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*Original Article*

# Long non-coding RNA GACAT1promotes proliferation and invasion of gastric cancercells by targeting miR-378

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**Abstract:** Long noncoding RNAs (lncRNAs), a class of non-coding RNA, is a new group of RNAs which is closely related to cancer tumorigenesis. It is suggested to be ideal candidate biomarker with potential diagnostic and therapeutic implications. However, there is little known about the function and mechanism of lncRNA-GACAT1 in human gastric cancer (GC). In the present study, qRT-PCR was performed to detect the expression of GACAT1 and miR-378 in GC tissues and GC cells. Luciferase reporter gene assay identified the regulation of GACAT1 on miR-378. MTT and colony formation assays were used to detect the effect of GACAT1 on the proliferation ability of GC cells. Transwell assay was performed to analyze the migration and invasion abilities of GC cells. Our results revealed that GACAT1 was highly expressed, and miR-378 was lowly expressed in GC tissues and cells. GACAT1 expression was negatively correlated with miR-378 expression in GC tissues. In addition, we found that GACAT1 directly regulated miR-378 in GC cells negatively. The effects of GACAT1 overexpression on the promotion of GC cell proliferation and invasion could be inhibited by miR-378. Therefore, our study demonstrated that GACAT1 promoted the progression of GC via miR-378, and could be a potential therapeutic target for treatment of GC.

**Keywords:** lncRNA-GACAT1, miR-378, gastric cancer, proliferation, migration and invasion

## Introduction

Gastric cancer (GC) is a high-incidence neoplastic disease particularly in Eastern Asia [1], and it is the third leading cause of cancer-related deaths in the world despite a downward trend of morbidity [2]. To date, although early GC patients could have an effective treatment and achieve clinical cure, most GC patients have to delay to metaphase or advanced GC because of the lack of sensitive biomarkers for early diagnose. Therefore, the molecular and functional mechanisms of gastric cancer are still extremely urgent.

Long noncoding RNAs (lncRNAs) are a new sort of non-coding RNA longer than 200 nucleotides [3]. A large number of studies have indicated that lncRNAs regulate gene expressions through the processes of transcription regulation, post-transcription regulation, chromatin modification and genomic imprinting [4, 5]. LncRNAs have been regarded as a new class of non-coding RNAs that contribute to cancer development and progression [6]. Studies have proved that lncRNA-GACAT1 is abundantly and specifically expressed in GC [7, 8]. And there are researches to show that it plays critical roles in the development and function of colorectal cancer (CRC) [9, 10], as well as the progression of breast cancer[11]. Recently, lncRNA-GACAT1 have been found up-regulateda wide variety of tumors, such as glioma [12], acute myeloid leukemia [13], gallbladder cancer [14], pancreatic cancer [15] etc. However, the function and mechanism studies of lncRNA-GACAT1 in GC are only just beginning. In this study, we analyzed the expression level of lncRNA-GACAT1 in human GC cell lines and tissues, and the effects on the progression and prognosis of GC.

MicroRNAs (miRNAs) are small, noncoding RNAs about 20 nucleotides in length with important functions in development, cell differentiation, and regulation of cell cycle and apoptosis [16]. They regulate gene expression generally by inhibiting translation or degrading mRNA transcript [17]. miRNA expression is mostly deregulated in cancers via a variety of mecha-

nisms such as amplification, deletion, mutation, and epigenetic silencing [16]. What is more, miRNAs are stably present in various biofluids, and the level of miRNAs in the body fluids reflects specific pathological states [18]. These characteristics made them new and effective biomarkers for human cancer diagnostics. To date, the inhibitory effect of miR-378 in tumor growth and migration as well as the dysregulation of miR-378 had been reported in various tumor types, such as renal cell carcinoma [19], prostate cancer [20], colorectal cancer [21], liver cancer [22], and myelodysplastic syndromes [23] etc. However, the exact mechanism of the miR-378 down-regulation in GC and the crosstalk between miR-378 and the lncRNA-GACAT1 remain unclear.

In our study, we will prove the effects of GACAT1 on GC, and the interactions between GACAT1 and miR-378. Our research also will demonstrate that GACAT1 could be a potential therapeutic target for treatment of GC.

## Materials and methods

### *Clinical specimens*

In this study, we collected 31 pairs of GC tissues and adjacent normal tissues samples from The Forth Affiliated Hospital of Harbin medical university between 2015 and 2016. No patients had undergone radiotherapy or chemotherapy before surgical resection. Informed consent was also got from each patient. Our study has got the Ethics committee approval from The Forth Affiliated Hospital of Harbin medical university. The GC histological diagnosis was evaluated based on World Health Organization (WHO). All tissue samples were store in -80°C.

### *Cell lines*

Human gastric epithelium GES-1 cells, GC cell lines (BGC-823, HGC-27, MGC-803 and AGS), and human embryonic kidney 293T (HEK293T) cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Human gastric epithelium GES-1 and HEK293T cells were routinely cultured in Dulbecco's modified Eagle's (DMEM) medium (Invitrogen, Carlsbad, CA, USA); GC cell lines were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA). All culture mediums were added with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin. All cells were maintained in a humidified incubator at 37°C with 5% $CO_2$. The culture medium was changed every day.

