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ORIGINAL PAPER

miR-196a-5p Correlates with Chronic Atrophic Gastritis Progression to Gastric Cancer and Induces Malignant Biological Behaviors of Gastric Cancer Cells by Targeting ACER2

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Abstract

Background As the prognosis of early gastric cancer (EGC) is significantly better than that of advanced gastric cancer (AGC), the development of biomarkers to monitor the progression of chronic atrophic gastritis (CAG) to gastric cancer (GC) is essential.

Methods Stomach tissue miRNA and mRNA sequences from patients with chronic non-atrophic gastritis (CNAG), CAG, precancerous lesions of gastric cancer (PLGC), and GC were analyzed. A publicly available GC-related miRNA microarray dataset was obtained from the Gene Expression Omnibus database. Spearman's correlation and differential gene analyses, and clinical validation were used to identify novel miRNAs correlating with CAG progression to GC. miRNA targets were predicted using weighted gene co-expression analysis and databases. A dual-luciferase reporter assay was performed to check for direct interaction between miR-196a-5p and ACER2. The CCK-8 and wound healing assays, and flow cytometry were performed to evaluate cell proliferation, migration, and apoptosis.

Results miR-196a-5p was correlated with CAG progression to GC. Overexpression of miR-196a-5p promoted GC cell proliferation and migration and inhibited apoptosis, whereas suppression of miR-196a-5p exerted the opposite effect. Based on the prediction and luciferase assays, ACER2 was identified as the target of miR-196a-5p. ACER2 was downregulated in GC cell lines. Knockdown of ACER2 increased GC cell proliferation rates and migration ability and inhibited apoptosis, while ACER2 overexpression led to the opposite effect.

Conclusions miR-196a-5p correlated with CAG progression to GC and induced malignant biological behaviors of GC cells by targeting ACER2, providing a novel monitoring biomarker and target for GC prevention.

Keywords miR-196a-5p · ACER2 · Chronic atrophic gastritis · Gastric cancer · Malignant behavior

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Introduction

Gastric cancer (GC), a heterogeneous and prevalent disease, remains the fifth leading cause of cancer and the third leading cause of cancer-related mortality worldwide [1]. GC is histologically classified into two major subtypes: intestinal and diffuse [2]. Intestinal-type GC (IGC) develops via Correa's pathway, a relatively fixed evolutionary paradigm starting with inflammation and progressing to atrophy, intestinal metaplasia, gastric precancerous lesions, and frank malignancy, which makes it possible to develop biomarkers to monitor its early onset [3]. As the prognosis of early gastric cancer (EGC) is significantly better compared with advanced gastric cancer (AGC) [4], the detection of EGC is essential.

MicroRNAs (miRNAs) are endogenous non-coding single-stranded small RNAs mainly derived from single-stranded RNA precursors, with a general length of 19–24 nucleotides [5, 6]. An estimated 30% of the protein-coding genes in the human genome are regulated by miRNAs [7]. miRNAs can cleave and degrade mRNA by binding to the 3'-untranslated region (UTR) [8]. As regulatory molecules of gene expression, miRNAs function as oncogenes or antioncogenes in tumor development by regulating cell proliferation, apoptosis, and differentiation [9]. Many studies have suggested that miRNAs are aberrantly expressed in cancer tissues compared with normal tissues [10]. It has also been demonstrated that miRNA imbalance plays a key role in the pathological progression of GC [11].

Herein, to discover novel miRNAs, we conducted miRNA and mRNA sequencing of stomach tissues from patients with chronic non-atrophic gastritis (CNAG), chronic atrophic gastritis (CAG), precancerous lesions of gastric cancer (PLGC), and GC, which were uploaded to the NCBI Sequence Read Archive (SRA) with the project numbers SRP234371 and SRP234584. A publicly available GC-related miRNA microarray dataset (GSE93415) was obtained to ensure screening stability [12]. Through bioinformatic methods and clinical validation, miR-196a-5p was found to be correlated with CAG progression to GC. By targeting ACER2 (alkaline ceramidase 2), miR-196a-5p induced the malignant biological behaviors of GC cells, which provided a new insight for GC prevention.

Materials and Methods

Participants and Samples

A total of 57 stomach tissues were collected, including 5 CNAG, 5 CAG, 5 PLGC, and 5 GC for RNA sequencing and 15 CNAG, 14 CAG, 9 PLGC, and 9 GC for clinical validation from the patients who underwent endoscopy or surgery at the First Affiliated Hospital of Guangzhou University of Chinese Medicine. All specimens were snap-frozen in liquid nitrogen after resection and stored at -80℃. All patients signed an informed consent form before the operation. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine.

RNA Extraction and RNA Sequencing Data Acquisition and Processing

Total RNA was extracted from 5 CNAG, 5 CAG, 5 PLGC, and 5 GC tissues using OMEGAE.Z.N. Total RNA Kit I (R6834-01–50 preps, OMEGAE, USA). RNA quality was assessed by spectrophotometry and denaturing agarose gel electrophoresis. Sequencing libraries for miRNAs and mRNAs were prepared, purified, qualified, and sequenced using the BGISEQ-500 platform. The sequencing data were uploaded to the NCBI SRA under project numbers SRP234371 and SRP234584.

Collection of miRNA Datasets for GC

A publicly available GC-related miRNA microarray dataset (GSE93415, 20 GC tissues, and 20 adjacent normal tissues) was obtained from the Gene Expression Omnibus database [12]. The gene expression matrix was extracted using R and used to screen for novel miRNAs.

Novel miRNA Identification

We defined "stage" as representing the severity of pathology; CNAG was represented by "1," CAG by "2," PLGC by "3," and GC by "4." In our sequence data, we conducted Spearman's correlation analysis between "stage" and each miRNA to screen for novel miRNAs with a p-value < 0.01 and $|R| > 0.5$ as the cutoff. Next, in the miRNA microarray dataset (GSE93415), we screened out genes with significantly differential expression using the "limma" package [13] with $|\log_2 FC| \geq 1.585$ and a false discovery rate (FDR) < 0.01 as the cutoff. After intersecting miRNAs were screened from our sequence data and the publicly available microarray dataset, the shared miRNAs were considered novel miRNAs.

