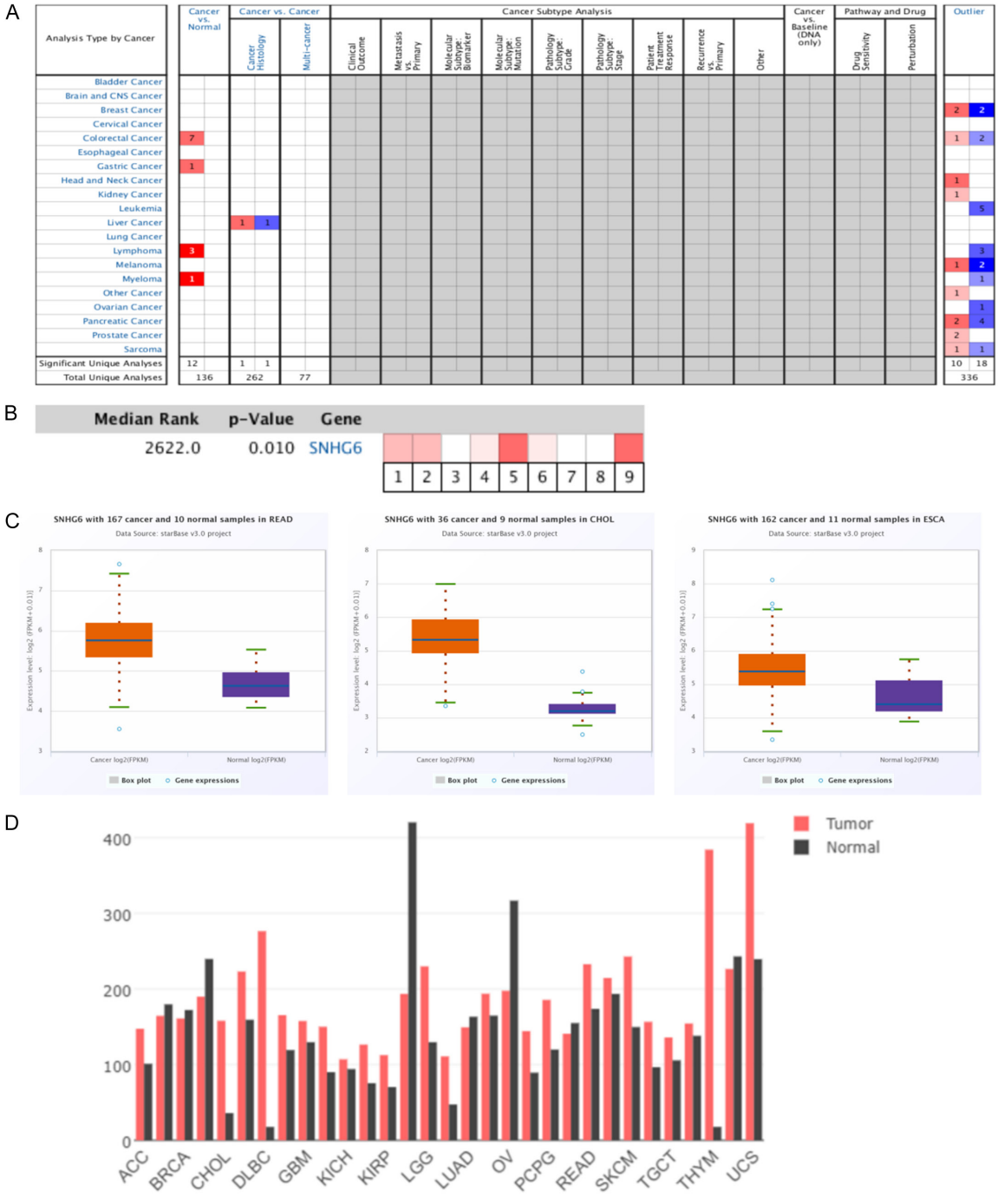

Figure 3. Expression of SNHG6 in online databases. A: SNHG6 expression in Oncomine database. B: Statistical analysis of differences in SNHG6 expression. C: SNHG6 expression in ENCORI database. D: SNHG6 expression in GEPIA database.