### *Lentiviral vector construction and transfection*

Human GACAT1 DNA was amplified by RT-PCR from BGC-823 cells, and then inserted in to a lentiviral vector, EGF was used as the control. Then HEK293T cells were co-transfected with packaging vectors (pCMV-VSVG, pMDLg/pRRE and pRSV-REV) and GACAT1 or EGF plasmids to package the lentivirus vectors. BGC-823 cells ($5 \times 10^4$ cells/well) were seeded in 24-well plates and transduced with lentivirus supplemented with 8 μg/mL of ploybrene (sigma-Aldrich Chemie, The Netherlands). The stable expression cell lines were screened using G418 (Life Technologies, 0.8 mg/mL).

### *SiRNA interference*

SiRNAs of GACAT1 were synthesized by GenePharma (GenePharma Co., Ltd., Shanghai, China). The sequence of selected regions to be targeted by siRNAs for si-GACAT1#1, si-GACAT1#2, si-GACAT1#3, and si-GACAT1#1-3 was 5': GGA GCA GAA UUA GAA CAA UUU (sense), 3': UUC CUC GUC UUA AUC UUG UUA (anti-sense); 5': GUA GCC UGC UCC AGA UUA UUU' (sense), 3': UUC AUC GGA CGA GGU CUA AUA (anti-sense); 5': GCUGGCUUCACCUCUCAAUUU (sense), 3': UUC GAC CGA AGU GGA GAG UUA (anti-sense). AGS cells were transfected with 50 nMsiRNA (Negative control, NC) or GACAT1-siRNA using Lipofectamine 3000 (Invitrogen) according to the manuscript protocol.

### *Transfection of miRNA mimics*

Cells ($1\times10^5$ cells/well) were seeded in 6-well plates and transfected with 200 μl mature miR-378 (100 nM) and negative control (NC) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) for 72 hrs according to the manufacturer's protocol.

### *Quantitative real-time reverse transcription PCR (qRT-PCR)*

The total RNA was extracted by using TRIzol Reagent (Invitrogen USA) according to the manufacturer's instructions and reversely trans-

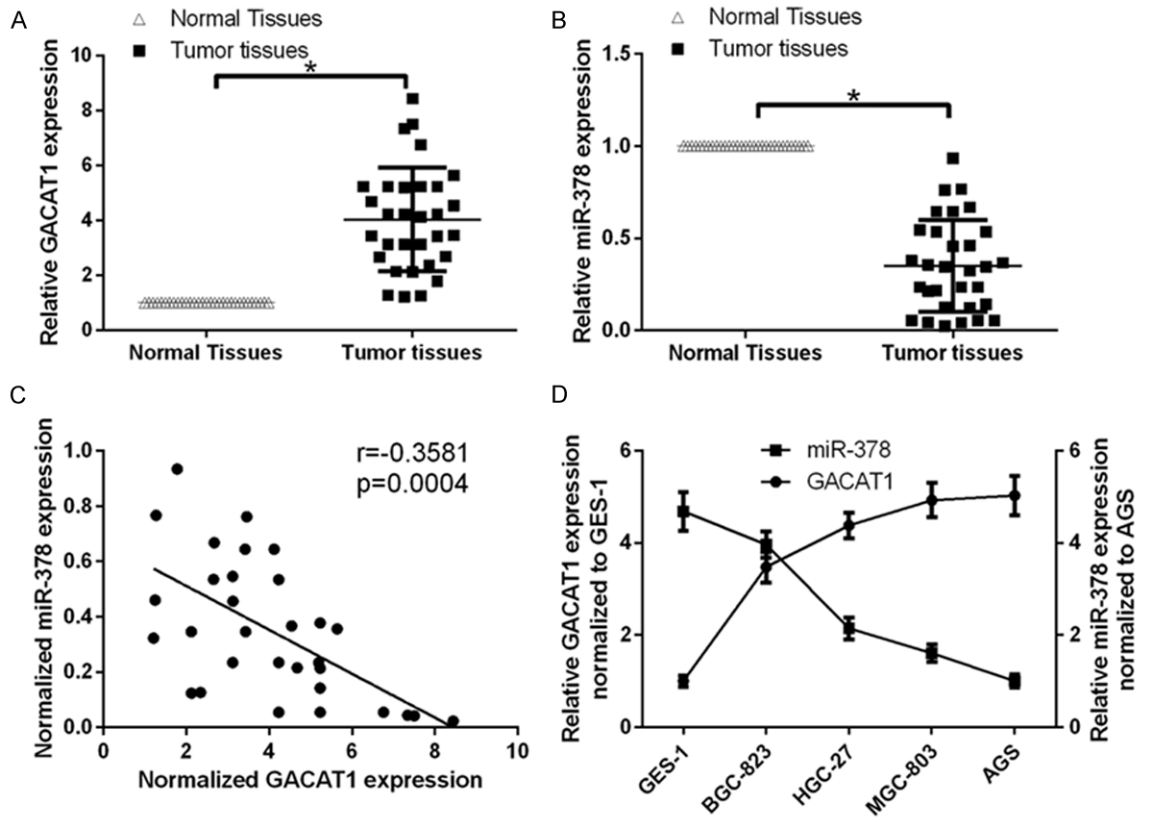


**Figure 1.** Expression of GACAT1 and miR-378 in GC tissues and cell lines. A. Expression of GACAT1 in 31 pairs of GC tissues and paired adjacent normal tissues. Expression levels are detected by qRT-PCR and shown as log2-fold change vs matching normal adjacent tissue (*$P < 0.05$). B. The expression level of miR-378 was detected by qRT-PCR in GC tissues (*$P < 0.05$). C. Correlation between GACAT1 and miR-378 expression in GC tissues. D. qRT-PCR was performed to detect the expression levels of GACAT1 and miR-378 in human gastric epithelium GES-1 cells and GC cell lines (BGC-823, HGC-27, MGC-803 and AGS).