Targets of Novel miRNA Prediction

To identify novel miRNA-targeted mRNAs, weighted gene co-expression analysis (WGCNA) was performed to construct an mRNA co-expression network and explore its correlation with the novel miRNA expression and pathological stage. Firstly, by using the "WGCNA" package [14] in R, a weighted mRNA co-expression network was established in

our sequenced mRNA dataset based on the top 25% genes with the largest variance differences. Subsequently, we built a similarity matrix based on the Pearson's correlation value between the paired genes. An adjacency matrix was then constructed using the formula $a_{mn} = |cmn|^{\beta}$ (amn: adjacency matrix between genes m and n, c_{mn} = Pearson's correlation between paired genes, β: soft power value) and subsequently transformed into a topological overlap matrix. The value of the soft threshold power was chosen with a scale-free topology scale, R^2, exceeding 0.90. Genes with similar expression patterns were categorized into modules by average linkage hierarchical clustering, with the module minimum size set as 100, and colors were used to label the modules. Those gene modules that were significantly negatively correlated with the novel miRNAs and correlated with "stage" were considered as potential targets of the novel miRNAs. We also used the miRDB (http://mirdb.org/), miRTarBase (http://miRTarBase.cuhk.edu.cn/), and TargetScan (http://www.targetscan.org/mamm_31/) databases to predict the target mRNAs of the novel miRNAs. The intersected mRNA from database predictions and WGCNA were considered as miRNA targets and sent for further analysis.

Cell Culture

The GC cell lines AGS, BGC-801, MNK-45, MGC-803, and the normal human gastric epithelial cell line GES-1 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in RPMI 1640 containing 10% FBS and 1% double antibiotics at 37 °C in a humidified atmosphere with 5% CO_2.

Cell Transfection

Mimics, inhibitors, and negative controls of miR-196a-5p and ACER2 were transfected into AGS and MGC-803 cells using Lipofectamine 2000 reagent (Invitrogen), according to the manufacturer's instructions.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted using TRIzol reagent (Invitrogen, China), according to the manufacturer's protocol. Reverse transcription was performed according to the manufacturer's instructions using the PrimeScript RT Reagent Kit (Takara, China). A SYBR PrimeScript RT-PCR Kit (Takara) was used for quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The $2^{-\Delta\Delta Ct}$ statistic was used to calculate gene expression levels.

Cell Proliferation Assay

AGS and MGC-803 cells were harvested with 0.25% trypsin–EDTA solution, sub-cultured in 96-well plates, and transfected with mimics, inhibitors, and negative controls of miR-196a-5p and ACER2. At 0, 24 and 48 h after transfection, 10% CCK-8 reagent was added, and the cells were cultured in the incubator for 1 h. The results were measured using a microplate reader at 450 nm (optical density [OD). GC cell proliferation rates were calculated based on the formula: Day n OD value/Day 0 average OD value.

Apoptosis Assay

Cell apoptosis was analyzed using the Annexin V PE/7-Amino-Actinomycin (7-AAD) Apoptosis Detection Kit (BD Pharmingen, San Diego, CA, USA), according to the manufacturer's protocol. At 24 h after transfection, cells were incubated with Annexin V antibody and 7-Amino-Actinomycin and analyzed by flow cytometry.

Migration Assay (Scratch Test)

AGS and MGC-803 were transfected with mimics and inhibitors, as well as negative controls of miR-196a-5p or ACER2, and cultured in 24-well plates for 24 h. After using a 10-μL pipette tip to create a scratch a line across the cells, the plates were incubated in 5% CO_2 at 37 °C. Wound healing was observed using an inverted microscope at 0, 24, and 48 h.

Statistical Analysis

The Student's t-test or Mann–Whitney test was used to compare the gene expression levels of different groups depending on the distribution of data. Pearson's or Spearman's correlation tests were performed to evaluate the statistical correlation, depending on the distribution of data. All statistical analyses were performed using the R software (version 3.6.3) or GraphPad Prism software (version 8.0). All p-values were two-tailed, and a p-value < 0.05 was considered statistically significant.

Results

miR-196a-5p is a Novel miRNA Correlated with CAG Progression to GC

Through Spearman's correlation analysis, we found that 45 miRNAs significantly correlated with "stage" in our sequence data. In addition, in the miRNA microarray dataset (GSE93415), differential expression analysis revealed

that 20 miRNAs were significantly differentially expressed between GC and normal tissues. After taking this intersection, miR-196a-5p was found to be a novel miRNA that correlated with CAG progression to GC (Fig. 1A). The expression of miR-196a-5p showed a gradual upward trend accompanied by CAG progression to GC (Fig. 1B) and was significantly correlated with tumorigenesis ($r = 0.6350$, $p = 0.0026$, Fig. 1C). In addition, miR-196a-5p was markedly upregulated in tumor tissues in the GSE93415 dataset ($p < 0.0001$, Fig. 1D).