SNHG6 and NC-SNHG6 groups, while cells in sh-SNHG6 group presented weaker viability and invasion activity and stronger apoptosis than cells in NC-SNHG6 group (all $P<0.05$, **Figure 6**).

Impact of SNHG6 on PI3K/AKT/mTOR pathway and autophagy

Cells in PcDNA3.1-SNHG6 group showed higher protein levels of PI3K, p-PI3K, AKT, p-AKT,

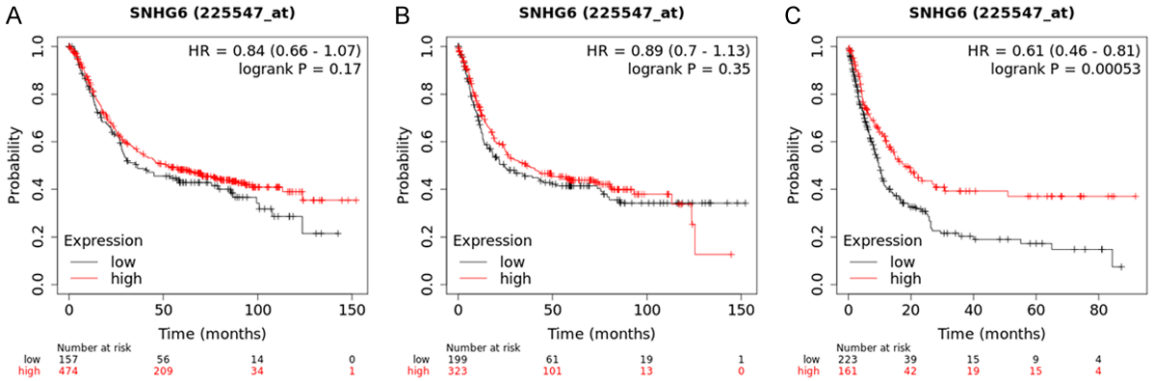

Figure 4. Association of SNHG6 with the survival of GC patients. A: The relationship between SNHG6 and OS. B: The relationship between SNHG6 and FP. C: The relationship between SNHG6 and PPS.