cribed to cDNA by using Super Script TM III Reverse Transcriptase (Invitrogen, USA) according to the manufacturer's protocols. The primers for reverse transcription of miR-378 is 5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GGC CTT C-3'; The primers for reverse transcription of U6 is 5'-AAA ATA TGG AAC GCT TCA CGA ATT TG-3'. The mRNA expression levels were analyzed by the SYBR-Green PCR Master Mix kit (Takara) in PCR reaction. The reactions were performed on ABI 7500 system (Applied Biosystems, Carlsbad, CA, USA) using the following program: 95°C for 30 s as the first step in a loop; 95°C for 5 s, 60°C for 34 s as the second step, a total of 40 cycles. The primer sequences for GAPDH are: 5'-TGT TCG TCA TGG GTG TGA AC-3' (the forward primer) and 5'-ATG GCA TGG ACT GTG GTC AT-3' (the reverse primer) (internal control); The primer sequences for GACAT1 are: 5'-ACC GGA GGA AAA TCC CTA GC-3' (the forward primer) and 5'-CCA TAA AAG GGG CGG CTG T-3' (the reverse primer); The primer sequences for U6 are: 5'-CTC GCT TCG GCA GCA CA-3' (the forward primer) and 5'-AAC GCT TCA CGA ATT TGC GT-3' (the reverse primer); The primer sequences for hsa-miR-378 are: 5'-GCA CUG GAC UUG GAG UCA-3' (the forward primer) and 5'-CAG TGC AGG GTC CGA GGT-3' (the reverse primer).

*Dual luciferase reporter assay*

Wild type and mutant GACAT1 sequences were constructed and cloned into pGI3-basic luciferase reporter vector (Promega, Madison, WI). BGC-823 cells were seeded at density of $5 \times 10^4$ cells/well in 24-well plates, and co-transfected with wild type GACAT1 or mutant type GACAT1, control or miR-378, and a renilla plasmid (RL-SV40) respectively using Lipofectamine 3000. After 48 hrs, Dual-Luciferase Reporter Assay System (Promega) was used to detected reporter gene activities according to the manufacturer's instructions.