Validation of miR-196a-5p Expression in Clinical Samples and GC Cell Lines

The expression of miR-196a-5p showed an increasing trend with CAG progression. Significant differences were observed between CAG and PLGC, as well as between PLGC and GC (Fig. 2A). We compared miR-196a-5p expression between gastric cancer cell lines (AGS, BGC-801, MNK-45, and MGC-803) and GES-1 using qRT-PCR. Compared with GES-1 cells, miR-196a-5p was significantly upregulated in AGS, BGC-801, MNK-45, and MGC-803 cells (Fig. 2B). As AGS and MGC-803 displayed the most

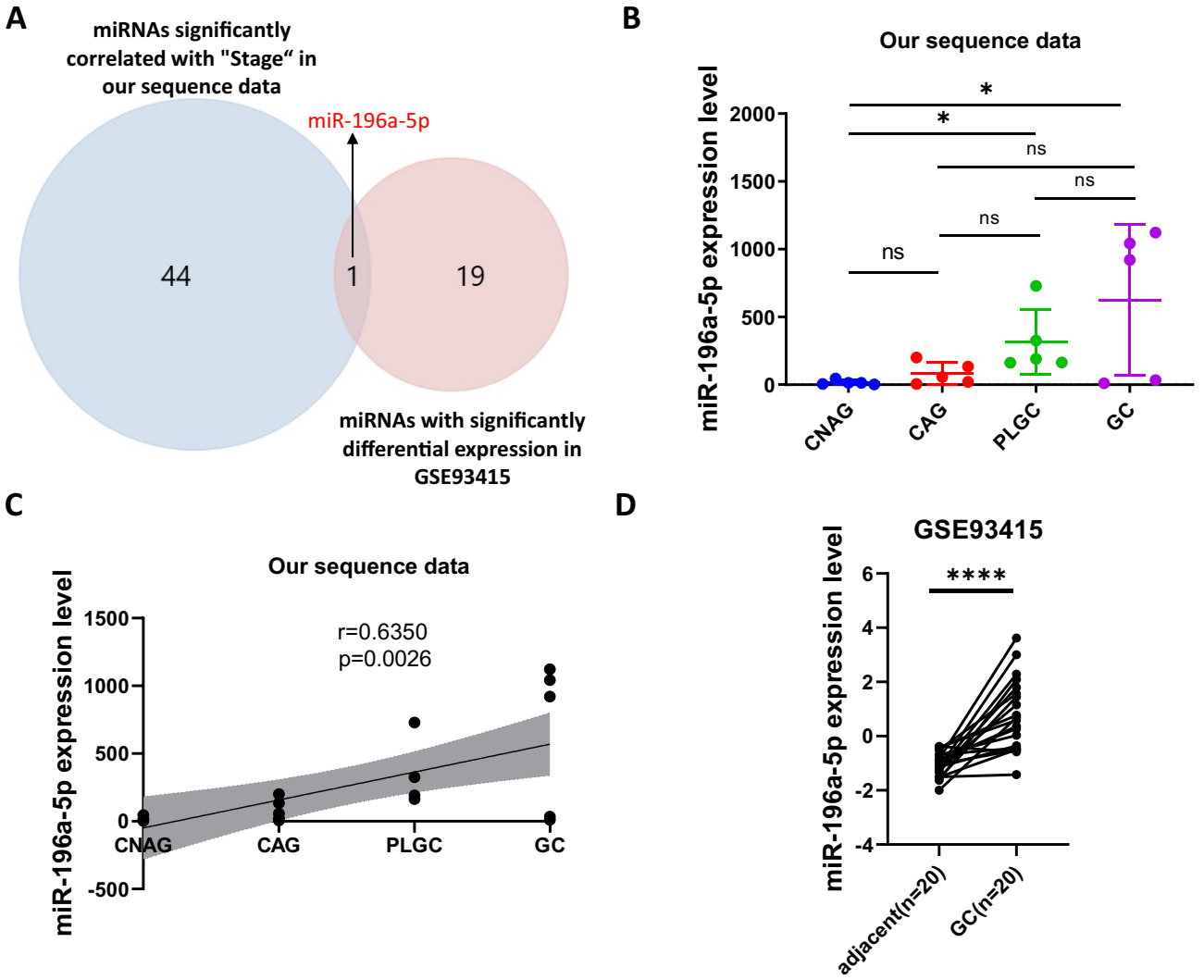


Fig. 1 miR-196a-5p is a novel miRNA correlated with CAG progression to GC. **A** miR-196a-5p was found in both miRNAs that significantly correlated with "stage" in our sequence data and miRNAs with significantly differential expression in GSE93415. It was regarded as a novel miRNA correlated with CAG progression to GC. **B** The expression of miR-196a-5p showed a gradually upward trend accompanied with CAG progression to GC in the RNA sequence data. **C** miR-196a-5p was significantly correlated with tumorigenesis in RNA sequence data ($r = 0.6350$, $p = 0.0026$). **D** miR-196a-5p was also remarkedly upregulated in tumor tissues in GSE93415 datasets. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