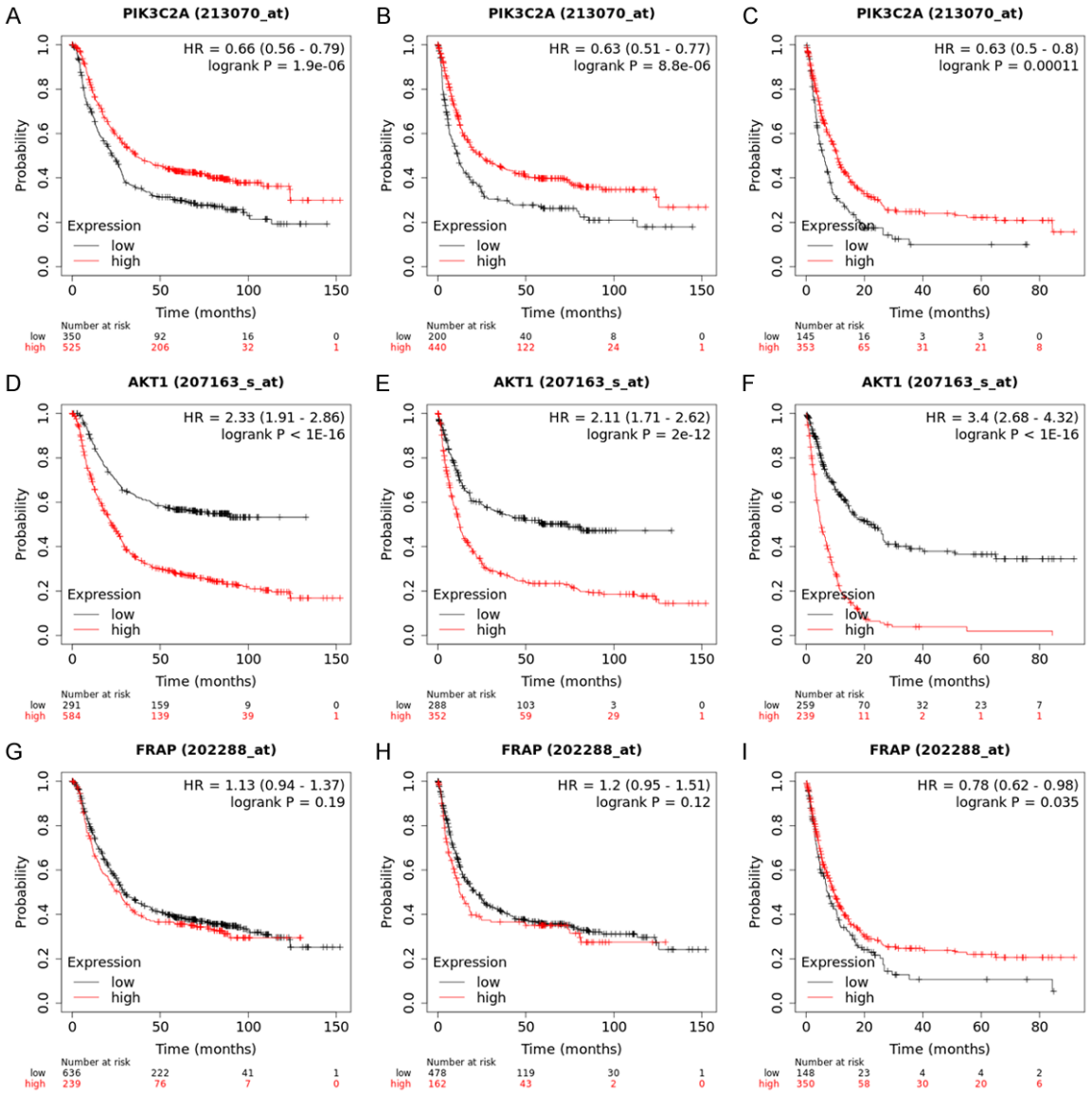

Figure 5. Association of PI3K/AKT/mTOR pathway with the survival of GC patients.

