



Original Research

CEACAM5 targeted by miR-498 promotes cell proliferation, migration and epithelial to mesenchymal transition in gastric cancer

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ABSTRACT

Objective: Recent studies have shown that carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) may serve as an independent predictor of advanced gastric cancer (GC). The purpose of this research is to explore the patterns of expression, functions, and upstream regulatory pathway of CEACAM5 in GC.

Methods: The levels of miR-498 and CEACAM5 expression in GC cells and tissues were measured via qRT-PCR. Wound-healing, CCK-8, and western blotting experiments were conducted for the evaluation of GC cell migration, proliferation, and epithelial-mesenchymal transition (EMT), respectively. The targeting relationship between miR-498 and CEACAM5 was validated via pull-down and luciferase reporter assays. Xenograft tumor mouse models were established to observe CEACAM5's influence on the growth of tumors *in vivo*.

Results: Elevated levels of CEACAM5 were detected among the GC cells and tissues. The results of the *in vitro* experiments revealed that the knockdown of CEACAM5 in GC cells significantly inhibited their proliferation, migration, and EMT. Moreover, CEACAM5 inhibition effectively hampered GC cell growth within the nude mice. Moreover, miR-498 directly targeted CEACAM5. MiR-498 downregulation had been observed among the cells and tissues of GC. The stimulation of GC cell proliferation, migration, and EMT, which had been engendered by CEACAM5 overexpression, was reversible through the overexpression of miR-498.

Conclusion: The outcomes of this research suggest that miR-498 is capable of repressing the proliferation, migration, and EMT of GC cells through CEACAM5 downregulation.

Introduction

Gastric cancer (GC) is a common malignant tumor that occurs within the digestive system [1]. GC has an extremely high mortality rate, and it has gravely affected the patients' quality of life and survival [1]. Existing treatment options for GC cannot appreciably improve the survival prognosis of patients. Hence, there is an urgent need for more in-depth studies on the biological mechanisms of GC. Some studies have demonstrated that the important causes of GC mortality are tumor invasion and metastasis, which may strictly be regulated by various genes that can either promote or suppress tumors [2]. Accordingly, activating tumor suppressor genes or downregulating the expression of oncogenes may be an effective approach of tumor therapy [3]. Therefore, exploring GC's metastasis and invasion mechanisms and searching for potential therapeutic targets are necessary for its early diagnosis, prevention, and treatment.

Over the last few years, epithelial-mesenchymal transition (EMT) has

become a research hotspot in oncology. Almost 90% of epithelial malignant tumor metastasis, including gastric cancer, may implicate EMT [4]. EMT is the process of transforming polar epithelial cells into active interstitial cells and gaining the ability to migrate and invade [5]. The loss of phenotype and the acquisition of interstitial characteristics are the important features of EMT occurrence. These include the decreased expression of epithelial cell adhesion molecules (ZO-1 and E-cadherin), increased expression of interstitial cell adhesion molecules (Vimentin and N-cadherin), and enhanced activity of transcription factors (Snail, Slug, and Twist). This phenotype conversion would loosen the connection between tumor cells, reorganize the cytoskeletal proteins, and reduce their ability of adhere [4,6]. A lot of studies have proven that EMT is aberrantly activated, and that it displays pro-tumorigenic properties in GC progression [7,8]. In light of that, research that address EMT may contribute knowledge that may help in elucidating the pathogenesis of GC and discovering novel biotherapeutic targets.

Carcinoembryonic antigen-related cell adhesion molecule 5

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(CEACAM5) is from the immunoglobulin (Ig) superfamily and CEA family, which are closely related to cell adhesion [9]. Research has shown that CEACAM5 was overexpressed in a variety of tumor tissues [10]. Moreover, its overexpression can change cell polarity and normal tissue structure, slow cell differentiation and maturation, and eventually lead to tumor formation [10]. Some studies suggested that CEACAM5 has a vital part in the differential diagnosis and prognosis of epithelial tumors, such as those in the gastrointestinal tract, respiratory tract, and breast [10]. Aberrant CEACAM5 expression is usually related to the progression of the abovementioned tumors [11]. Notably, a study has reported that CEACAM5 can function as an independent indicator for the pathological diagnosis of GC [12]. Nonetheless, no study so far has shown the specific role and mechanisms of CEACAM5 in GC.

With an understanding of GC pathogenesis, the abnormal expression of microRNAs (miRNAs) has become a research hotspot in oncology. A growing number of research has revealed that miRNA may serve as therapeutic targets or tumor markers to participate in tumor occurrence and development [13,14]. Previous research has documented that miRNA may operate as an oncogenes or tumor suppressor in GC to exert the corresponding biological function. For example, miR-148a boosts GC cell proliferation and metastasis targeting p27 [15]. Another study has suggested that miR-29b suppresses GC cell migration and growth via the downregulation of MMP2 expression [16]. Through bioinformatics analysis, miR-498 has been predicted to be an upstream target of CEACAM5. Moreover, it has been confirmed to serve as an inhibitor of a number of tumors, including liver cancer [17], cervical cancer [18], and ovarian cancer [19]. Even so, there are only a few studies on the application of miR-498 in GC. Its downstream regulatory pathway in GC needs to be studied further.

In this work, we explore the specific function of CEACAM5 in GC. Also, we aim to uncover whether miR-498 influences GC progression through CEACAM5. Our findings may contribute new perspectives on targeted GC therapy.

Materials and methods

Clinical samples

Gastric tissue specimens were gathered from GC patients who received resection surgery at our hospital. All patients signed a written informed consent, and none had received radiotherapy or chemotherapy prior this surgery. A total of 40 GC tissues and 40 adjacent normal tissues were obtained. All the tissues were confirmed via gastroscopy and pathologic examinations. The Ethics Committee of the The Sixth Hospital of Wuhan, The Affiliated Hospital of Jiangnan University granted approval to this research protocol.

