Tumor Biol. (2016) 37:10545–10551
DOI 10.1007/s13277-016-4942-0

ORIGINAL ARTICLE

miR-448 suppressed gastric cancer proliferation and invasion by regulating ADAM10

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Received: 16 January 2016 / Accepted: 29 January 2016 / Published online: 8 February 2016

Abstract MicroRNAs (miRNAs) are a class of short, noncoding RNAs that act a crucial role in tumor development. Previous studies showed that miR-448 expression was deregulated in many tumors. However, the role of miR-448 in gastric cancer (GC) remains unknown. In our study, we demonstrated that miR-448 expression was downregulated in GC tissues compared with the corresponding nontumor tissues. We also showed that miR-448 expression was downregulated in GC cell lines. Ectopic expression of miR-448 suppressed GC cell proliferation, colony formation, and invasion. Moreover, we identified A Disintegrin And Metalloproteinases 10 (ADAM10) as a direct target gene of miR-448 in GC cell. ADAM10 expression was upregulated in GC tissues and cells. Furthermore, the expression level of miR-448 was negatively correlated with the expression level of ADAM10 in GC tissues. Moreover, ADAM10 overexpression rescued the effect of miR-448-mediated GC cell proliferation, colony formation, and invasion. These results demonstrated that miR-448 might play as a tumor suppressor miRNA partly through targeting ADAM10 expression.

Keywords Gastric cancer · microRNAs · miR-448 · ADAM10

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Introduction

Gastric cancer (GC) is one of the most common tumors and the second leading death-related tumor worldwide [1–4]. Despite recent advances in therapeutic strategies such as radiotherapy, surgery, and adjuvant chemotherapy, the prognosis of GC remains poor [2, 5–8]. Most GC patients are diagnosed with the late stage [9–12]. Therefore, it is crucial to identify novel biomarkers for diagnosis and therapy in GC patients.

MicroRNAs (miRNAs) are a group of short, small, noncoding RNAs that typically regulate gene expression through binding to the 3'-untranslated region (3′-UTR) of the target genes [13–15]. MiRNA plays crucial roles in many biological processes such as development, inflammation, differentiation, cell proliferation, apoptosis, and invasion [16–20]. Emerging evidences have demonstrated that miRNA are deregulated in various cancers including breast cancer, gladder cancer, bladder cancer, cutaneous squamous cell carcinoma, laryngeal cancer, rhabdomyosarcoma, and GC [21–27]. miRNA can act as a tumor suppressor gene or oncogene in the initiation and development of tumors [19, 28, 29]. Recently, increasing studies have demonstrated that miR-448 plays an important role in various tumors [30–32]. However, the expression and functional role of miR-448 in GC is still unknown.

In this study, we demonstrated that miR-448 expression was downregulated in the GC tissues and cell lines and overexpression of miR-448 suppressed GC cell proliferation, colony formation, and invasion. We identified A Disintegrin And Metalloproteinases (ADAM 10) as a direct target gene of miR-448 in the GC cell.

Materials and methods

Tissue samples

Primary GC tissues and their corresponding non-tumor tissues were collected from our department between 2010 and 2014. Written informed consent was collected from all patients, and this study was approved by the Ethics Committee of The Second Affiliated Hospital of Kunming Medical University. These samples were immediately frozen and stored in liquid nitrogen.

Cell lines cultured and transfection

Human GC cell lines (SGC-7901, MGC-803, HGC-27, and BGC-823) and normal gastric mucosa cells line (GES-1) were purchased from Cell Bank of Chinese Academy of Sciences (Beijing, China). The cell lines were cultured in RPMI 1640 medium (Gibco, USA) containing 10 % fetal bovine serum (FBS). miR-448 mimic and scramble, ADAM10 vector, and control were synthesized by RiboBio (Guangzhou, China). Cell transfection was performed by using Lipofectamine 2000 kit (Invitrogen, USA) following to the manufacturer's information.