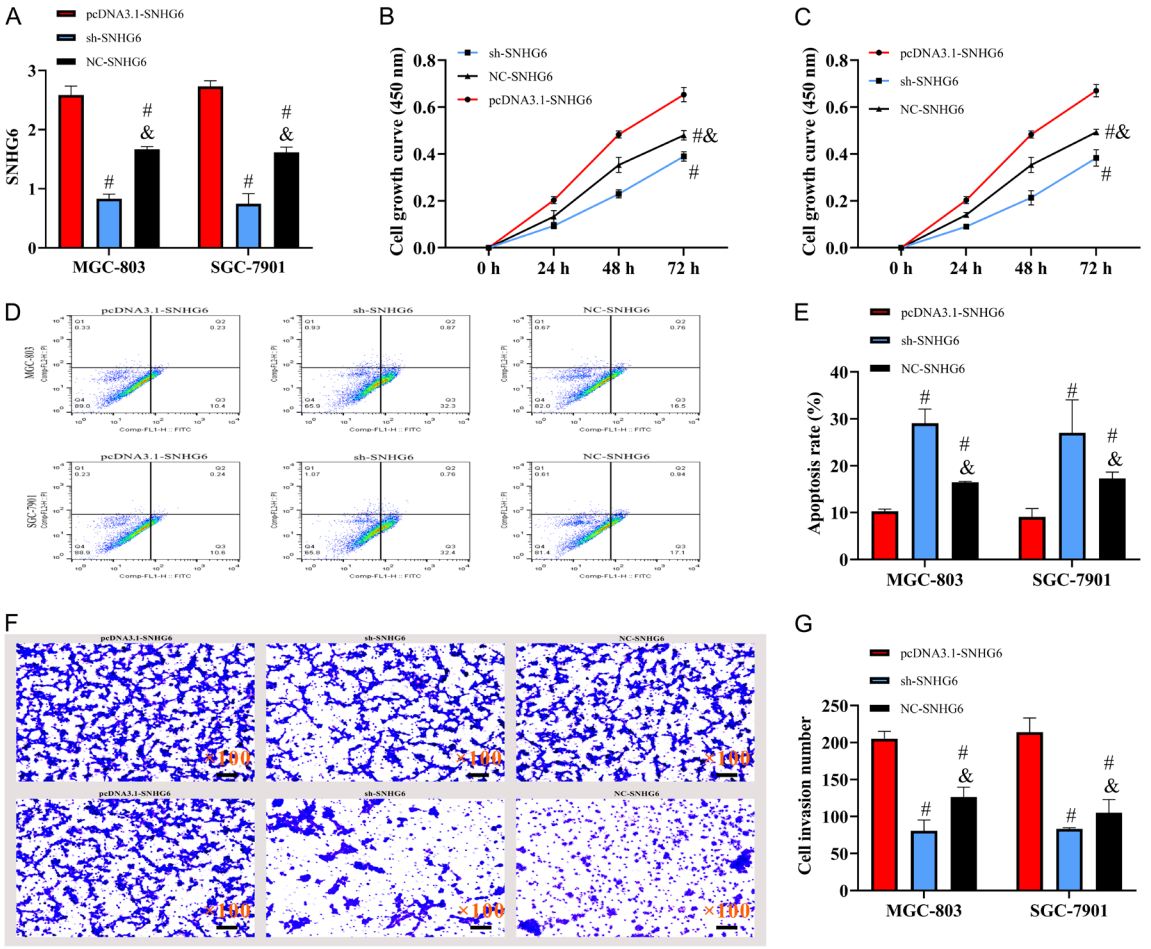

Figure 6. Impact of SNHG6 on GC cell biological behaviors. A: The transfection efficiency determined by PCR. B: MGC-803 cell growth curve. C: SGC-7901 cell growth curve. D: Flow cytometry. E: Apoptosis rate. F: Staining of transmembrane cells. G: Cell invasion. #P<0.05 *vs.* pcDNA3.1-SNHG6; &P<0.05 *vs.* sh-SNHG6.

p-mTOR and mTOR and lower protein levels of Beclin1 and LC3 than cells in sh-SNHG6 and NC-SNHG6 groups; while cells in sh-SNHG6 group showed lower protein levels of PI3K, p-PI3K, AKT, p-AKT, p-mTOR and mTOR and higher protein levels of Beclin1 and LC3 than cells of NC-SNHG6 group (all P<0.05, **Figure 7**).

Impact of PI3K/AKT/mTOR on GC cells

Cells in NVP-BEZ235 group presented weaker viability and invasion activity and stronger apoptosis than the blank group (all P<0.05, **Figure 8**).

Rescue assay

Groups A and C showed no notable difference in cell viability, invasion and apoptosis (all P>0.05, **Figure 9**). However, Group B showed stronger cell viability and invasion and weaker apoptosis (all P<0.05, **Figure 9**).

Discussion

GC is a life-threatening malignant tumor with a high incidence, and finding a novel diagnosis and treatment is the key to protecting the life and health of patients [19]. At the current stage, the application value of lncRNA in GC has captured attention from scholars at home and abroad. Its value in diagnosis and therapy is as follows: 1. LncRNA, a human genetic material, can be detected in many human body samples such as blood, tissues and cells, and possesses a higher specificity than traditional tumor markers, so it can be adopted as a major reference for early diagnosis of tumors in the future [20]. 2. LncRNA is an agent strongly correlated with cell biological behaviors, and monitoring