Cell culture

The GC cells (AGS, MKN45, and HGC-27) and the normal gastric epithelial cell (GES-1) were sourced from the Institute of Cell Research, Shanghai Academy of Sciences, China. These above cells were cultivated at 37 °C with 5% CO₂ in a Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, USA) that had 100 U/ml streptomycin, 100 U/ml penicillin, and 10% fetal bovine serum (FBS; Gibco, USA). When 90% cell fusion was attained, subculturing was performed. Cells at the logarithmic phase were chosen for the succeeding experiments.

Cell transfection

Small interfering RNA (siRNA) that target CEACAM5 (si-CEACAM5), siRNA negative control (si-NC), miR-498 mimic, and its negative control (miR-NC) were constructed by RiboBio (Guangzhou, China). CEACAM5 overexpression (OE) plasmid (CEACAM5-OE) and its corresponding empty vector (NC-OE) were purchased from OBIO Technology (Shanghai, China). Strictly following the manufacturer's instructions, a

Lipofectamine 3000 (Invitrogen, USA) was utilized for the transfection of the abovementioned plasmids into the AGS and HGC-27 cells. Forty-eight hours after the transfection, RT-PCR was carried out to evaluate the transfection efficiency.

qRT-PCR analysis

The relative miRNA (miR-498) and mRNA (CEACAM5) expressions in GC cells were estimated via qRT-PCR. The total RNAs in transfected cells were extracted with the aid of a TRIzol reagent (Invitrogen, USA). Afterward, Master Mix kit (Takara, USA) and miRNA First-Strand cDNA Synthesis Kit (Invitrogen, USA) were employed to synthesize cDNA from mRNAs and miRNAs, respectively, through reverse transcription. The ABI 7500 detection system, SYBR Green PCR Kit (Takara, Japan), and TaqMan specific microRNA probe (Life technology, USA) were utilized for the qRT-PCR. The reaction was carried out with the following conditions: 94 °C for 10 s, 60 °C for 30 s, 68 °C for 2 min, 40 cycles. GAPDH and U6 were adopted to normalize the data. The relative expressions were calculated by applying the 2^{-ΔΔCt} approach [20]. We listed the primer information in Table 1.

CCK-8 assay

Each of the wells of 96-well culture plates were inoculated with 1 × 10⁴ transfected cells and incubated for 0, 24, 48, and 72 h. After incubating for their specified durations, each well was supplied with 10 μl of the CCK-8 solution then cultured for two more hours. A microplate reader (Bio-Rad, USA) with a 450 nm filter was utilized to read the absorbance in each well, which reflects the cell proliferation activity. The cell growth curve was drawn after recording all results.

Wound-healing assay

The 6-well culture plates were inoculated with 3 × 10⁵ cells/well and cultured overnight. On the following day, a sterilized 100 μl pipette gun head was wielded to straightly scrape a line on the middle of each well. The shed cells were rinsed with PBS and then cultivated for 24 h in a serum-free RPMI-1640 medium. At 0 and 24 h, images of the wounded sites were captured through the microscope. The migratory abilities of the cells were then evaluated.

Animal experiments

Ten 4- to 6-week-old female BALB/C nude mice that weighed 18–20 g were randomly designated to the sh-CEACAM5 (n = 5) or sh-NC (n = 5) groups after adaptive rearing under standard conditions. Short harpin RNA (shRNA) that target CEACAM5 (Sh-CEACAM5) and the control (Sh-NC) was acquired from OBIO Technology (Shanghai, China) and then transfected together with lentiviruses. Sh-NC- or Sh-CEACAM5-transfected HGC-27 cells were subcutaneously delivered into each mouse, depending on their group. The nude mice and their tumors were weighed every 4 days, starting from the 4th day after their injection. Euthanasia of the mice via CO₂ inhalation was carried out after 28 days. The xenograft tumors were then acquired and weighed. The Animal

Table 1
The sequences of primers.

Gene name	Sequence (5' - 3')
CEACAM5	F: GCCTCAATAGGACCACAGTCAC R: CAGGTTAAGGCTACAGCATCCTC
miR-498	F: TTTCAAGCCAGGGGGCGTTTTTC R: GCTTCAAGCTCTGGAGGT GCTTTTC
GAPDH	F: AAGCCGATCTACGCCGGAGAGCGCC R: CCTAGCGACGGCGGCTTAGCGAACC
U6	F: GCACATTCTCCCAGTTATGA R: TCACAAATTTGCATGTCATCT

Ethics Committee of The Sixth Hospital of Wuhan, The Affiliated Hospital of Jiangnan University approved all the procedures that had been executed in this animal experiment.

Luciferase reporter assay

TargetScan (www.targetscan.org) and starBase (<http://starbase.sysu.edu.cn/index.php>) were utilized in the prediction of miR-498's target gene, CEACAM5. Wild-type (CEACAM5-WT) and mutated CEACAM5 (CEACAM5-Mut) 3'-UTR luciferase reporters were constructed, with the genomic DNA of human GC cells as the template. The density of GC cells at logarithmic phase was adjusted to 2×10^5 cells/well with the RPMI-1640 culture medium. CEACAM5-WT and CEACAM5-Mut were each introduced simultaneously into the AGS and HGC-27 cells along with a miR-NC or miR-498 mimic. Lipofectamine 3000 (Invitrogen, USA) was utilized to accomplish the transfections. The cells were maintained for 48 h in the following conditions: 37 °C and 5% CO₂. Finally, the luciferase activities were assessed with the aid of a Promega luciferase assay kit (USA).