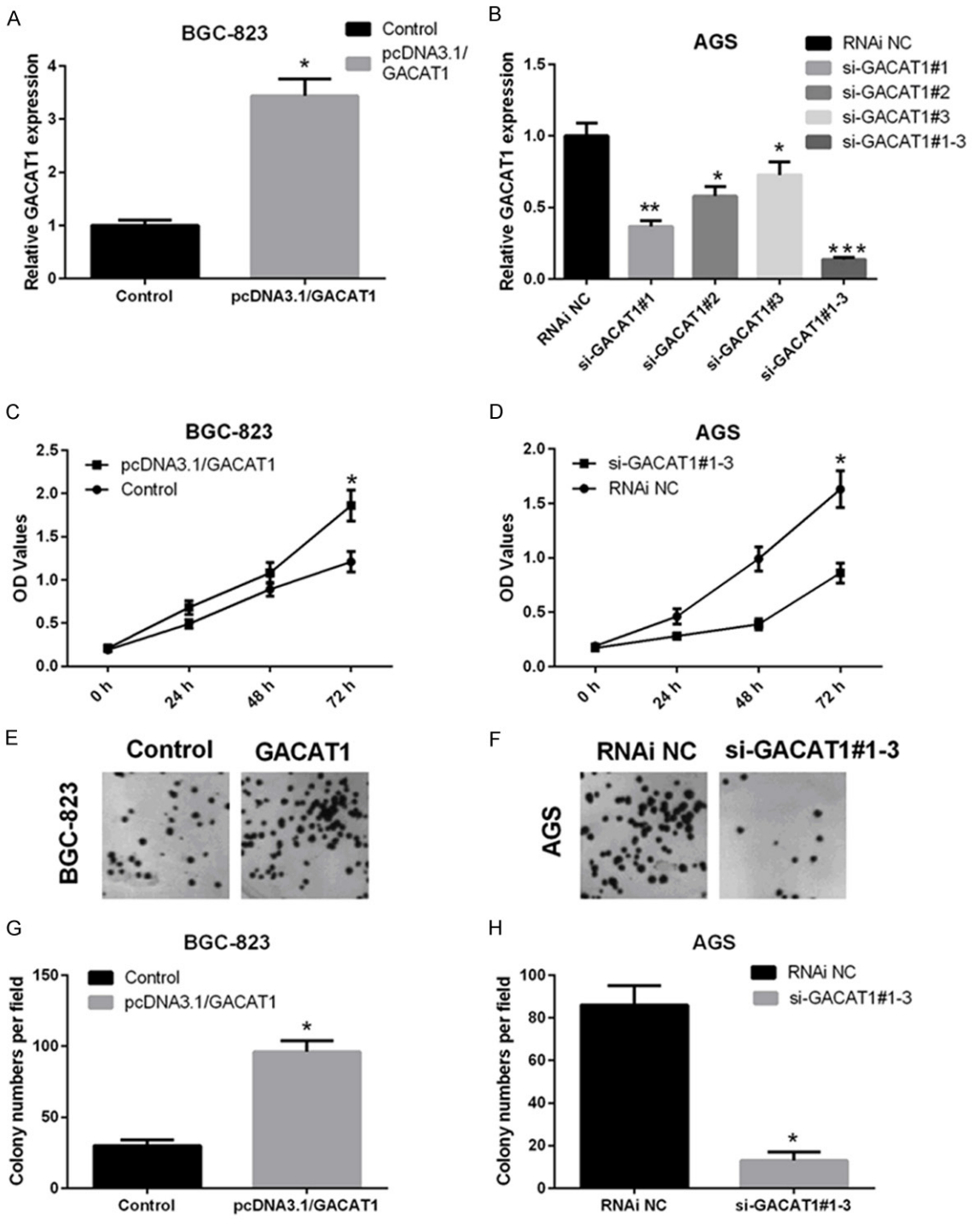


**Figure 2.** GACAT1 promotes proliferation ability of GC cells. A. The mRNA expression level of GACAT1 was measured by qRT-PCR in BGC-823 cells transfected with control and Lenti-GACAT1 (*$P$ < 0.05). B. Silence of GACAT1 in AGS cells after siRNA transfection. GACAT1 expression was detected by qRT-PCR in AGS cells transfected with negative control (NC), si-GACAT1#1, si-GACAT1#2, si-GACAT1#3, and si-GACAT1#1-3 (*$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001). C. MTT assay was performed to detect the proliferation ability of BGC-823 cells transfected with control and Lenti-GACAT1 (*$P$ < 0.05). D. MTT assay was performed to detect the proliferation ability of AGS cells transfected with NC and si-GACAT1#1-3 (*$P$ < 0.05). E. Overexpression of GACAT1 inhibited colony formation as demonstrated by colony formation assays in BGC-823 cells. F. Knockdown of GACAT1accelerated colony formation as demonstrated by colony formation assays in AGS cells. G. Changes in cell colony formation as affected by overexpression of GACAT1 (*$P$ < 0.05). H. Changes in cell colony formation as affected by knockdown of GACAT1 (*$P$ < 0.05).

*Methylthiazoletetrazolium (MTT) assay*

The transfected BGC-823 and AGS cells (3 × $10^3$ cells/well) were seeded in 96-well plates with 100 μLRPMI 1640 medium (10% FBS) at 37°C, 20 μL of MTT solution (5 mg/ml) were added into each 96-well at 0, 24, 48, and 72 hrs. After 4 hrs, 200 μL of dimethyl sulfoxide (DMSO) were added into each 96-well to dissolve the precipitates. The absorbance (A) was detected at 490 nm using Elx800 Reader (Bio-Tek Instruments Inc., Winooski, VT, USA).

*Colony formation assay*

The transfected BGC-823 and AGS cells (800 cells/well) were seeded in a six-well plate with 2 ml RPMI 1640 medium (10% FBS) at 37°C. After 14 days, cells were fixed with methanol and stained with 0.1% crystal violet. The number of visible colonies are counted.

*Transwell assay*

For migration assay, the transfected BGC-823 and AGS cells (3 × $10^3$ cells/well)were seeded in thetranswell chamber (Millipore, USA) without serum. For invasion assay, matrigel (BD biosciences) was plated in transwell inserts for 30 min at 37°C. 600 μl of RPMI 1640 including 10% FBS was added to the lower chamber. After 24 hrs, migrated cells were fixed with 100% methanol, and stained with crystal violet. The cells that did not migrate or invade were removed using cotton swabs. Migrative or invasive cells were photographed using a phase-contrast microscope (Olympus, Tokyo, Japan), and were then counted.

*Statistical analysis*

The data were analyzed by the Student's t-test and variance (ANOVA) using GraphPad (GraphPad Prism Software, La Jolla, CA, USA) and SPSS 15.0 software (SPSS, Chicago, IL, USA). Each experiment was repeated at least three times. All results were summarized and presented as means ± SD. $P < 0.05$ was considered statistically significant.

## Results

*Expression pattern of GACAT1 and miR-378 in GC tissues and cell lines*

We sorted out the GC tissues and paired adjacent normal tissues from 31 patients randomly. The mRNA expression level of GACAT1 was analyzed by qRT-PCR. Our results showed that the mRNA expression level of GACAT1 was up-regulated in GCtissues compared to adjacent normal tissues ($P < 0.05$, **Figure 1A**). The expression level of miR-378 was then detected by qRT-PCR in GC tissues. We found that miR-378 was down-regulated in GC tissues compared to adjacent normal tissues ($P < 0.05$, **Figure 1B**). GACAT1 expression was negatively correlated with miR-378 expression in 31 GC tissues (P = 0.004, **Figure 1C**). Moreover, we analyzed the expression levels of GACAT1 and miR-378 using qRT-PCR assay in human gastric epithelium GES-1 cells and GC cell lines (BGC-823, HGC-27, MGC-803 and AGS). As shown in **Figure 1D**, the results showed that GACAT1 expression was significantly upregulated in GC cell lines compared with that in human gastric epithelium GES-1 cells; The miR-378 expression level was significantly decreased in GC cell lines compared with that in human gastric epithelium GES-1 cells.