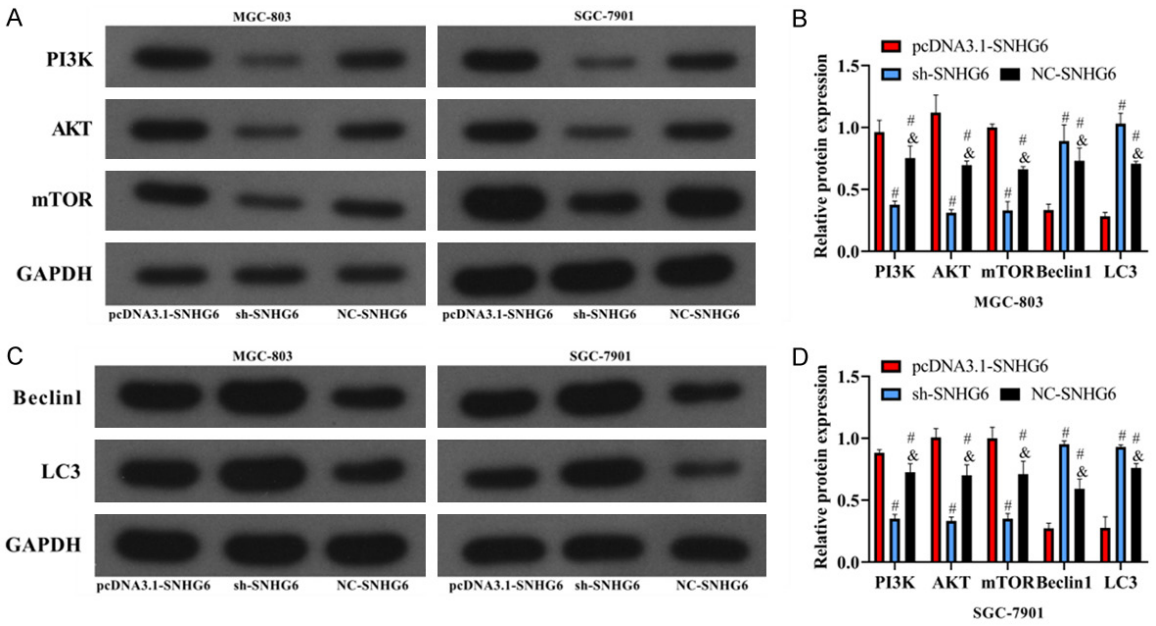

Figure 7. Impact of SNHG6 on PI3K/AKT/mTOR pathway and autophagy. A: Western blot images of PI3K/AKT/mTOR pathway. B: Protein expression in MGC-803 cells. C: Western blot images of autophagy-associated proteins. D: Protein expression in SGC-7901 cells. #P<0.05 *vs.* pcDNA3.1-SNHG6; &P<0.05 *vs.* sh-SNHG6.