Pull-down assay

The Pierce Magnetic RNA-Protein Pull-Down Kit (Pierce Biotechnology, USA) detected the binding site of miR-498 and CEACAM5. Bio-labeled miR-223-3p (Bio-miR-223-3p), Bio-labeled miR-552-3p (Bio-miR-552-3p), Bio-labeled miR-498 (Bio-miR-498), Bio-labeled miR-335-5p (Bio-miR-335-5p), Bio-labeled miR-361-5p (Bio-miR-361-5p), Bio-labeled miR-148a-3p (miR-148a-3p), Bio-labeled miR-148b-3p (Bio-miR-148b-3p), Bio-labeled miR-152-3p (Bio-miR-152-3p), and Bio-NC were all sourced from OBIO Technology (Shanghai, China). Magnetic streptavidin beads (ThermoFisher, USA) were incubated with the lysed cells that had been transfected with either Bio-miR-498 or Bio-NC. Subsequently, RNA was extracted from cells after the addition of DNase A and protein K. Finally, RT-PCR was conducted to evaluate CEACAM5 mRNA expression.

Western Blot

A RIPA lysis buffer (Beyotime, Shanghai, China) was utilized for the isolation of proteins from the transfected cells. The BCA protein quantitative kit (Beyotime, Shanghai, China) was then employed to quantify the concentration of the isolated proteins. The protein samples (20 µg) were separated with the aid of a 12% SDS-PAGE gel before being electroblotted onto the PVDF membranes. A blocking solution (5% skim milk) was then applied and maintained for 4 h at 4 °C to seal the membranes. Afterward, the proteins were incubated overnight at 4 °C with the following primary antibodies from Abcam: E-cadherin (cat. no. ab233611; 1:1000 dilution), CEACAM5 (cat. no. ab133633; 1:1000 dilution), Snail (cat. no. ab216347; 1:1000 dilution), Vimentin (cat. no. ab8978; 1:1000 dilution), and GAPDH (cat. no. ab9485; 1:1000 dilution). The membranes were rinsed with TBST before they were maintained at 37 °C for 3 h with an anti-rabbit IgG (cat. no. ab6721; Abcam). An ECL reagent (GE Healthcare, USA) was applied to detect the protein bands. Finally, the grayscale values of the protein bands were assessed using the Image J software. GAPDH was adopted as the internal control.

Statistical analysis

All data gathered in this study were analyzed in IBM SPSS Statistics 20.0 (Chicago, IL, USA). The figures were written in the form of the mean ± standard deviation (SD). One-way ANOVA with Tukey's test was applied for multiple comparisons, whereas student's t-test was for the pairwise comparisons. Spearman's rank correlation coefficient was applied to analyze the association of miR-498 expression with that of CEACAM5. A $P < 0.05$ is indicative of statistical significance.

Results

GC cells and tissues exhibited CEACAM5 upregulation

We evaluated CEACAM5's differential expression in GC. Based on the GEPIA analysis (<http://gepia.cancer-pku.cn/>), the GC samples exhibited upregulated CEACAM5 expressions (Fig. 1A). Considering that result, the CEACAM5 mRNA levels in the 40 pairs of tumoral and adjacent normal tissue samples from GC patients were detected via qRT-PCR. The GC tissues manifested significantly higher CEACAM5 expression than the control (Fig. 1B). QRT-PCR was performed to assess CEACAM5 levels among the normal gastric cell (GES-1) and GC cells (HGC-27, MKN45 and AGS). The outcome revealed that, unlike the GES-1 cells, the GC cells had elevated CEACAM5 levels. AGS and HGC-27 exhibited the highest CEACAM5 expressions (Fig. 1C), thus they were selected for the succeeding experiments. Finally, CEACAM5-specific siRNA was introduced into the AGS and HGC-27 cells to decrease CEACAM5 levels within them. Western blotting and qRT-PCR were conducted to determine the transfection efficiency. The outcomes of the qRT-PCR revealed that the Si-CEACAM5 group exhibited lower CEACAM5 levels than the NC group (Fig. 1D). Moreover, the western blot analysis showed that the Si-CEACAM5 group also had significantly lower levels of CEACAM5 protein than the NC group (Fig. 1E). These results prove that the transfection was successful. Generally speaking, these findings indicate that the downregulation of CEACAM5 may be related to the development of GC.

CEACAM5 knockdown repressed GC cell proliferation, migration, and EMT

We tried to explore the specific biological functions of CEACAM5 in GC. We performed CCK-8, wound-healing, and western blotting experiments to evaluate cell proliferation, cell migration, and the levels of proteins related to EMT (E-cadherin, Vimentin, and Snail), respectively. CCK-8 results demonstrated that knocking-down CEACAM5 significantly inhibited the viability of AGS and HGC-27 cells compared with the NC group (Fig. 2A). Furthermore, the outcome of the wound-healing experiment revealed that knocking-down CEACAM5 considerably diminished the migratory abilities of HGC-27 and AGS (Fig. 2B). Finally, western blotting analysis uncovered that the Si-CEACAM5 group had high E-cadherin expression while having significantly lower levels of Vimentin and Snail (Fig. 2C). These results suggest that CEACAM5 depletion can effectively repress GC cell viability, migration, and EMT.

CEACAM5 knockdown repressed GC cell growth in mice

Having demonstrated CEACAM5's tumor-promoting effect in GC cells, we then verified its role in GC tumor xenograft growth. The tumor growth rate of shRNA-CEACAM5 transfected mice notably slowed down (Fig. 3A). Moreover, the tumor volume of shRNA-CEACAM5 transfected mice was significantly smaller than that of the control (Fig. 3B). The results of the tumor weight test revealed that the knockdown of CEACAM5 considerably decreased tumor weight (Fig. 3C). These results suggest that CEACAM5 knockdown inhibited *in vivo* GC growth.