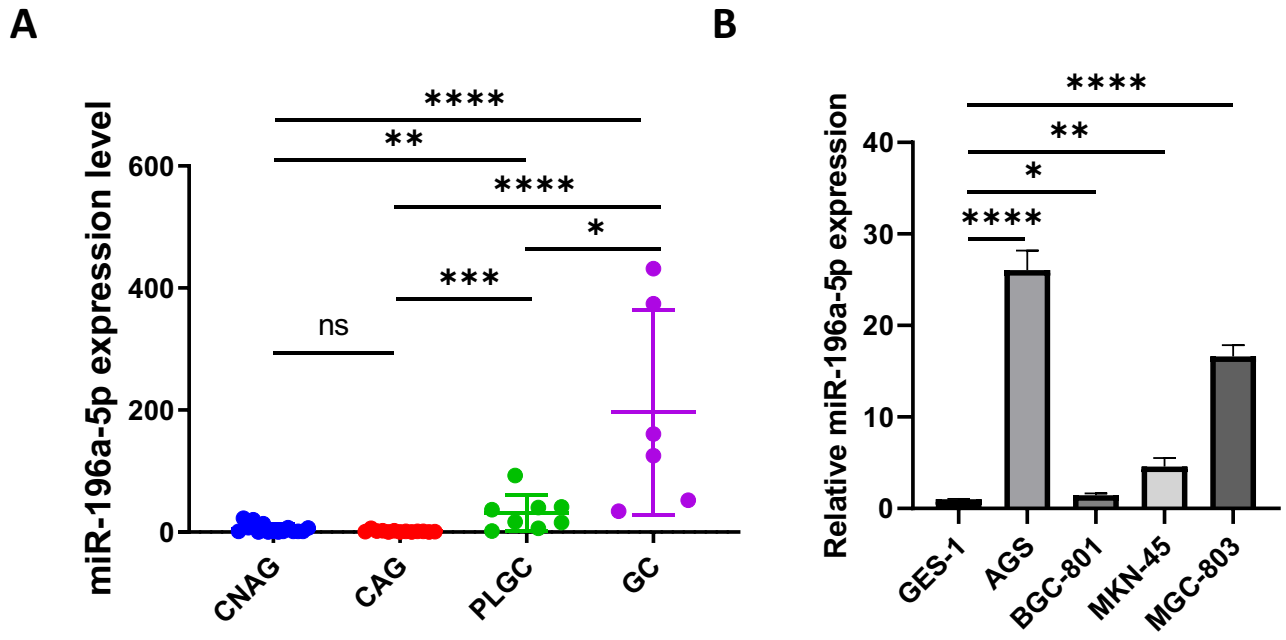


Fig. 2 Validation of miR-196a-5p expression in clinical samples and GC cell lines. **A** miR-196a-5p showed a gradual upward trend with CAG progression to GC confirmed by qPCR. **B** miR-196a-5p was significantly upregulated in AGS, BGC-801, MNK-45, and MGC-803 compared with GES-1. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$

remarkable alterations, we chose them for subsequent experiments.

miR-196a-5p Possessed the Greatest Potential in Targeting ACER2

A total of 4573 mRNAs in our sequence data with the highest expression variance (top 25%) were selected for subsequent WGCNA. $\beta=8$ (scale-free $R^2=0.91$) was the lowest power fit scale-free index, over 0.90, which was determined as the soft-thresholding power parameter to ensure a scale-free network (Fig. 3A). Genes with similar expression patterns were grouped into 12 co-expression modules. We found that only the green module, containing 260 genes, was significantly negatively correlated with miR-196a-5p expression ($R=-0.65$, $p=0.002$) and pathological stage ($R=-0.53$, $p=0.02$) (Fig. 3B). For database prediction, there were 370 and 303 genes from miRDB, miRTarBase, and 375 from TargetScan. After intersecting, only ACER2 remained a candidate target of miR-196a-5p (Fig. 3C). We compared ACER2 expression between GC cell lines and GES-1 cells and found that the expression of ACER2 was significantly downregulated in GC cell lines ($p<0.001$, Fig. 3D), which was in line with the upregulation of miR-196a-5p in GC cells.

Validation of miR-196a-5p Targeting ACER2

AGS and MGC-803 cells were transfected with the miR-196a-5p inhibitor or mimic for downregulation (Fig. 4A) and overexpression (Fig. 4B) of miR-196a-5p. After transfection with the inhibitor, ACER2 expression was upregulated (Fig. 4C), whereas after transfection with mimics, the expression of ACER2 was decreased (Fig. 4D), indicating that miR-196a-5p exerted a negative effect on the regulation of ACER2 in GC lines. Upon transfection of the empty psiCHECK2 plasmid or psiCHECK2 plasmid cloned with the mut-ACER2 gene, there was no significant difference in luciferase activity between the groups (Fig. 4E, G). After transfecting the psiCHECK2 plasmid cloned with the ACER2 gene, luciferase activity was significantly downregulated in the miR-196a-5p mimics group compared with the mimics NC group, whereas it was markedly upregulated in the miR-196a-5p inhibitor group compared with the inhibitor NC group ($p<0.001$, Fig. 4F). Overall, miR-196a-5p targets ACER2 to reduce its expression.