Quantitative real-time RT-PCR

Total RNA from cell or tissue collected was extracted by using TRIzol (Invitrogen, USA). Quantitative real-time PCR (qRT-PCR) analysis was performed to measure the mRNA and miRNA expression. PCR primers used were as follows: ADAM10, forward 5′-TCGAACCATCACCCTGCAACCT-3′ and reverse 5′-GCCCACCAATGAGCCACAATCC-3′; GAPDH, forward 5′-AATGGGCAGCCGTTAGGAAA-3′ and reverse 5′-TGAAGGGGTCATTGATGGCA-3′. The relative miR-448 expression was normalized to U6 SnRNA and the expression of ADAM10 was normalized to GAPDH.

Dual-luciferase reporter assay

Cells were cultured in the 96-well plate and were tranfected with pLuc-3'-UTR, renilla, and miR-448 mimic or scramble by using Lipofectamine 2000 kit (Invitrogen, USA) following to the manufacturer's information. After 48 h, luciferase activities were analyzed by using a dual-luciferase reporter analysis system (Promega, USA). The relative luciferase activity was normalized to renilla luciferase activity.

Western blot

Protein was isolated from cell or tissue using RIPA buffer. Total protein was electrophoresed by 12 % SDS and transferred to PVDF membranes (Millipore, USA). After blocking with 10 % nonfat milk, the membrane was incubated with the primary antibody. The antibody was shown as the following: ADAM10, GAPDH (Abcam, England). Blots were measured by using the ECL system.

Cell growth and colony formation

Cell proliferation was detected using the Cell Counting Kit-8 kit (CCK-8 kit, Dojindo, Japan). The cell was cultured in the 96-well plate, and cell proliferation was detected on 0, 24, 48, and 72 h. The absorbance was detected at 450 nm. For cell colony formation, cells were seeded in the 6-well plate and kept in RPMI 1640 medium for 2 weeks. Colonies were stained with crystal violet and counted.

Cell invasion

Cell invasion was performed using transwell chambers. Cells were plated in the upper of the membranes coated with Matrigel (BD Biosciences, MD). The upper chamber was not supplemented with FBS, and the 10 % FBS was added to the lower membrane. After incubating for 48 h, the cells in the lower membrane were stained with 1 % crystal violet (Sigma, USA) and then counted.

Statistical analysis

Results presented are expressed as mean ± SD (standard deviation). Student's t test was performed to measure the difference between two groups, and differences between more than two groups were assessed using one-way ANOVA. $P < 0.05$ was considered significant.

Results

miR-448 expression was downregulated in the GC cell and tissue

We firstly measured the expression of miR-448 in the 40 pairs of GC tissues. miR-448 expression was downregulated in GC tissues compared with the corresponding nontumor tissues (Fig. 1a). Moreover, miR-448 expression was downregulated in 28 cases (28/40; 70 %) compared with adjacent tissues (Fig. 1b). We also demonstrated that miR-448 expression was decreased in the GC cell lines (SGC-7901, MGC-803, HGC-27, and BGC-823) compared with normal gastric mucosa cells line (GES-1) (Fig. 1c).

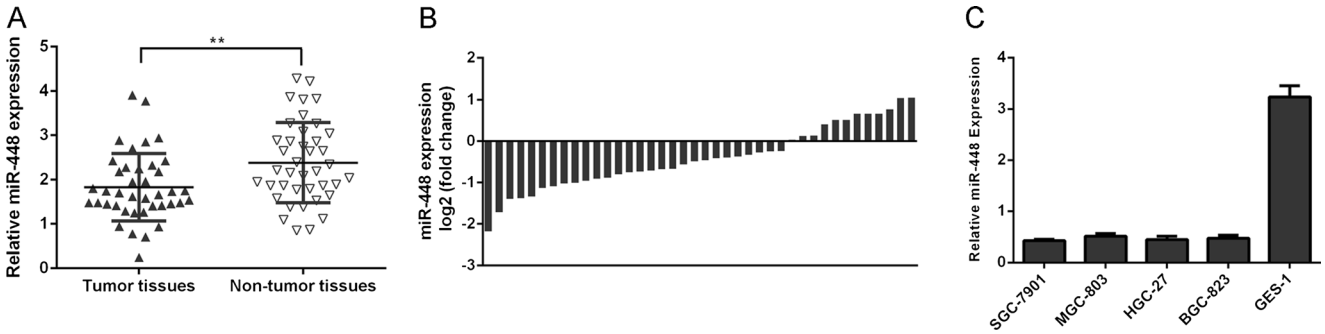

Fig. 1 miR-448 was downregulated in the GC cell and tissue. **a** The expression of miR-448 was measured by qRT-PCR. **b** miR-448 was downregulated in 28 cases (28/40; 70 %) compared to adjacent tissues. **c** The expression of miR-448 was decreased in the GC cell lines (SGC-7901, MGC-803, HGC-27, and BGC-823) compared with normal gastric mucosa cells line (GES-1). $^{**}p < 0.01$