CEACAM5 was predicted as a miR-498 target

After observing CEACAM5's tumor suppressive effect on GC *in vivo* and *in vitro*, we further studied its upstream regulatory miRNAs. StarBase and TargetScan were employed to identify the upstream miRNAs of CEACAM5, discovering that eight common miRNAs (miR-148b-3p, miR-148a-3p, miR-152-3p, miR-223-3p, miR-335-5p, miR-361-5p, miR-498, and miR-552-3p) were overlapping (Fig. 4A). The results of the pull-down assay showed that, among those miRNAs, CEACAM5 had the higher enrichment in miR-498 (Fig. 4B). Hence, miR-498 was selected as the upstream gene that target CEACAM5. StarBase database suggested

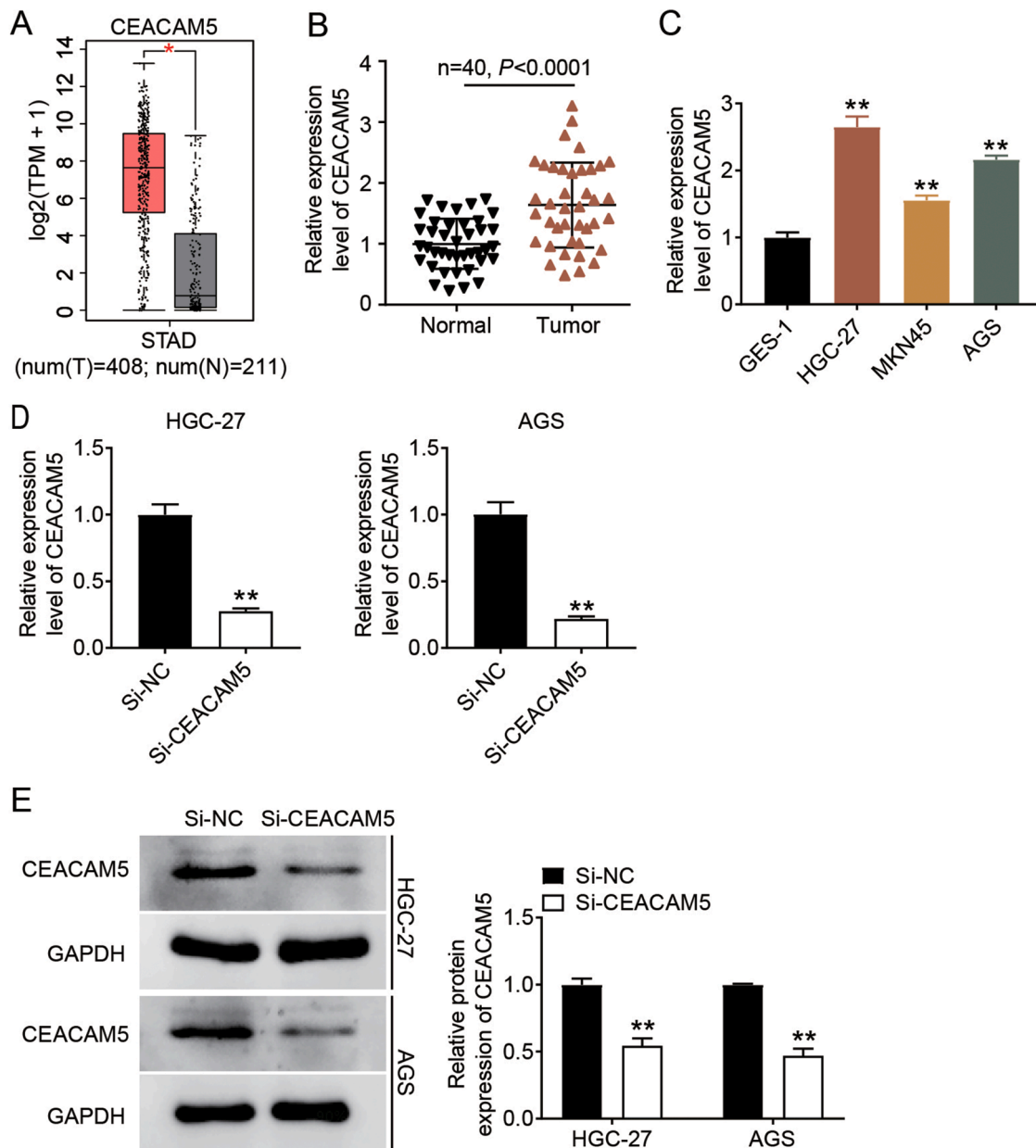


Fig. 1. CEACAM5 was upregulated in GC tissues and cells. (A) GEPIA showed the high expression of CEACAM5 in GC samples. STAD, stomach adenocarcinoma. $*p < 0.05$. (B) CEACAM5 mRNA levels in 40 sets of tumoral and adjacent normal tissue samples from GC patients were quantified via RT-PCR. (C) CEACAM5 mRNA expressions among the GC cells (HGC-27, MKN45 and AGS) and in the normal gastric cell (GES-1) were estimated via RT-PCR. $**p < 0.001$ vs. GES-1. (D) CEACAM5 knockdown efficiencies after the siRNA transfection of AGS and HGC-27 were assessed by means of qRT-PCR. $**p < 0.001$ vs. Si-NC. (E) CEACAM5 knockdown efficiencies after siRNA transfection in HGC-27 and AGS cells were detected by western blotting. $**p < 0.001$ vs. Si-NC.

that CEACAM5 and miR-498 had a potential binding site (Fig. 4C). Nonetheless, that required further verification. The luciferase experiment revealed that GC cells that underwent combined transfection of CEACAM5-WT and miR-498 mimic exhibited significantly lower luciferase activities. Moreover, those carrying CEACAM5-Mut remained almost unchanged (Fig. 4D). These results prove that CEACAM5 was miR-498's target gene. The PCR results showed the GC tissues poorly expressed miR-498, unlike normal tissues (Fig. 4E). Furthermore, western blotting analysis also revealed that AGS and HGC-27 had significantly poorer levels of miR-498 than GES-1 (Fig. 4F). Spearman's correlation analysis revealed an inverse relationship between the expressions of CEACAM5 and miR-498 in GC (Fig. 4G). Overall, these findings suggest that miR-498 targeted CEACAM5 directly and negatively modulated CEACAM5 expression in GC.