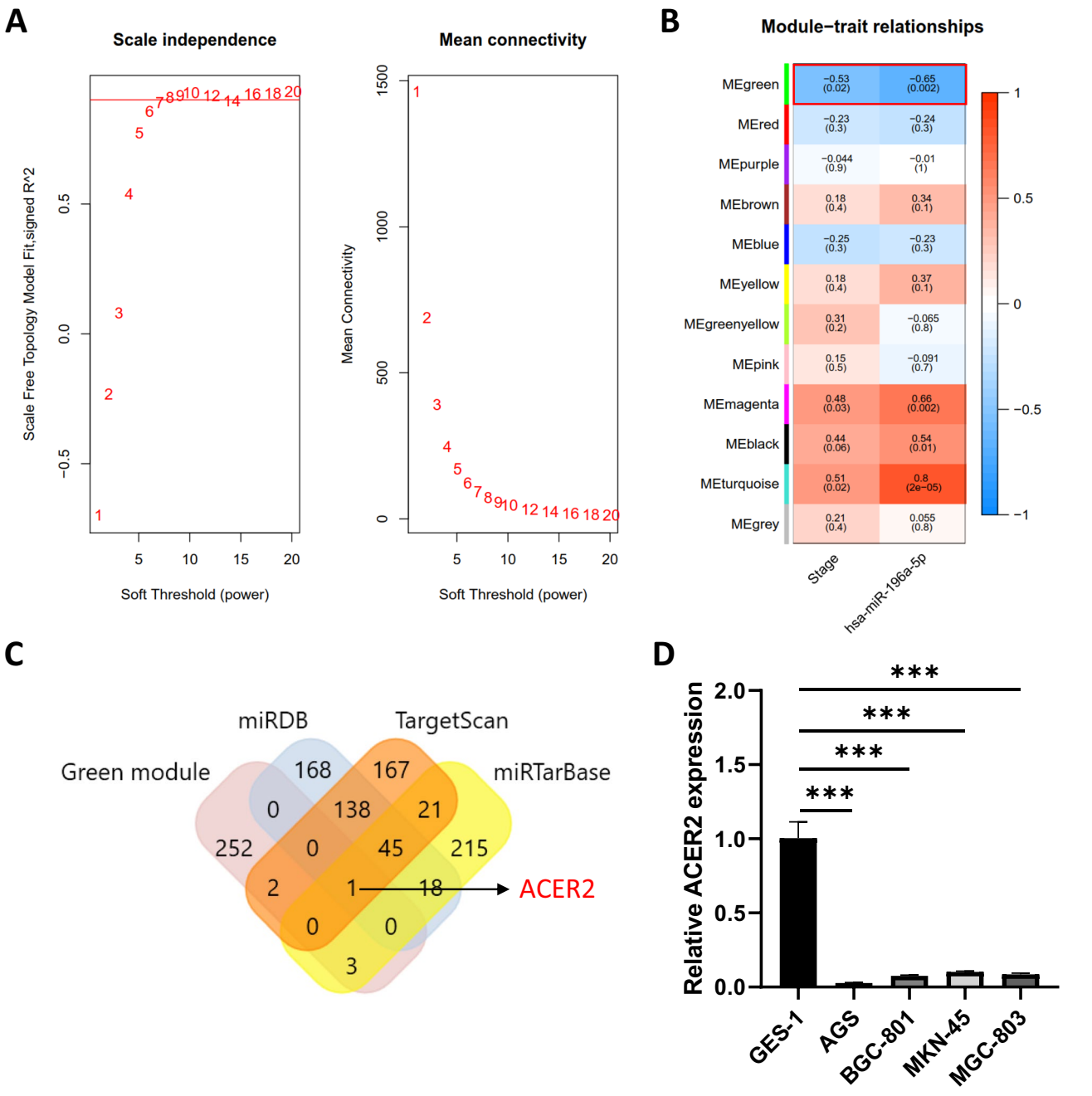


Fig. 3 miR-196a-5p possessed the greatest potential in targeting ACER2. **A** $\beta = 8$ (scale-free $R^2 = 0.91$) was the lowest power fit scale-free indices over 0.90 and was determined as the soft-thresholding power parameters to ensure a scale-free network. **B** Genes with similar expression patterns were grouped into 12 co-expression modules. The green module, containing 260 genes, was significantly negatively correlated with miR-196a-5p expression ($R = -0.65$, $p = 0.002$) and pathology stage ($R = -0.53$, $p = 0.02$). **C** By taking the intersection of the green module, miRDB, miRTarBase, and TargetScan, ACER2 remained as the only candidate target of miR-196a-5p. **D** ACER2 was significantly downregulated in GC cell lines. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

Overexpression of miR-196a-5p Promotes Malignant Behavior of GC Cells

As miR-196a-5p is associated with CAG progression to GC, its impact on the malignant behavior of GC cells was evaluated. AGS and MGC-803 cells were transfected with the miR-196a-5p inhibitor or mimics, and their proliferation rates were evaluated using CCK-8. miR-196a-5p knockdown