ADAM10 expression was upregulated in the GC cell and tissue

ADAM10 expression was upregulated in the GC tissues compared with the corresponding nontumor tissues (Fig. 2a). Moreover, ADAM10 expression was upregulated in 27 cases (27/40; 67 %) compared with the adjacent tissues (Fig. 2b). There was a negative relationship between the expression level of miR-448 and ADAM10 in the GC tissues (Fig. 2c). We also showed that the expression level of ADAM10 was increased in the GC cell lines (SGC-7901, MGC-803, HGC-27, and BGC-823) compared with GES-1 (Fig. 2d).

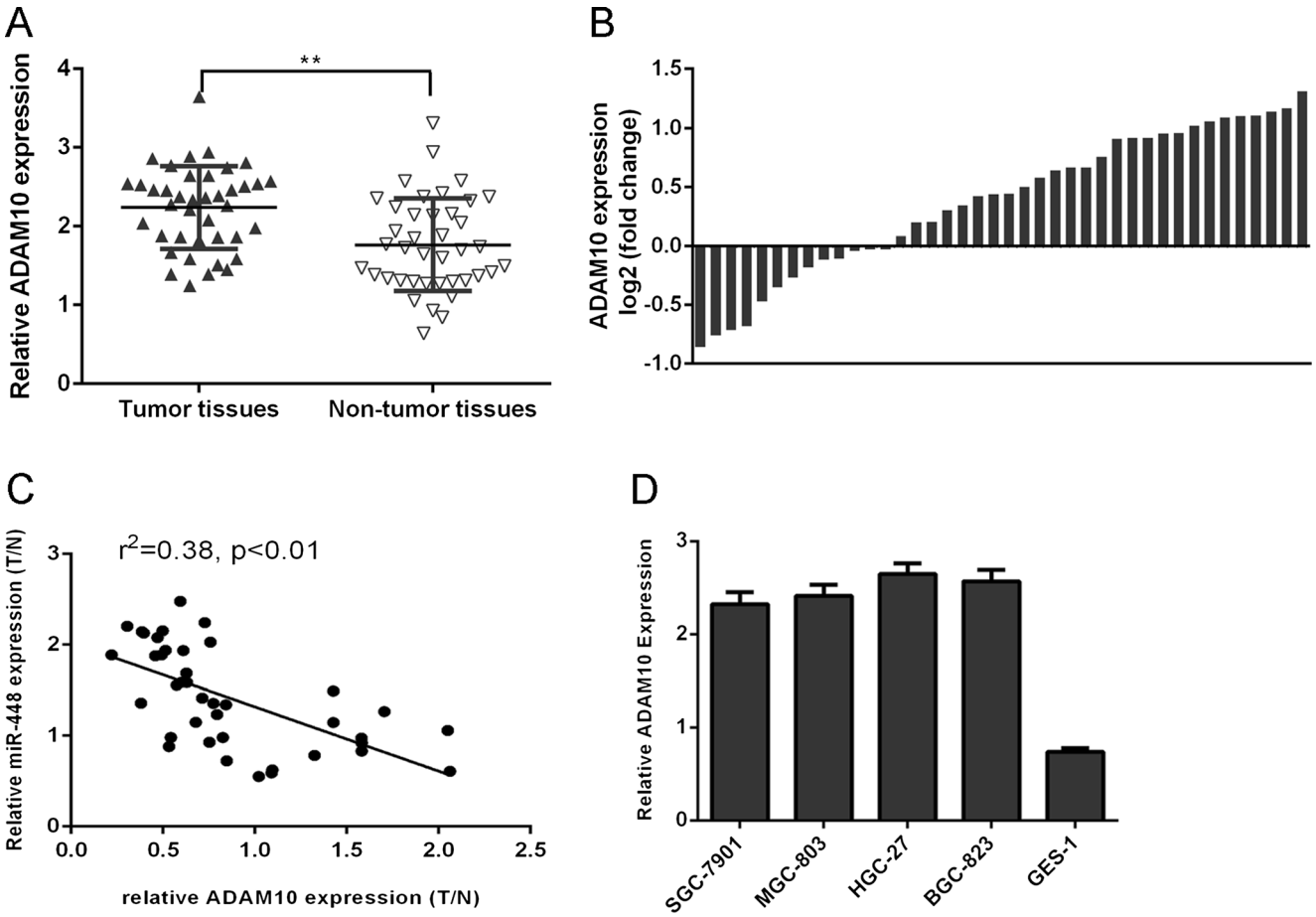

Fig. 2 ADAM10 was upregulated in the GC cell and tissue. **a** The expression of ADAM10 was measured by qRT-PCR. **b** ADAM10 was upregulated in 27 cases (27/40; 67 %) compared to adjacent tissues. **c** There is a negative relation between the expression of miR-448 and the expression of ADAM10 in the GC tissues. **d** The expression of ADAM10 was upregulated in the GC cell lines (SGC-7901, MGC-803, HGC-27, and BGC-823) compared with normal gastric mucosa cells line (GES-1). $^{**}p < 0.01$

miR-448 suppressed the GC cell proliferation, colony formation, and invasion

miR-448 expression was upregulated in the GC cell HGC-27 after being treated with miR-448 mimic, and this result confirmed that the efficiency of miR-448 mimic was high (Fig. 3a). Overexpression of miR-448 suppressed the GC cell HGC-27 proliferation (Fig. 3b). Moreover, ectopic expression of miR-448 inhibited the HGC-27 cell colony formation and invasion (Fig. 3c, d).