CEACAM5 upregulation's effect on the proliferation, migration, and EMT of GC cells were reversible via miR-498 overexpression

To further evaluate miR-498's role, via regulating CEACAM5 in GC cells, we constructed a CEACAM5-overexpression plasmid. Compared with the empty vector group, the CEACAM5-OE group had significantly higher CEACAM5 levels. CEACAM5 overexpression blocked the repressive effect of miR-498 upregulation on the expression of CEACAM5. These results indicate that the recombinant plasmid was successfully constructed (Fig. 5A). Subsequently, functional experiments *in vitro* demonstrated that CEACAM5 overexpression significantly facilitated the activity and migration of AGS and HGC-27 cells, increased the expressions of Vimentin and Snail, and reduced the expression of E-cadherin. These changes, however, were partially reversible by the addition of miR-498 mimic (Fig. 5B-D). Altogether, our results show that miR-

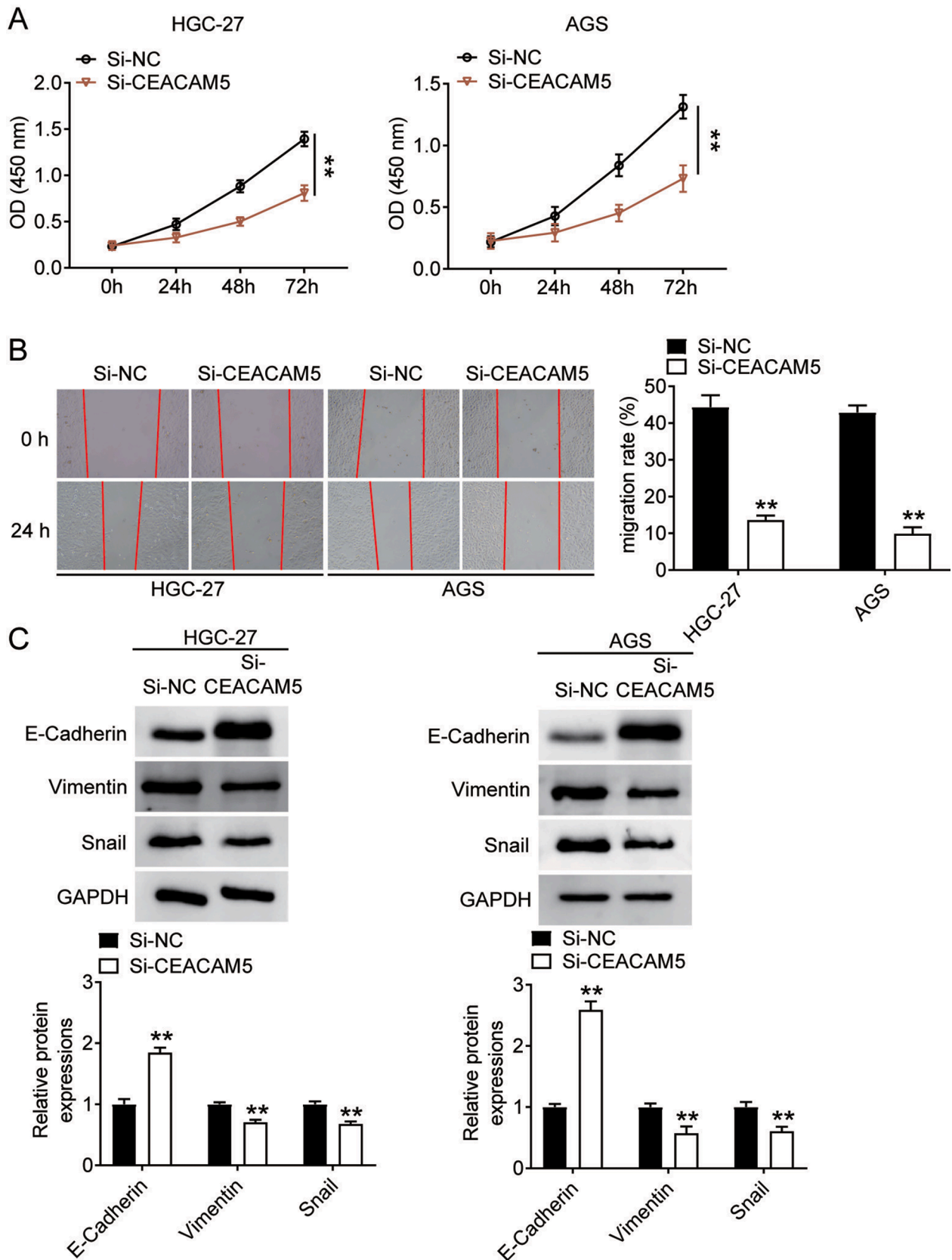


Fig. 2. CEACAM5 knockdown repressed GC cell migration, proliferation, and EMT. (A) Effects of CEACAM5 knockdown on proliferation of HGC-27 and AGS cells was detected by CCK-8 assay. (B) Effect of CEACAM5 knockdown on migration of HGC-27 and AGS cells was detected by wound-healing assay. (C) Effect of CEACAM5 knockdown on protein expression of EMT-related protein (E-cadherin, Vimentin and Snail) in HGC-27 and AGS cells was detected by western blotting. $^{**}p < 0.001$ vs. Si-NC.