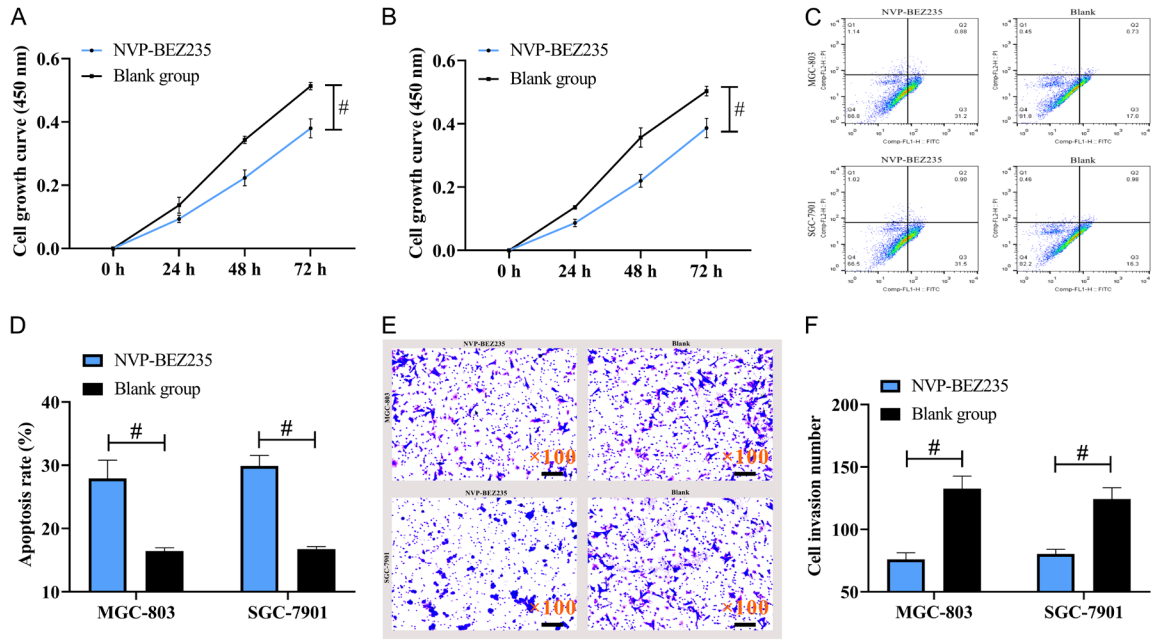

Figure 8. Impact of PI3K/AKT/mTOR on GC cells. A: MGC-803 cell growth curve. B: SGC-7901 cell growth curve. C: Flow cytometry. D: Apoptosis rate. E: Staining of transmembrane cells. F: Cell invasion. #P<0.05 in terms of intergroup comparison.

its changes probably helps judge the clinical development of tumors to facilitate the early and timely treatment of patients in clinical practice [21]. 3. Similarly, molecular targeted therapy based on lncRNA may deliver better clinical therapy effect than the current radical tumor surgery and can better improve patients' prognosis [22]. Currently, research on SNHG6 in GC is still relatively rare, and its specific mechanism requires deeper exploration.