*GACAT1 promotes proliferation ability of GC cells*

To evaluate the effects of GACAT1 on GC cell proliferation ability, BGC-823 cells were respectively transfected with control and Lenti-GACAT1 to construct stably transfected cell lines. qRT-PCR analysis was applied to detect GACAT1 expression and validate the efficiency of GACAT1 overexpression. The results indicated that the expression level of GACAT1 was higher in the GACAT1 group than that in control group of BGC-823 cells ($P < 0.05$, **Figure 2A**). AGS cells were respectively transfected with negative control (NC), si-GACAT1#1, si-GACAT1#2, si-GACAT1#3, and si-GACAT1#1-3. qRT-PCR analysis was used to measure GACAT1 expression and validate the efficiency of GACAT1 knockdown. The results indicated that the expression level of GACAT1 was lower in the si-GACAT1#1, si-GACAT1#2, si-GACAT1#3, and si-GACAT1#1-3 group than that in NC group of AGS cells ($P < 0.05$, **Figure 2B**). The growth curves detected by MTT assay indicated that GACAT1 overexpression significantly increased BGC-823 cell growth ($P < 0.05$, **Figure 2C**); silence of GACAT1 by siRNAs significantly decreased AGS cell growth ($P < 0.05$, **Figure 2D**). Consistent with the results of MTT assay, colony-formation assay revealed that GACAT1 overexpression significantly increased anchor-

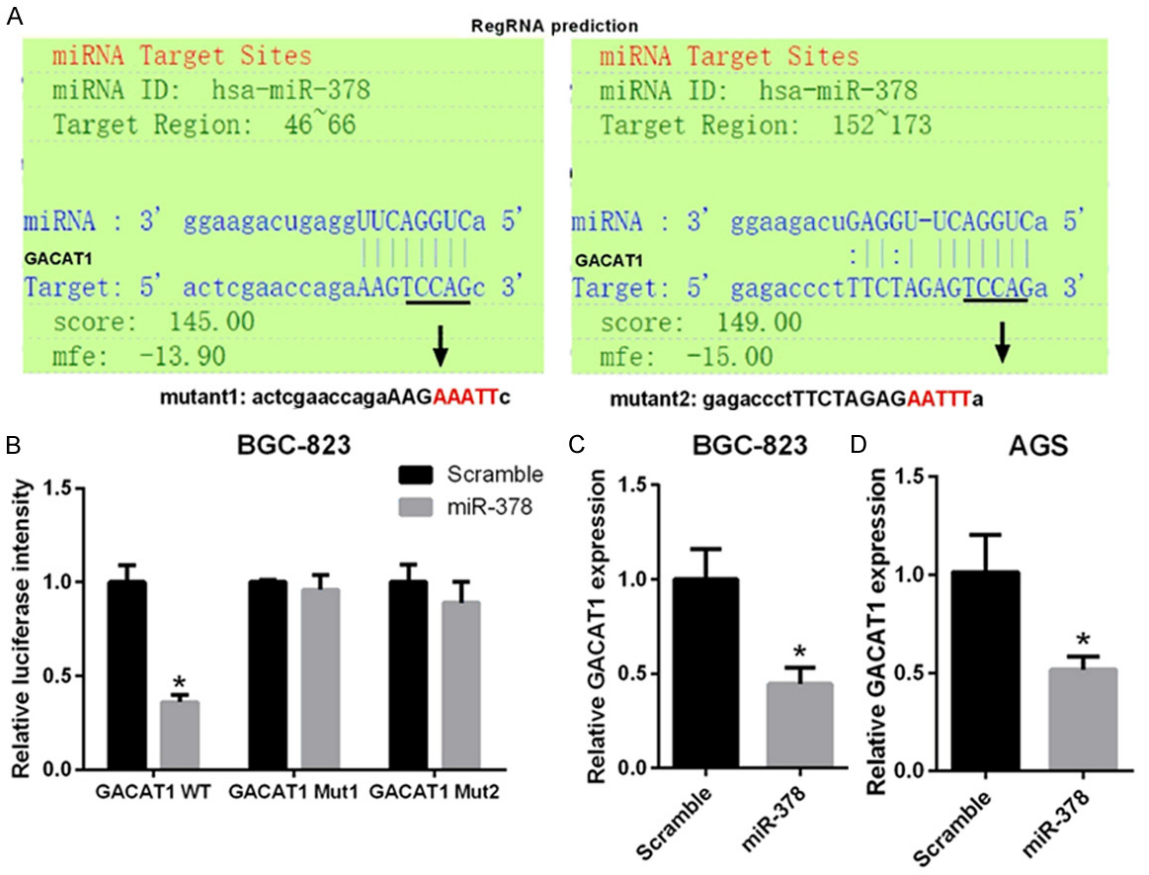


**Figure 3.** GACAT1 regulates miR-378 transcription negatively. A. Use of the StarBase v2.0 (http://starbase.sysu.edu.cn/ mirLncRNA.php) databases showed that the 46nt-66nt site and 152nt-173nt of GACAT1, which is conserved in humans and a potential binding site for miR-378. We designed mutation sequence according to two binding sites. B. Wild type and mutant GACAT1 sequences were cloned into pGL3 basic vectors and co-transfected with miR-378 into BGC-823 cells. Luciferase activity was detected using the dual-luciferase assay (*$P < 0.05$). C. qRT-PCR was used to analyze the expression level of GACAT1 in BGC-823 cells transfected with scramble and miR-378 respectively (*$P < 0.05$). D. qRT-PCR was used to analyze the expression level of GACAT1 in AGS cells transfected with scramble and miR-378 respectively (*$P < 0.05$).

age-independent growth of BGC-823 cells, as indicated by the formation of more and larger colonies in soft agar ($P < 0.05$, **Figure 2E** and **2G**); GACAT1 knockdown significantly decreased anchorage-independent growth of AGS cells, as indicated by the formation of fewer and smaller colonies in soft agar ($P < 0.05$, **Figure 2F** and **2H**). Therefore, we suggested that GACAT1 promotes proliferation ability of GC cells.