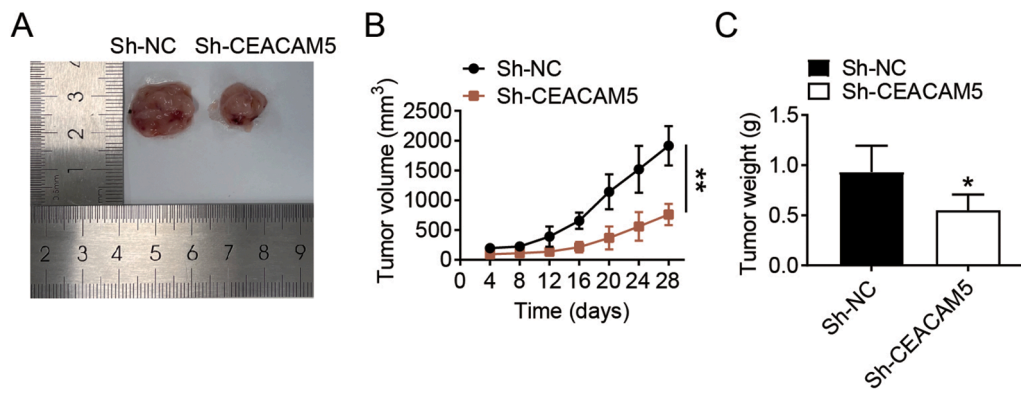


Fig. 3. Knockdown of CEACAM5 suppressed the growth of GC cells in mice with transplanted tumor. (A) Tumor size between Sh-NC and Sh-CEACAM5 groups. (B) Tumor volumes from the Sh-NC and Sh-CEACAM5 groups were measured every 4 days. (C) After 28 d, the nude mice were sacrificed by CO₂ inhalation. Tumor weights were evaluated in Sh-NC and Sh-CEACAM5 groups. ** $p < 0.001$ vs. Sh-NC.

498 may hamper the growth of GC cells and tumor in mice by modulating CEACAM5.

Discussion

GC is a malignant tumor characterized by early metastasis and poor prognosis. GC has seriously affected the quality of life and mortality of GC patients [21]. In light of this, looking for new treatments and tumor markers for its early diagnosis is the key to solve this issue. In the present study, we found elevated expressions of CEACAM5 in GC cells and tissues. The knockdown of CEACAM5 repressed the migration, proliferation, and EMT of GC cells *in vitro* and impeded GC tumor growth *in vivo*. Additionally, miR-498 could regulate GC cell growth by means of targeting CEACAM5. These findings suggest that the miR-498/CEACAM5 pathway can be utilized as a novel prognostic indicator for GC.

Belonging to the carcinoembryonic antigen (CEA) family, CEACAM5 executes a vital role in tumorigenesis and tumor development and is extensively investigated as a tumor marker in clinical research [9, 22–24]. As reported, the upregulation of CEACAM5 was positively associated with the poor prognosis of colorectal cancer patients [25]. Furthermore, its inhibition considerably suppressed colorectal cancer metastasis [25,26]. Another study reported that the elevated CEACAM5 expression in circulating tumor cells (CTCs) originating from breast cancer patients pre-surgery was closely associated with worse prognosis [23]. This suggests that CEACAM5 may be used to guide the prognostic detection and treatment of breast cancer patients [23]. Zhang *et al.* have proven that CEACAM5 could strengthen the migration and proliferation of non-small cell lung cancer (NSCLC) cells [10]. Notably, in GC, a few studies have reported the positive connection between the abnormally high CEACAM5 expressions and poor prognoses of patients, thus illuminating CEACAM5's potential as a clinical diagnostic marker in GC [12,27]. Nevertheless, CEACAM5's mechanism and specific role in GC have yet to be elucidated fully. In this study, *in vitro* cell assays revealed the elevated CEACAM5 levels in GC cells and tissues. Furthermore, CEACAM5 knockdown significantly impaired GC cell migration and proliferation. The outcomes of the *in vivo* experiments also demonstrated that silencing CEACAM5 significantly suppressed GC tumor growth. These results show that CEACAM5 is actively involved in GC progression and exerts a cancer-promoting effect.

We also scrutinized the influence of CEACAM5 on the expressions of three EMT-associated transcription factors in GC: E-cadherin, Snail, and Vimentin. A previous study reported that CEACAM5 accelerate tumor growth at metastatic sites of breast cancer through the stimulation of MET [28]. Furthermore, CEACAM5 overexpression promoted the aggressive loco-regional tumor growth of pancreatic cancer via EMT regulation [29]. Based on the abovementioned findings, we speculate that CEACAM5 may affect GC growth by modulating EMT. Our results

demonstrate that CEACAM5 knockdown significantly upregulated Vimentin and Snail, while downregulating E-cadherin. This indicates that CEACAM5 is capable of promoting GC metastasis through EMT.

MiRNAs are endogenous non-coding single RNAs with 18–26 nucleotides. Although miRNAs do not have the function to encode proteins, they can be regulated by targeting mRNA 3'- or 5'-UTR at the post-transcriptional level and ultimately modulate biological functions by controlling mRNA expression [13,14]. Over the past few years, many miRNAs have been identified as oncogenes or tumor suppressors in GC. Additionally, aberrant miRNA expression is closely involved in GC occurrence and development. MiR-498 is a tumor-suppressor which regulates a variety of genes and proteins related to tumor growth, invasion, and metastasis in order to reduce tumor progression including liver cancer [17], cervical cancer [18], ovarian cancer [19]. In GC, Zhao *et al.* have revealed that the overexpression of miR-498 contributed to the inhibition of GC cell proliferation and chemotherapy resistance [30]. At present, only a few studies have shown that miR-498 has a tumor-suppressive role in GC, and its downstream regulatory pathway needs to be investigated further. Overexpression of miR-498 has been shown to inhibit gastric cancer cell proliferation, migration, and invasion [31]. Another recent study has demonstrated that miR-498 impeded the GC cell metastasis and proliferation through blocking EMT and AKT pathway by targeting BMI-1 [32]. In this study, miR-498 and CEACAM5's binding sites were identified in TargetScan and starBase. Their binding relationship has been validated further via pull-down and luciferase experiments. In addition, miR-498 overexpression could block the stimulating influence of CEACAM5 overexpression on GC cell proliferation, migration, and EMT. In brief, miR-498 inhibited GC development by targeting CEACAM5. Our findings were consistent with previous reports indicating that miR-498 plays a tumor-suppressive role in GC.