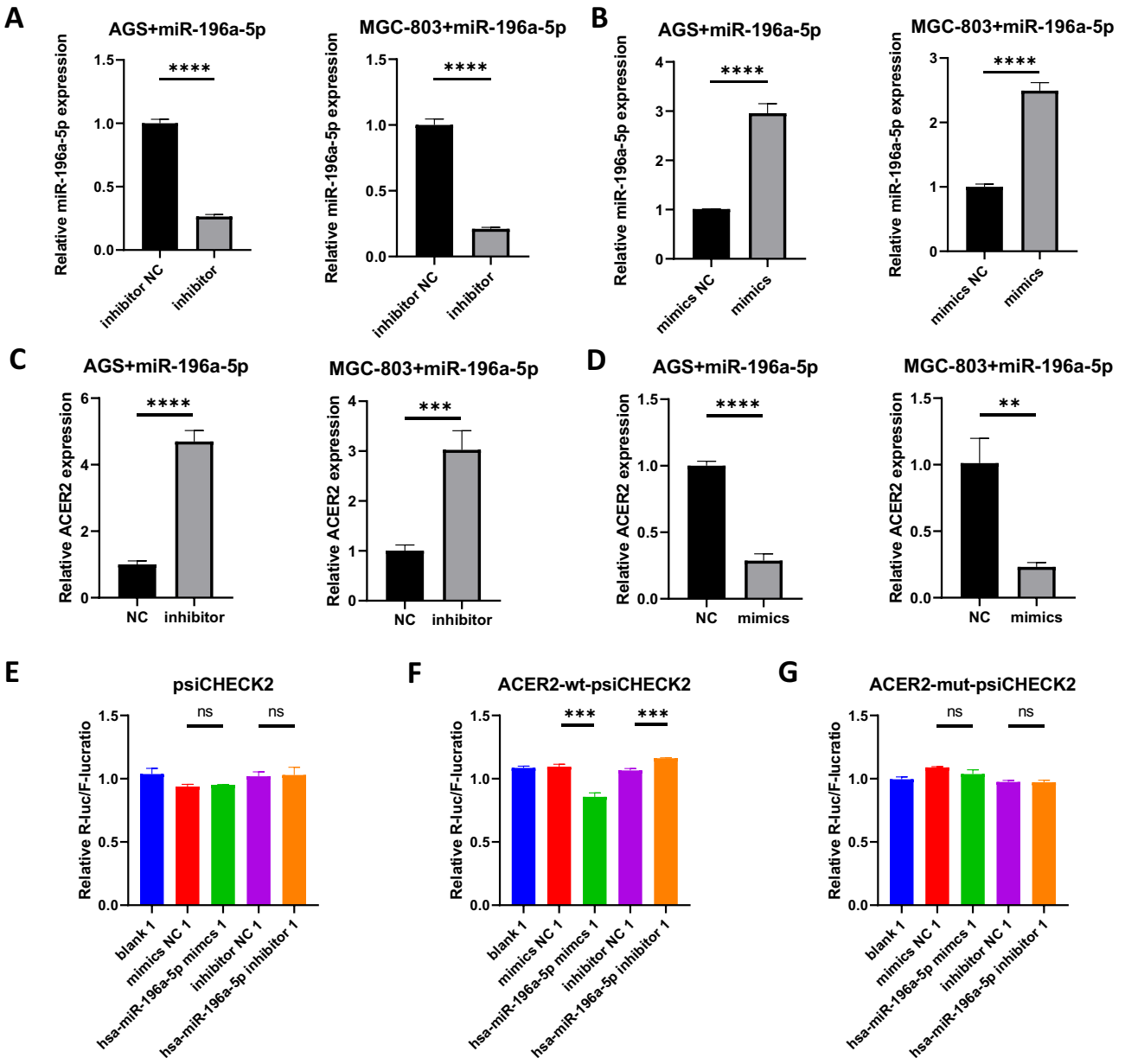
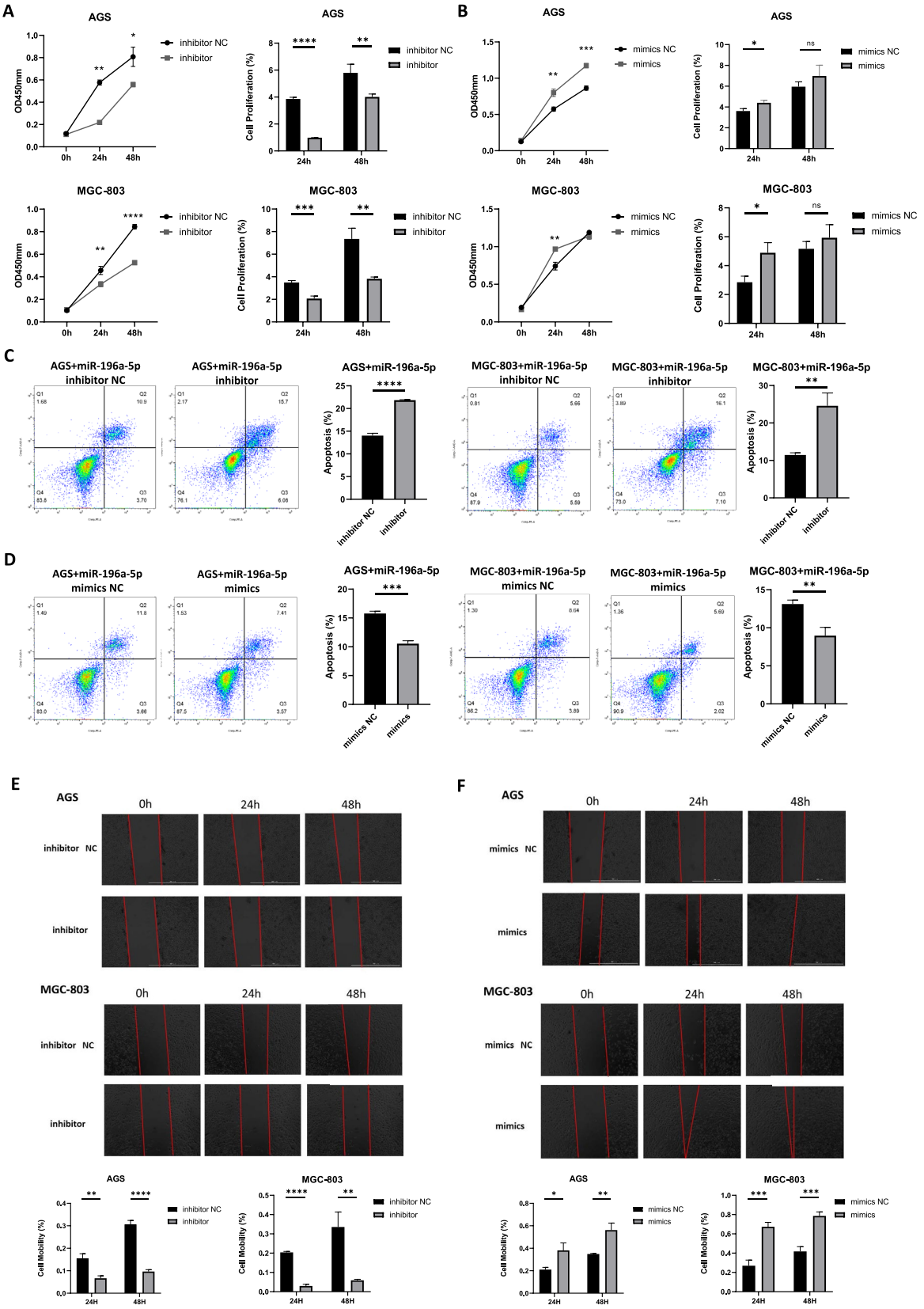


Fig. 4 Validation of miR-196a-5p targeting ACER2. **A** The expression of miR-196a-5p in AGS and MGC-803 was significantly downregulated after transfection with the miR-196a-5p inhibitor. **B** The expression of miR-196a-5p in AGS and MGC-803 was significantly upregulated after transfection with the miR-196a-5p mimic. **C** ACER2 expression was significantly upregulated after transfection with the miR-196a-5p inhibitor. **D** ACER2 expression was significantly downregulated after transfection with the miR-196a-5p mimic. **E** There was no significant difference in luciferase activity between each group upon transfection of the empty psiCHECK2 plasmid. **F** Luciferase activity was significantly downregulated in the miR-196a-5p mimics group, whilst it was remarkedly upregulated in the miR-196a-5p inhibitor group compared with the inhibitor NC group. **G** There was no significant difference in luciferase activity between each group when transected with the psiCHECK2 plasmid cloned with mut-ACER2 gene. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

suppressed the proliferation rates of AGS and MGC-803 cells (Fig. 5A), whereas miR-196a-5p overexpression conferred the opposite effect (Fig. 5B). As confirmed by flow cytometric analysis, miR-196a-5p knockdown promoted apoptosis in AGS and MGC-803 cells (AGS: $p < 0.0001$; MGC-803: $p < 0.01$, Fig. 5C), while miR-196a-5p overexpression induced the opposite effect (AGS, $p < 0.001$; MGC-803, $p < 0.01$; Fig. 5D). Using a cell scratch test, we examined the impact of miR-196a-5p on GC cell migration; miR-196a-5p knockdown suppressed the migration ability of AGS and MGC-803 cells (Fig. 5E), while overexpression boosted these effects (Fig. 5F).