*GACAT1 regulates miR-378 expression in GC cells negatively*

We next predicted the binding sites between GACAT1 and miR-378 using StarBase v2.0 (http://starbase.sysu.edu.cn/mirLncRNA.php) databases. As shown in **Figure 3A**, we found that there were two binding sites (the 46nt-66nt site and 152nt-173nt) of GACAT1, which is conserved in humans and a potential binding site for miR-378, and designed mutation sequence according to two binding sites. Wild type and mutant GACAT1 sequences were cloned into pGL3 basic dual luciferase reporter vector and co-transfected with miR-378 into BGC-823 cells. Luciferase activity was detected using the dual-luciferase assay. The results showed a decrease in luciferase activity in cells transfected with wt-GACAT1 and miR-378 ($P < 0.05$); Co-transfection of mut-GACAT1 and miR-378 had no effect on reporter activity, which may due to the disrupt of miR-378 binding in the MUT construct ($P < 0.05$, **Figure 3B**). Because GACAT1 served as a sponge for miR-378

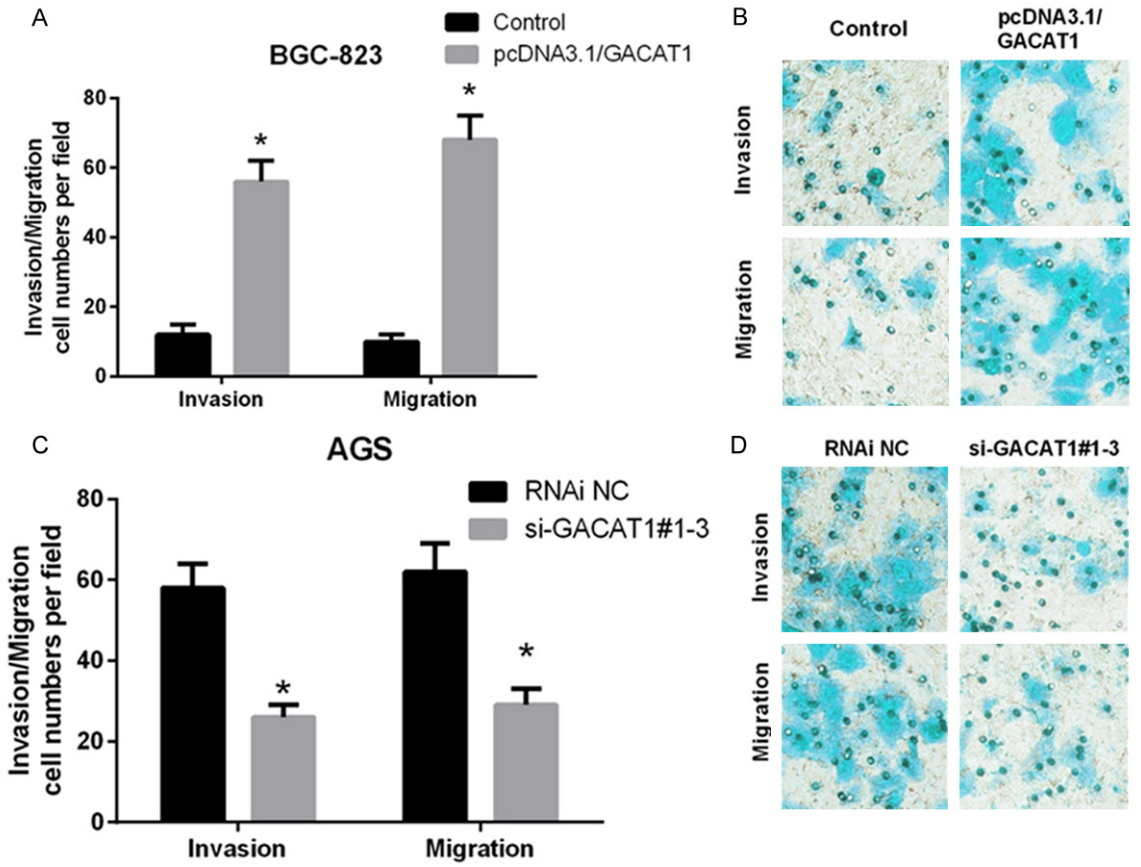


**Figure 4.** GACAT1 promotes migration and invasion of GC cells. A, B. Cell migration and invasion abilities were detected by Transwell in BGC-823 cells transfected with control and Lenti-GACAT1 respectively (*$P$ < 0.05). C, D. Cell migration and invasion abilities were detected by Transwell in AGS cells transfected with NC and si-GACAT1#1-3 respectively (*$P$ < 0.05).

in GC, we speculated that miR-378 could effectively modulate GACAT1 expression. qRT-PCR was used to analyze the expression level of GACAT1 in BGC-823 and AGS cells transfected with scramble and miR-378 respectively. As expected, the expression level of GACAT1 was decreased with miR-378 overexpression in BGC-823 ($P$ < 0.05, **Figure 3C**) and AGS cells ($P$ < 0.05, **Figure 3D**).

*GACAT1 promotes migration and invasion of GC cells*

Malignant cancer cells could acquire the abilities of migration and invasion. In the present study, the Transwell assay was used to determine the effects of GACAT1 on GC cell migration and invasion. The results revealed that the migration and invasion abilities of BGC-823 cells were increased after GACAT1 overexpression ($P$ < 0.05, **Figure 4A** and **4B**); The migration and invasion abilities of AGS cells were also inhibited after GACAT1 knockdown ($P$ < 0.05, **Figure 4C** and **4D**). These results indicated that GACAT1 could be a positive regulatory factor of GC cell migration and invasion.