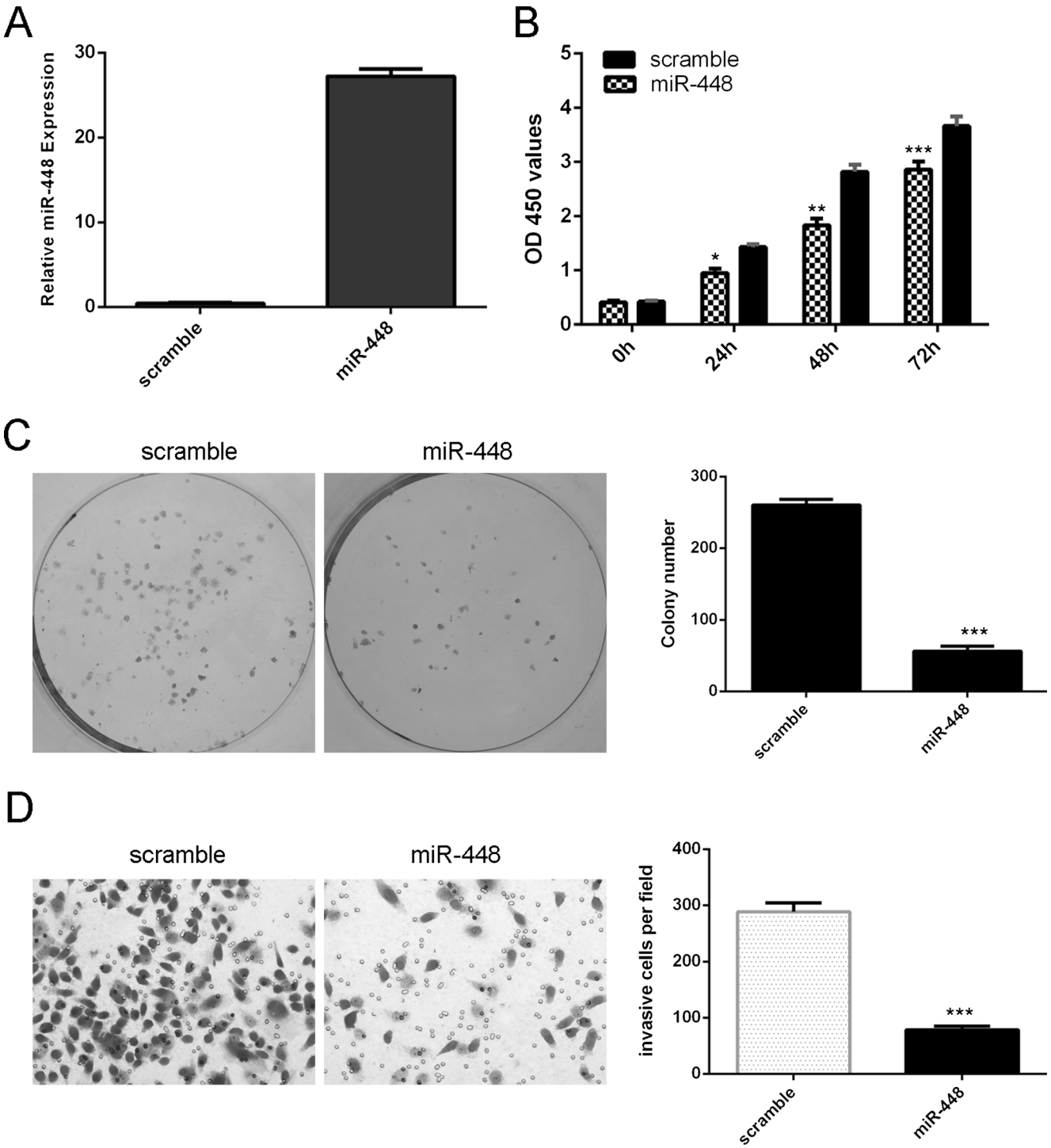

Fig. 3 miR-448 suppressed the GC cell proliferation, colony formation, and invasion. **a** The expression of miR-448 in the HGC-27 cell after tranfected with miR-448 mimic using qRT-PCR. **b** Overexpression of miR-448 suppressed the HGC-27 cell proliferation. **c** Ectopic expression of miR-448 inhibited the HGC-27 cell colony information. **d** miR-448 overexpression suppressed the HGC-27 cell invasion. $*p < 0.05$,$**p < 0.01$,$***p < 0.001$

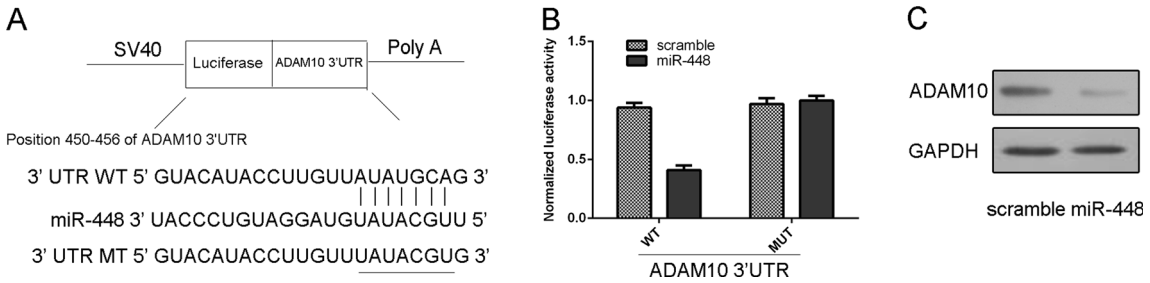

Fig. 4 ADAM10 was a direct target gene of miR-448 in GC cell. **a** Bioinformatics assay with TargetScan algorithms predicted ADAM10 as a hypothetical target gene of miR-448. **b** Luciferase activity assays of luciferase vectors with wild-type or mutant ADAM10 3′-UTR were performed after co-transfection with miR-448 mimic or scramble. **c** Overexpression of miR-448 suppressed the ADAM10 protein expression in the HGC-27 cell

ADAM10 was a direct target gene of miR-448 in GC cell

Bioinformatics assay with TargetScan algorithms predicted ADAM10 as a hypothetical target gene of miR-448 (Fig. 4a). In HGC-27 cells co-transfected with miR-448 mimic or scramble and the reporter plasmid, the relative luciferase activity of this reporter with WT ADAM10 3′-UTR was inhibited by miR-448 mimic; however, this luciferase activity of MT reporter was unaffected (Fig. 4b). Furthermore, overexpression of miR-448 suppressed the ADAM10 protein expression in the HGC-27 cell (Fig. 4c).