A
AGS
OD450mm
0h
24h
48h
inhibitor NC
inhibitor
Cell Proliferation (%)
MGC-803
B
mimics NC
mimics
ns
C
AGS+miR-196a-5p
inhibitor NC
inhibitor
MGC-803+miR-196a-5p
Apoptosis (%)
D
mimics NC
mimics
E
F
Cell Mobility (%)

◂**Fig. 5** Overexpression of miR-196a-5p promoted malignant behavior of GC cells. **A** miR-196a-5p knockdown suppressed the proliferation rates of AGS and MGC-803 cells. **B** miR-196a-5p overexpression increased the proliferation rates of AGS and MGC-803 cells. **C** miR-196a-5p knockdown promoted the apoptosis rates of AGS and MGC-803 cells. **D**miR-196a-5p overexpression suppressed the apoptosis rates of AGS and MGC-803 cells. **E** miR-196a-5p knockdown suppressed the migration ability of AGS and MGC-803 cells according to the cell scratch test. **F** miR-196a-5p overexpression increased the migration ability of AGS and MGC-803 cells according to the cell scratch test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

Inhibition of ACER2 Promotes Malignant Behavior of GC Cells

Inhibition of ACER2 has been reported to be associated with malignant behavior. Therefore, we transfected cells with ACER2 inhibitors or mimics to explore their effects on GC cells. AGS and MGC-803 cells were transfected with ACER2 inhibitors or mimics, and their proliferation rates were evaluated using the CCK-8 assay. ACER2 knockdown increased the proliferation rates of AGS and MGC-803 cells (Fig. 6A), whereas ACER2 overexpression had the opposite effect (Fig. 6B). Flow cytometric analysis confirmed that ACER2 knockdown inhibited apoptosis in AGS and MGC-803 cells (AGS: $p < 0.001$; MGC-803: $p < 0.01$, Fig. 6C), whereas ACER2 overexpression induced the opposite effect (AGS: $p < 0.01$; MGC-803: $p < 0.0001$, Fig. 6D). The cell scratch test indicated that ACER2 knockdown promoted the migration ability of AGS and MGC-803 cells (Fig. 6E), whereas overexpression impaired this ability (Fig. 6F).

Discussion

Because the prognosis of EGC is much better than that of AGC, early diagnosis and treatment are crucial [15]. Recent studies have shown that miRNAs, serving as tumor suppressor genes or oncogenes, play a vital role in GC development [16]. miRNAs participate in important biological activities, including cell proliferation, apoptosis, and differentiation [17]. As abnormal cell proliferation and apoptosis often occur in tumors, abnormal deletions, mutations, or overexpression of miRNAs are associated with tumorigenesis [18]. In this study, through high-throughput and clinical validation, miR-196a-5p correlated with CAG progression to GC and could be utilized as a novel biomarker for monitoring CAG progression. Overexpression of miR-196a-5p promoted GC cell proliferation and migration and inhibited apoptosis, whereas suppression of miR-196a-5p exerted the opposite effect. miR-196a-5p, located on chromosome 11, is known to regulate various critical cellular processes, such as proliferation, apoptosis, and differentiation, by targeting various transcription factors, including HOXB8, HMGA2, and annexin A1 [19, 20]. In GC, miR-196a-5p is upregulated and modulates GC stem cell characteristics by targeting Smad4 [21]. It also has been found to be overexpressed in other cancers and promotes malignant behaviors. In non-small cell lung cancer, tumor-associated macrophages secrete exosomal miR-196a-5p to promote metastasis [22]. In colorectal cancer, miR-196a-5p promotes metastasis by targeting IκBα [23]. These observations indicate that miR-196a-5p is a pivotal oncogene in various tumors, not only in GC.

miRNAs can interact with the 3′-UTR, 5′-UTR, coding sequence, and gene promoter to regulate gene expression; the most common is targeting the 3'-UTR to inhibit gene expression [24]. In this study, using prediction and luciferase assays, miR-196a-5p was confirmed directly targeting the 3'UTR of the ACER2 mRNA. Using qRT-PCR, ACER2 was found to be downregulated in the GC cell lines. Knockdown of ACER2 increased GC cell proliferation rates and migration ability and inhibited apoptosis, while ACER2 overexpression led to the opposite effect. ACER2 belongs to a family of ceramidases with the ability to remove fatty acids from the lipid molecule ceramide, thereby producing sphingosine (SPH), which can be phosphorylated to form sphingosine-1-phosphate (S1P) [25]. Ceramides, SPH, and S1P are bioactive lipids that mediate various biological processes. Ceramides have been reported to have antiproliferative, pro-apoptotic, and pro-senescent effects [26, 27]. SPH has also been shown to mediate cell cycle arrest [28], differentiation [29], and programmed cell death (PCD) [30]. In contrast, S1P mainly promotes cell proliferation and survival and inhibits senescence [31]. It was found that when cells express low levels of ACER2, the proliferative effect of S1P could counterbalance the anti-proliferative effect of low levels of SPH, thus promoting cell proliferation, whereas in cells expressing high levels of ACER2, the apoptotic effect of high levels of SPH could override the anti-apoptotic effects of S1P, thus resulting in PCD [32]. This mechanism can explain why knockdown of ACER2 increased GC cell malignancy. Furthermore, Wang et al. found that ACER2 can produce ROS and induce autophagy and apoptosis, further illustrating the antitumor effect of ACER2 [33].

Conclusion

In conclusion, our study revealed that miR-196a-5p was upregulated from the progression of CAG to GC and could be utilized as a novel biomarker for monitoring CAG progression. miR-196a-5p enhanced the malignant biological behaviors of GC cells by downregulating ACER2, thus resulting in CAG progression to GC, which provides a novel target for GC prevention.