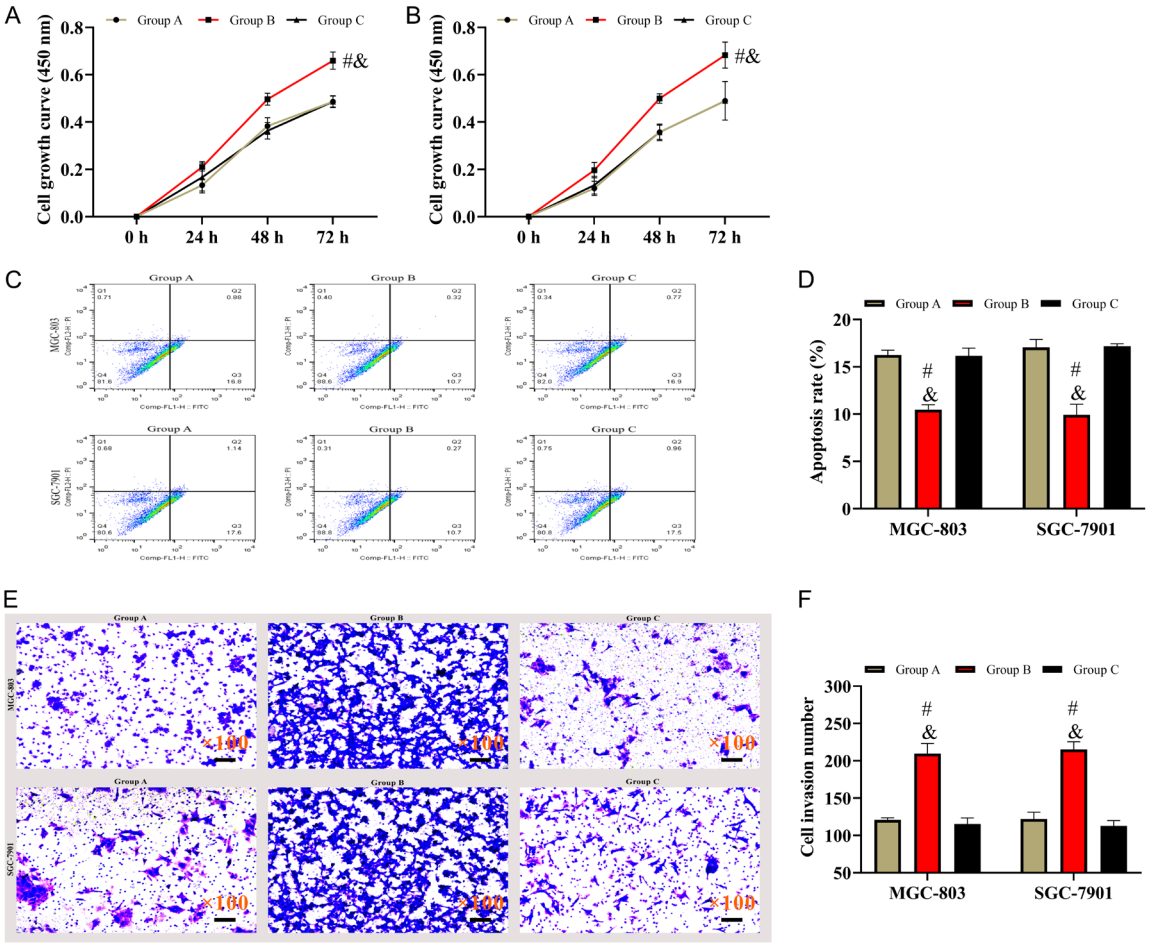

Figure 9. Rescue assay verified the relationship between SNHG6 and PI3K/AKT/mTOR. A: MGC-803 cell growth curve. B: SGC-7901 cell growth curve. C: Flow cytometry. D: Apoptosis rate. E: Staining of transmembrane cells. F: Cell invasion. #P<0.05 *vs.* Group A; &P<0.05 *vs.* Group C.

Accordingly, this study determined the impacts of SNHG6 on GC to more deeply understand its clinical value and the related mechanism to provide crucial reference and guidance for future diagnosis and treatment of GC.

In our study, we first analyzed the clinical value of SNHG6. We found higher serum SNHG6 in GC patients than healthy individuals, which implied the high SNHG6 expression in GC. Prior research has revealed a high SNHG6 level in tumor diseases such as colorectal cancer and cervical cancer [23, 24], which suggests that its expression is consistent in various diseases and also supports the accuracy of our experimental results. Afterwards, we made analysis based on ROC curves, and found excellent sensitivity and specificity of SNHG6 for predicting the development of GC, which also laid a foundation for SNHG6 as a diagnostic marker of GC. At the current stage, the early diagnosis of tumor diseases in clinical practice still mainly depends on the comprehensive evaluation of tumor markers and imaging examination, and the gold standard for tumor diagnosis is still pathological biopsy [25]. The complexity of diagnostic techniques greatly hinders the early diagnosis rate of GC. SNHG6 can be determined with blood specimens, so it can be screened comprehensively on a large scale. In addition, it possesses a higher specificity than tumor markers. We believe SNHG6 screening in the future can effectively improve the early diagnosis rate of GC and patients' outcomes. In our study, GC patients presented a notable decrease in SNHG6 expression after therapy, which suggested that SNHG6 was strongly related with changes of GC and SNHG6 can help evaluate disease development. In the follow-up, we also found a favorable value of SNHG6 in predicting the survival of GC patients

and a positive correlation of its increase with a high risk of death. The data further illustrated the strong association of SNHG6 with GC and the clinical application value of SNHG6. Moreover, similar to a prior study [26], we discovered higher serum mRNA expression of PI3K/AKT/mTOR related molecules in GC patients than that in healthy individuals, which preliminarily suggested the possible obvious activation of PI3K/AKT/mTOR signaling pathway in cases with GC. However, it is shown in the Plotter database that SNHG6 has nothing to do with the OS and FP of GC, which is different from our research results. As we all know, the prognosis and survival of cancer patients are closely correlated with the pathological stages of diseases. The difference between the results from Plotter database and our results may be due to the inconsistency of pathological stages between the patient data collected in the database and our research subjects, as well as the contingency of statistical calculation due to too few research objects.