ADAM10 rescued the effect of miR-448-mediated GC cell proliferation, colony formation, and invasion

ADAM10 expression was upregulated in the pCDNA-ADAM10-treated HGC-27 cell (Fig. 5a, b). We rescued ADAM10 expression in the miR-448-overexpressing HGC-27 cell. CCK8 analysis demonstrated that ADAM10 overexpression promoted the miR-448-overexpressing HGC-27 cell proliferation (Fig. 5c). Colony formation analysis showed that ADAM10 overexpression increased the miR-448-overexpressing HGC-27 cell colony formation (Fig. 5d). Invasion analysis demonstrated that ectopic expression of ADAM10 promoted the miR-448-overexpressing HGC-27 cell invasion (Fig. 5e).

Discussion

In this study, we demonstrated that miR-448 expression was downregulated in the GC tissues compared with the corresponding nontumor tissues. We also showed that miR-448 expression was downregulated in GC cell lines compared with normal gastric mucosa cell line. Ectopic expression of miR-448 suppressed GC cell proliferation, colony formation, and invasion. Moreover, we identified ADAM10 as a direct target gene of miR-448 in GC cell, and ADAM10 expression was upregulated in GC tissues and cells. Furthermore, the expression level of miR-448 was negatively correlated with the expression level of ADAM10 in the GC tissues. ADAM10 overexpression rescued the effect of miR-448-mediated GC cell proliferation, colony formation, and invasion. These results demonstrated that miR-448 played as a tumor suppressor miRNA partly through targeting ADAM10 expression.

Previous studies demonstrated that miR-448 played an important role in the development of tumors [30–32]. For example, Lv et al. showed that miR-448 acted as a tumor suppressor gene through inhibiting the CXCL12 expression in ovarian cancer [30]. Zhu et al. demonstrated that miR-448 expression was downregulated in hepatocellular carcinoma (HCC) tissues and inhibition of miR-448 increased HCC cell invasion and epithelial-mesenchymal transition (EMT) through targeting ROCK2 expression [31]. Li et al. showed that miR-448 suppression promoted EMT by inhibiting special AT-rich sequence-binding protein-1 (SATB1) expression in breast cancer. However, the role of miR-448 in GC remains still unknown. In our study, we firstly measured the expression of miR-448 in the GC tissues [32]. Our results showed that miR-448 expression was downregulated in the GC tissues compared with the corresponding nontumor tissues. Moreover, miR-448 was downregulated in 28 cases (28/40; 70 %) compared with adjacent tissues. Furthermore, overexpression of miR-448 suppressed the GC cell proliferation, colony formation, and invasion.

ADAM10 is one member of the ADAM family, and ADAM-mediated shedding was essential for many cell processes including cell development, determination, invasion, migration, proliferation, and wound healing [33–36]. Recent studies have found that ADAM10 plays important roles in the GC development. For example, Yoshimura et al. demonstrated that *H. pylori* infection was associated with high expression of ADAM10 and high expression of ADAM10 was present in gastric tumor [37]. Tanida et al. showed that IL-8 induced EGFR ligand shedding through regulating the ADAM10-