*GACAT1 promotes proliferation ability of GC cells via miR-378*

According to the negative correlation between GACAT1 expression and miR-378 expression, we further speculated GACAT1 affected the progression of GC cells by targeting miR-378. BGC-823 cells were co-transfected miR-378 or miR-NC and pcDNA3.1 (empty vector) or pcDNA3.1/GACAT1 to study the effects of miR-378 on cell proliferation and invasion mediated by GACAT1. Firstly, qRT-PCR was performed to detect the expression level of GACAT1. The results indicated that miR-378 could significantly decrease the GACAT1 expression in BGC-823 cell lines,

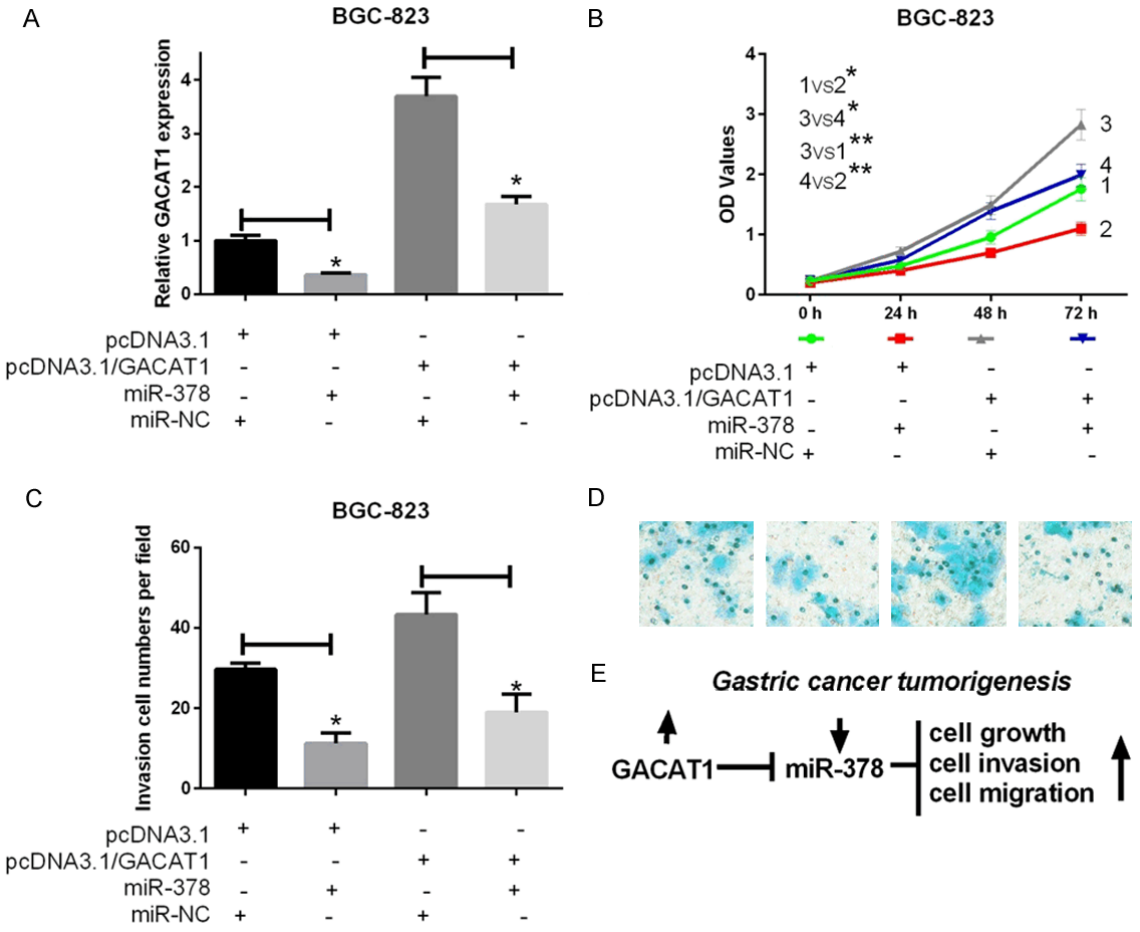


**Figure 5.** GACAT1 promotes proliferation ability of GC cells via miR-378. A. miR-378 (1 μg) or miR-NC (1 μg) was co-transfected into BGC-823 cells with pcDNA3.1 (empty vector, 1 μg) or pcDNA3.1/GACAT1 (1 μg). The expression level of GACAT1 was analyzed by qRT-PCR and GAPDH was used as an internal control (*$P$ < 0.05). B. Cell growth viability was assayed in miR-NC, miR-378 transfected or miR-378+pcDNA3.1 or miR-378+pcDNA3.1/GACAT1 co-transfected BGC-823 cells by MTT assay (*$P$ < 0.05, **$P$ < 0.01). C, D. Cell invasion was measured in miR-NC, miR-378 transfected or miR-378+pcDNA3.1 or miR-378+pcDNA3.1/GACAT1 co-transfected BGC-823 cells by Transwell assay (*$P$ < 0.05). E. The network model of GACAT1 in GC. GACAT1 decreased miR-378 expression. GACAT1 promoted the growth, migration and invasion abilities of GC cells through down-regulating miR-378.