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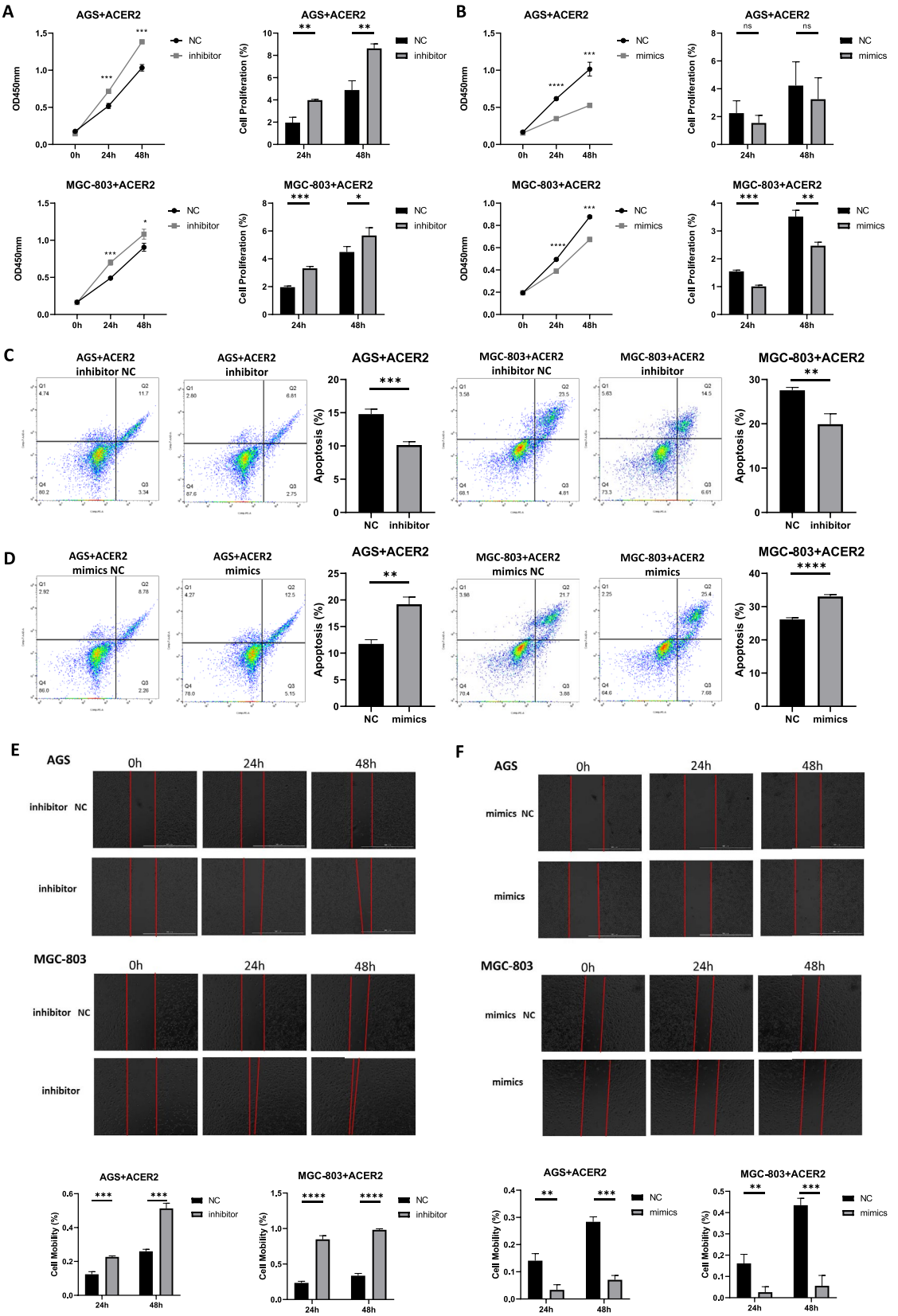
A
AGS+ACER2
OD450nm
0h
24h
48h
NC
inhibitor
Cell Proliferation (%)
MGC-803+ACER2
B
mimics
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C
AGS+ACER2 inhibitor NC
AGS+ACER2 inhibitor
MGC-803+ACER2 inhibitor NC
MGC-803+ACER2 inhibitor
Apoptosis (%)
D
AGS+ACER2 mimics NC
AGS+ACER2 mimics
MGC-803+ACER2 mimics NC
MGC-803+ACER2 mimics
E
AGS
MGC-803
inhibitor NC
inhibitor
Cell Mobility (%)
F
mimics NC
mimics

◂**Fig. 6** Inhibition of ACER2 promoted malignant behavior of GC cells. **A** ACER2 knockdown increased proliferation rates of AGS and MGC-803 cells. **B** ACER2 overexpression suppressed proliferation rates of AGS and MGC-803 cells. **C** ACER2 knockdown inhibited the apoptosis rates of AGS and MGC-803 cells. **D** ACER2 overexpression increased the apoptosis rates of AGS and MGC-803 cells. **E** ACER2 knockdown promoted the migration ability of AGS and MGC-803 cells according to the cell scratch test. **F** ACER2 overexpression suppressed the migration ability of AGS and MGC-803 cells according to the cell scratch test. $*p<0.05$; $**p<0.01$; $***p<0.001$; $****p<0.0001$

Author Contributions FL, YW, and PL designed this study. XJ, KJ, and JZ performed the experiments and wrote the manuscript. YY and JP coordinated the study and interpreted the results. KJ collected data. All authors have read and approved the final manuscript.

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Data Availability The datasets used and/or analyzed during the current study are available from the Gene Expression Omnibus database (GSE93415) and the NCBI Sequence Read Archive with project numbers SRP234371 and SRP234584.

Declarations

Conflict of interest None.

Ethical Approval and Consent to Participate All patients signed an informed consent form before the operation. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine.

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