This research still has some deficiencies. First of all, there is no further exploration of the upstream target genes of miR-498 to construct lncRNA-miRNA-mRNA ceRNA networks. Second, the validation of classical and non-classical pathways is not involved in this study. Finally, no detailed functional analyses of CEACAM5 in tumor tissues have been performed *in vivo*. These shortcomings shall be undertaken in future studies.

Conclusion

Our study has demonstrated the role of CEACAM5 in promoting the migration, proliferation, and EMT of GC cells. Furthermore, it has been uncovered that miR-498 is capable of controlling the growth of GC cells by downregulating CEACAM5. Our findings enrich the ceRNA network of lncRNA-miRNA-mRNA in GC.

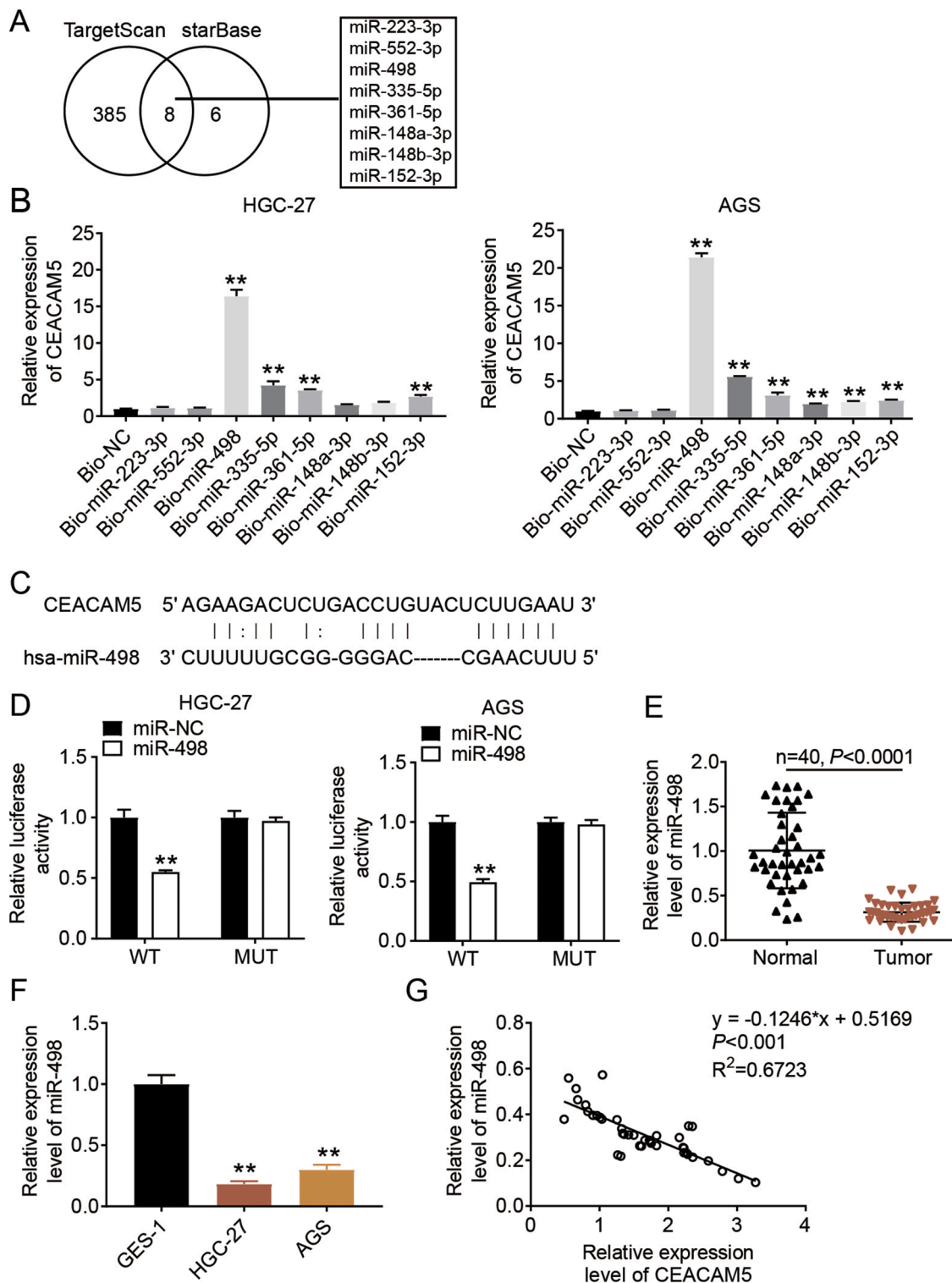


Fig. 4. CEACAM5 was predicted as a miR-498 target. (A) Eight common miRNAs (miR-223-3p, miR-552-3p, miR-498, miR-335-5p, miR-148a-3p, miR-361-5p, miR-148b-3p, and miR-152-3p) were overlapping in the two miRNA prediction tools (TargetScan and starBase). (B) Enrichment of CEACAM5 by eight common miRNAs (miR-223-3p, miR-552-3p, miR-498, miR-335-5p, miR-148b-3p, miR-148a-3p, miR-361-5p, and miR-152-3p) were detected by pull-down assay. $**p < 0.001$ vs. Bio-NC. (C) The potential binding sequence between miR-498 and CEACAM5 was predicted in starBase (<http://starbase.sysu.edu.cn/index.php>). (D) The relationship between miR-498 and CEACAM5 was determined by luciferase reporter assay. $**p < 0.001$ vs. miR-NC. (E) Relative miR-498 levels in 40 sets of GC tissues and adjacent normal tissues were detected via RT-PCR. (F) The relative miR-498 expressions among the GC cells (HGC-27 and AGS) and normal gastric cell (GES-1) were detected via RT-PCR. $**p < 0.001$ vs. GES-1. (G) Spearman correlation coefficient was applied to test the association between the expressions of miR-498 and CEACAM5 in GC.