and dramatically down-regulated high-expressed GACAT1 mediated by pcDNA3.1/GACAT1 ($P$ < 0.05, **Figure 5A**). Then, MTT proliferation assays revealed that miR-378 suppressed cell proliferation and GACAT1 promoted BGC-823 cell proliferation, while co-transfection of miR-378 and GACAT1 expression plasmid showed that miR-378 could abolish the effect of GACAT1 in inducing cell proliferation ($P$ < 0.05, **Figure 5B**). Furthermore, transwell invasion assay indicated that miR-378 inhibited and GACAT1 accelerated BGC-823 cell invasion, while co-transfection of miR-378 and GACAT1 expression vector showed that miR-378 decreased cell invasion promoted by GACAT1 ($P$ < 0.05, **Figure 5C** and **5D**). Therefore, we suggested that the effects of GACAT1 overexpression on the promotion of BGC-823 cell proliferation and invasion could be inhibited by miR-378. GACAT1 promoted the growth, migration and invasion abilities of GC cells through down-regulating miR-378 (**Figure 5E**).

## Discussion

LncRNAs are transcribed by RNA polymerase II [24] with greater than 200 nucleotides in length, and have noprotein-coding potential. However, they play important roles in regulating protein-coding genes at epigenetic, transcrip-

tional, post-transcriptional and translational levels [25-27]. A large study showed that they are frequently aberrantly expressed in cancers, and served as oncogenes or tumor suppressor genes to affect various physiological processes, such as proliferation, apoptosis, migration, metastasis, tumorigenesis, prognosis or diagnosis [28-33]. Therefore, the biological and molecular mechanisms of lncRNAs need more efforts to study in cancer.

In this study, we detected the expression of a novel long noncoding RNA GACAT1 in GC tissues and cells. We found that GACAT1 was upregulated in GC tissues compared to adjacent normal tissues (**Figure 1A**). At the same time, we found GACAT1 expression was significantly upregulated in GC cell lines compared with human gastric epithelium GES-1 cells (**Figure 1D**). In addition, we identified the function of GACAT1 in GC cells by applying overexpression and loss-of-function approaches. The results showed that GACAT1 overexpression significantly increased the proliferation ability of BGC-823 cels (**Figure 2C**, **2E** and **2G**); Silence of GACAT1 by siRNAssignificantly decreased the proliferation ability of AGS cells (**Figure 2D**, **2F** and **2H**). In addition, we revealed that GACAT1 overexpression promoted the migration and invasion abilities of BGC-823 cells (**Figure 4A** and **4B**); GACAT1 knockdown inhibited the migration and invasion abilities of AGS cells (**Figure 4C** and **4D**). Therefore, GACAT1 may act as a novel diagnostic marker and therapeutic target for GC.

MiRNAs are small, noncoding RNAs ~22 nucleotides in length, have important biological functions in the process of cancer development, such as cell differentiation, inflammation, metastasis and regulation of cell cycle and apoptosis [16, 34, 35]. At present, more new miRNAs are found dysregulated in various tumor types, and detection of their levels promises to be a new indicator for cancer diagnose. In this study, we detected the expression of miR-378 in GC tissues and cells. We found that miR-378 was down-regulated in GC tissues compared to adjacent normal tissues (**Figure 1B**). Simultaneously, we found miR-378 was significantly down-regulated in GC cell lines compared with human gastric epithelium GES-1 cells (**Figure 1D**).

At present, most of lncRNAs have been proved to play important roles in biological functions, such as proliferation, development [36], apoptosis [37] and differentiation [38], are deregulated in various human cancers [39-41]. The specific molecular mechanisms by which lncRNAsregulate tumor progressarenot fully understood. On the contrary, miRNAs have been identified to play important roles in the progression of in human tumor, the regulatory mechanisms are more diverse and complicated compared with lncRNAs. Recent studies have demonstrated the lncRNAs, served as miRNA sponges or miRNA inhibitors, interact with miRNAs and modulate the expression level of miRNA target genes [42, 43]. LncRNA-associated competing endogenous RNAs (ceRNA) network have been studied in gastric cancer [7], breast cancer [44], and glioblastoma multiforme [45]. In this study, we found that GACAT1 expression negatively correlated with miR-378 expression in 31 GC tissues (**Figure 1C**). The dual-luciferase assay results also indicated that there was a decrease in luciferase activity in cells transfected with wt-GACAT1 and miR-378, suggesting that GACAT1 regulated miR-378 transcription negatively (**Figure 3B**). At the same time, the expression level of GACAT1 was decreased with miR-378 overexpression in BGC-823 (**Figure 3C**) and AGS cells (**Figure 3D**). Furthermore, we revealed that miR-378 suppressed cell proliferation and invasion, and GACAT1 promoted cell proliferation and invasion, while co-transfection of miR-378 and GACAT1 expression plasmid showed that miR-378 could abolish the effect of GACAT1 in inducing cell proliferation and invasion (**Figure 5B** and **5C**). Therefore, GACAT1 promoted the growth and invasion abilities of GC cells through inhibiting miR-378.

In summary, our results indicated that GACAT1 was up-expressed, and miR-378 was down-expressed in GC tissues and cells. GACAT1 was negatively correlated with miR-378 expression in GC tissues. GACAT1 directly regulated miR-378 transcription negatively. The effects of GACAT1 on the promotion of GC cell proliferation and invasion can be suppressed by miR-378. Therefore, our study proved that GACAT1 could be a potential therapeutic target for treatment of GC.

## Acknowledgements

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## Disclosure of conflict of interest

None.

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