To verify the accuracy of our experimental results, we preliminary analyzed SNHG6 based on databases, and found that the current research on SNHG6 mainly focused on CRC, and SNHG6 was upregulated in other tumor diseases. Prognosis analysis based on follow-up results also revealed a strong correlation of SNHG6 with PPS of patients with GC. The prognostic follow-up results in this study revealed a strong relationship between SNHG6 and the overall survival rate of GC patients, which could once again emphasize the close relationship between SNHG6 and GC. The expression of PI3K, AKT, as well as mTOR also had strong correlations with the prognosis of GC patients, which fully verified the importance of PI3K/AKT/mTOR signaling pathway in GC. PI3K is a dimer composed of regulatory subunit p85 and catalytic subunit p110. After binding with growth factor receptor, it is able to change the protein structure of AKT and activate it and can also activate or suppress the activity of a series of downstream substrates such as Bax and Caspase-9 via phosphorylation. In this way, it can impact cell apoptosis, proliferation, differentiation, and migration [27, 28]. mTOR is the downstream target of PI3K/Akt and a pivotal molecule to regulate cell growth and metabolism [29]. The involvement of PI3K/AKT/mTOR pathway in tumor diseases has been broadly studied [30, 31]; so, it was omitted in this study.

Based on the above, we can have a rudimentary knowledge of the value of SNHG6 in GC, but its specific mechanism is still under exploration. Accordingly, we conducted *in vitro* analysis to more deeply explore the mechanism of action of SNHG6. According to the results, upregulating SNHG6 intensified viability and invasion activity of GC cells and weakened their apoptosis, while silencing it gave rise to opposite results. These results imply that SNHG6 plays an oncogenic role in GC. According to the determination results of PI3K/AKT/mTOR pathway and autophagy-associated proteins in cells, the expression of the pathway increased with the increase of SNHG6, while Beclin1 and LC3 decreased with it, suggesting that SNHG6 was able to activate PI3K/AKT/mTOR signaling pathway and inhibit autophagy. As we know, autophagy is a crucial link in cell apoptosis and metabolism, and the primary mechanism of chemotherapy is to kill tumor cells by activating autophagy [32, 33]. Thus, autophagy of tumor cells is also a crucial factor for rehabilitation of patients. Our assay results also fully imply that targeted silencing of SNHG6 can effectively improve the autophagy ability of GC cells, and SNHG6 is a new potential target for the treatment of GC in the future. Of course, this needs more research to confirm. Moreover, we inactivated the PI3K/AKT/mTOR pathway. As a result, suppressing the pathway weakened the viability and invasion activity of cells and intensified their apoptosis, suggesting that inhibiting the pathway can also accelerate GC cell apoptosis and alleviate GC development. Lastly, according to the rescue assay, the impact of increasing SNHG6 on GC cells was completely reversed by suppressing the PI3K/AKT/mTOR pathway, which confirmed our initial conjecture that SNHG6 was involved in the development of GC via the PI3K/AKT/mTOR pathway.

Of course, this study has many limitations. For instance, because of the short follow-up period for GC patients, we were unable to evaluate the effect of SNHG6 on the long-term prognosis of GC patients. In addition, due to the lack of a nude mouse tumorigenesis assay, we were unable to evaluate the specific involvement of SNHG6 in tumorigenesis. The changes of SNHG6 involved in the biological behavior of GC cells may not only be achieved through the PI3K/AKT/mTOR pathway, which also needs confirmation with more basic experiments. And due to limited experimental funds, we did not

conduct experiments such as tumor formation and phosphorylation detection in nude mice, which are also shortcomings that we need to supplement as soon as possible. The above limitations are the focuses and directions of our follow-up research. We will conduct a more detailed and comprehensive experimental analysis on SNHG6 to provide more reliable reference opinions for clinical practice in the future.

Conclusion

With high expression in GC patients, SNHG6 can affect the development of GC by activating the PI3K/AKT/mTOR signaling pathway and suppressing the autophagy of cells. Therefore, it is a potential breakthrough in the diagnosis and treatment of GC in the future.

Acknowledgements

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Disclosure of conflict of interest

None.

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