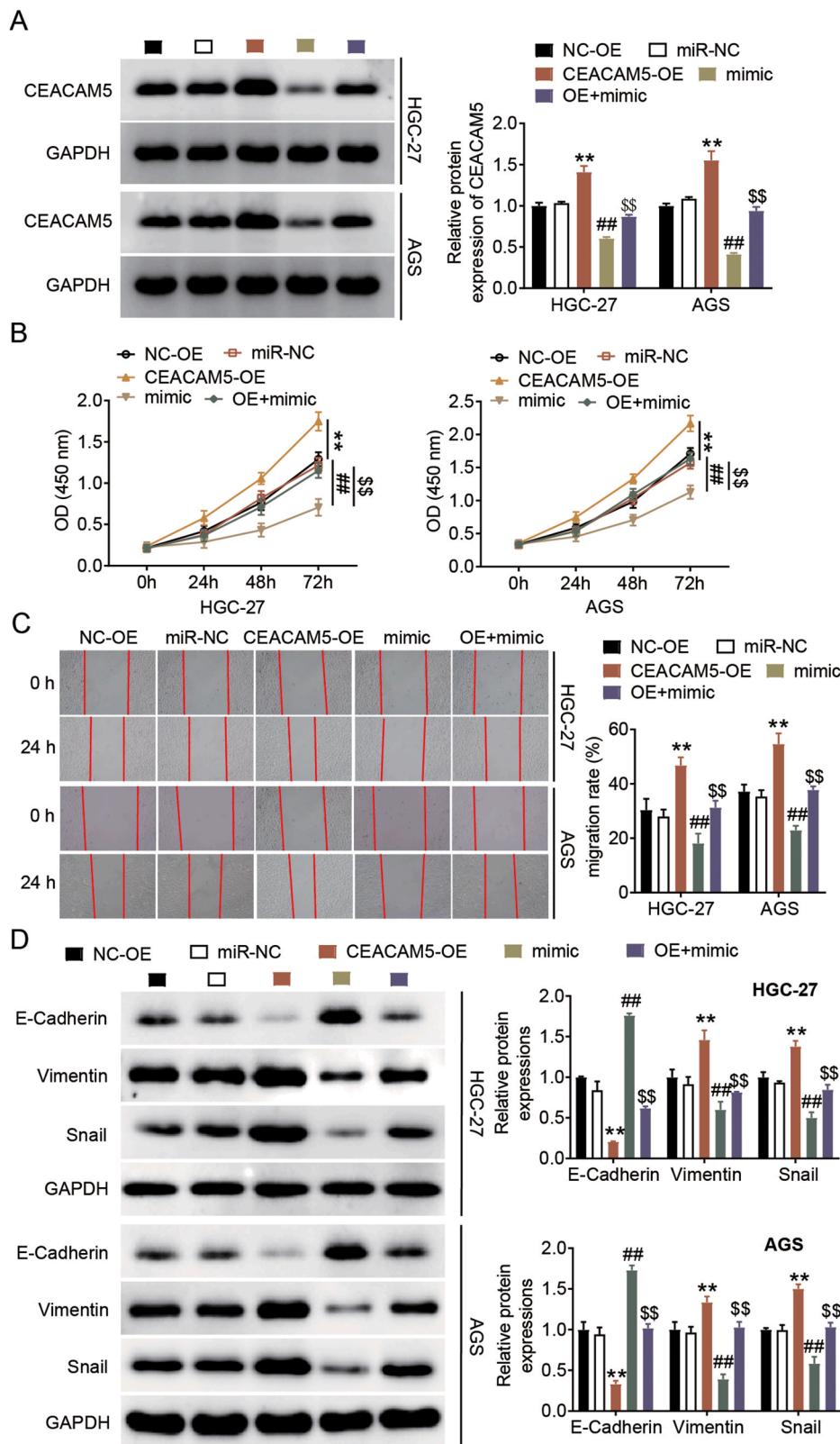


Fig. 5. MiR-498 reduced GC cell migration, proliferation, and EMT by inhibiting CEACAM5. (A) Overexpression efficiency of CEACAM5 expression was detected by western blotting. (B) Cell proliferation in AGS and HGC-27 with miR-498 mimic and CEACAM5-OE transfection was evaluated by means of the CCK-8 assay. (C) Cell migration in AGS and HGC-27 cells that carry miR-498 mimic and CEACAM5-OE was assessed through wound-healing assays. (D) Protein expressions of EMT-related protein (E-cadherin, Vimentin and Snail) in HGC-27 and AGS cells that underwent miR-498 mimic and CEACAM5-OE transfection were determined via western blotting. $**p < 0.001$ vs. NC-OE; $##p < 0.001$ vs. miR-NC; $$$p < 0.001$ vs. mimic.

Ethics Committee Approval and Patient Consent

This present study was performed based on the principles expressed in the Declaration of Helsinki. All experiments have been approved by the Ethics Committee of The Sixth Hospital of Wuhan, The Affiliated Hospital of Jiangnan University (Wuhan, China). All participants

provided a written informed consent to participate in the study and a consent for publication.

The Animal Care and Use Committee of the The Sixth Hospital of Wuhan, The Affiliated Hospital of Jiangnan University approved all procedures executed in the animal study. All the animal experiments were carried out in accordance with the National Institutes of Health

(NIH) Guide for the Care and Use of Laboratory Animals.

Funding information

No funding was received.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Authors' contributions

LZ and CZ performed the experiments and data analysis. LZ conceived and designed the study. NL made acquired the data. LZ, CZ, and NL performed the analysis and interpretation of data. All authors have read and approved this manuscript.

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Not applicable.

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