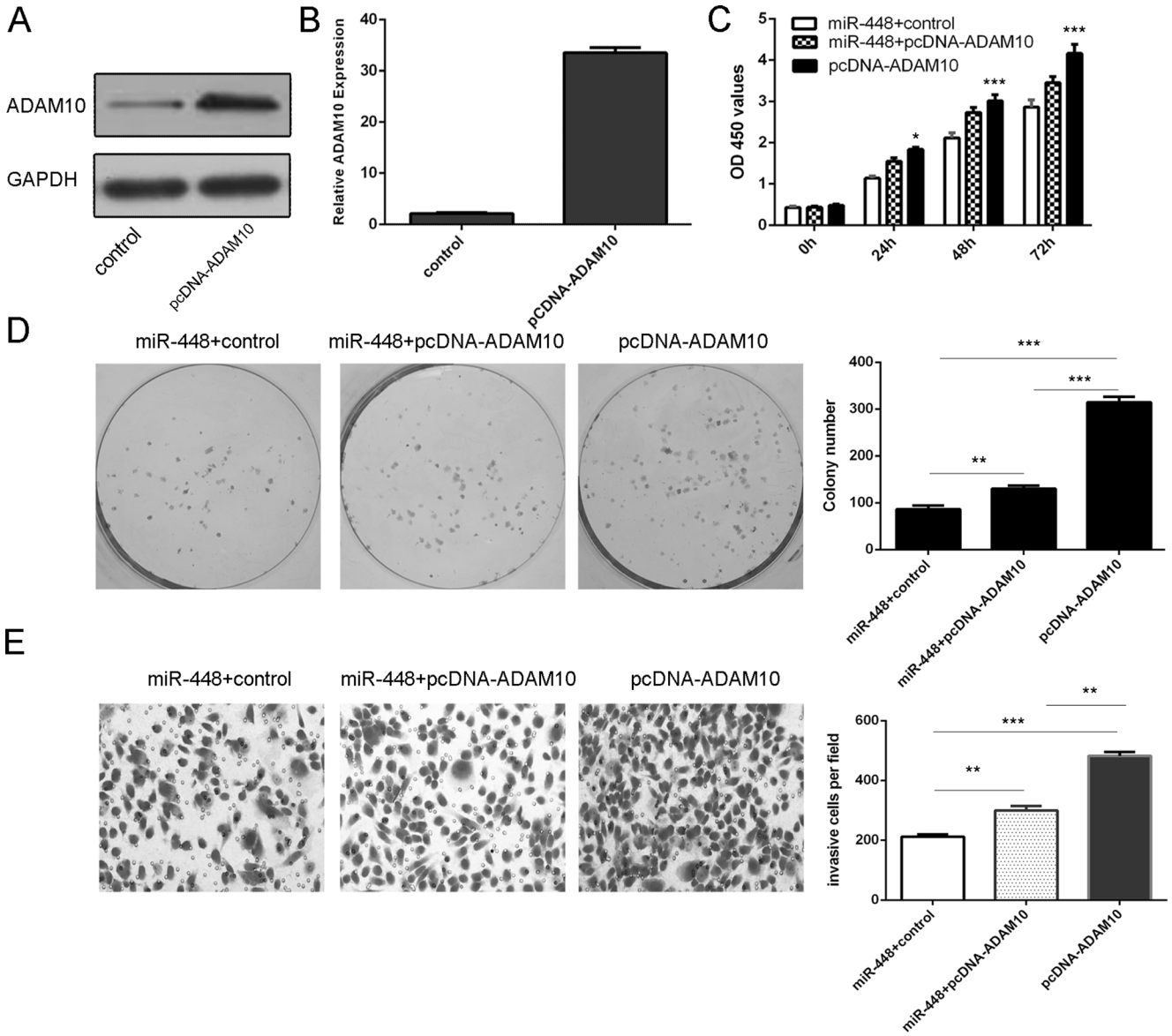

Fig. 5 ADAM10 rescued the effect of miR-448-mediated GC cell proliferation, colony formation, and invasion. **a** The protein expression of ADAM10 was measured by western blot. **b** The mRNA expression of ADAM10 was measured by qRT-PCR. **c** The cell proliferation was measured by CCK-8 analysis. **d** Colony formation analysis showed that ADAM10 overexpression increased the miR-448-overexpressing HGC-27 cell colony formation. **e** Invasion analysis demonstrated that ectopic expression of ADAM10 promoted the miR-448-overexpressing HGC-27 cell invasion. $*p < 0.05, **p < 0.01, ***p < 0.001$

dependent pathway in GC cells [38]. High ADAM10 expression was associated with the location and size of tumor, as well as vessel invasion, depth of invasion, TNM stage, and lymph node. However, the underlying mechanisms about deregulated expression of ADAM10 remain unclear. In line with previous data, we also demonstrated that ADAM10 expression was upregulated in GC tissues and cell lines. Moreover, we identified ADAM10 as a direct target gene of miR-448 in GC cells. ADAM10 overexpression rescued the effect of miR-448-mediated GC cell proliferation, colony formation, and invasion. These results suggested might provide one potential mechanism of post-transcriptional regulation of ADAM10.

In conclusion, our results suggested that miR-448 expression was upregulated in GC tissues and miR-448 acted as a tumor suppressor miRNA in the development of GC through inhibiting ADAM10 expression. Restoration of miR-448 may be a potential therapeutic strategy for GC patients in the future.

Compliance with ethical standards

Conflicts of interest None

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