European Review for Medical and Pharmacological Sciences 2019; 23: 7913-7919

MiR-885-5p promotes gastric cancer proliferation and invasion through regulating YPEL1

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Abstract. – OBJECTIVES: Dysregulation of microRNAs (miRNAs) was found to play crucial roles in the carcinogenesis of multiple human cancers. This study was aimed to investigate the biological function of miR-885-5p and associated mechanisms in gastric cancer (GC).

MATERIALS AND METHODS: Reverse Transcription-quantitative Polymerase Chain Reaction was used to measure miR-885-5p level in GC cell lines and normal cell line. The effects of miR-885-5p expression on cell proliferation, colony formation, and invasion were investigated by Cell Counting Kit-8 (CCK-8) assay, colony formation assay, and transwell invasion assay, respectively. Furthermore, Luciferase activity reporter assay and Western blot were conducted to validate Yippee-like-1 (YPEL1) as a direct target of miR-885-5p.

RESULTS: We found that miR-885-5p expression level was elevated in GC cell lines compared with normal cell line. Additionally, the knockdown of miR-885-5p inhibits GC cell proliferation, colony formation, and cell invasion *in vitro*. Notably, rescue experiments demonstrated that the knockdown of YPEL1 partially reversed the effects of miR-885-5p on GC cell growth and invasion.

CONCLUSIONS: The present study suggested that miR-885-5p regulates GC proliferation, colony formation, and invasion *via* targeting YPEL1.

Key Words
MiR-885-5p, YPEL1, Gastric cancer, Cell growth, Cell invasion.

Introduction

Gastric cancer (GC) ranks as the fifth most commonly diagnosed cancer type worldwide[1]. GC solely accounts for about 12% of all cancer-related deaths annually[2]. Despite great improvements in diagnosis or treatment methods, the overall survival for GC remains less than 30% in most countries[3]. Hence, investigations on abnormally expressed molecules that closely associated with disease development are of great importance.

Many studies have suggested that microRNAs (miRNAs) are dysregulated in cancer[4]. Therefore, identifying abnormally expressed miRNAs in GC has attracted extensive attentions[5]. Unfortunately, our knowledge regarding the role of miRNAs in GC is still limited. In a previous work[6], miR-1284 was found as downregulated expression in GC tissues and associated with large tumor size and worse metastasis. MiR-1284 overexpression inhibited GC cell proliferation and metastasis via targeting eukaryotic translation initiation factor 4A1[6]. Xie et al[7] showed low miR-381 expression in GC was correlated with large tumor size, advanced tumor node metastasis stage, and worse metastasis. In addition, the restoration of miR-381 inhibited GC progression by targeting Rho-associated coiled-coil containing protein kinase 2[7].

MiR-885-5p has been reported to have a role in cancer progression, including hepatocellular carcinoma, colorectal cancer, and papillary thyroid carcinoma. In hepatocellular carcinoma, miR-885-5p expression was found inversely correlated with cancer invasive and metastatic capabilities, and poor overall survival of cancer patients[8]. In addition, miR-885-5p overexpression was shown to inhibit the malignancy behaviors of hepatocellular carcinoma cells *via* regulating Wnt/β-catenin signaling pathway[8]. Su et al[9] showed that miR-885-5p elevated the expression in colorectal cancer and promoted tumor progression by targeting the suppressor of cytokine signaling. Moreover, miR-885-5p was found to be regulated by hsa_circ_0004458 to regulate papillary thyroid carcinoma progression[10].

Yippee-like-1 (YPEL1) is a nuclear protein and was reported to be involved in the mesenchymal to the epithelial-like transition process in

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human. YPEL1 expression level was shown to be decreased in pancreatic cancer tissues compared with normal tissues[11]. Recent bioinformatic analysis results[12] revealed the upregulate expression status of YPEL1 in erlotinib-treated epidermal growth factor receptor-mutant non-small cell lung cancer samples.

Here, we investigated the expression level of miR-885-5p in GC cell lines and normal cell line. Moreover, we analyzed the connection of miR-885-5p and YPEL1 using Luciferase activity reporter assay and Western blot assay. In addition, functional assays were conducted to explore the biological roles of miR-885-5p and YPEL1 on GC.

Materials and Methods

Cell Lines

GC cell lines (SGC7901 and BGC823) and gastric epithelial cell line GES-1 purchased from Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) were maintained in Roswell Park Memorial Institute-1640 (RPMI-1640; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The incubation atmosphere was kept at 37°C and contained 5% of CO_2.

Cell Transfection

MiR-885-5p inhibitor and negative control miRNA (miR-NC) were purchased from GenePharma (Shanghai, China). Small interfering RNA targeting YPEL1 (si-YPEL1) and negative control (siR-NC) were also purchased at GenePharma. For transfection, the cells were plated in 6-well plate at the density of 5×10^4 cells/well. The transfection experiment was mediated by Lipofectamine 2000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the provided protocols.

Quantitative Reverse Transcription PCR (qRT-PCR)

RNA from cultured cells was isolated using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and quantified with NanoDrop-1000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA). RNA was reverse transcribed into complementary DNA using reverse transcription kit (TaKaRa, Dalian, Liaoning, China) with the procedure of 37°C for 15 min and 85°C for 5 s. qRT-PCR was conducted at ABI 7500 system (Applied Biosystems, Foster City, CA, USA) using SYBR Green Mix (TaKaRa, Dalian, Liaoning, China) according to the manufacturer's instructions. Primers used were as follows: miR-885-5p forward 5'-GTCCATTACACTACCCTGCCTC-3' and reverse 5'-CGCGAGCACAGAATTAATACG-3'; U6 snRNA forward 5'-CTCGCTTCGGCAGCACA-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3'. Expression level and miR-885-5p was normalized to U6 snRNA and calculated with the comparative cycle threshold ($2^{-\Delta\Delta CT}$) method. The following procedures were employed: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, and 60°C for 30 s.

Cell Counting Kit-8 (CCK-8) Assay

The cells were seeded in 96-well plate with the density of 3,000 cells/well and incubated for the indicated time. Subsequently, 10 µl of CCK-8 reagent (Beyotime, Haimen, Jiangsu, China) was added to each well at this time, and further incubated for 4 h. At last, absorbance at 450 nm was measured using the microplate reader.

Colony Formation Assay

3×10^4 cells in serum-free medium were seeded into 6-well plate and incubated for 14 days. The colonies formed were fixed with 4% paraformaldehyde, stained with 0.5% crystal violet, and counted under a light microscope (Olympus, Tokyo, Japan).

Transwell Invasion Assay

1×10^5 cells in serum-free medium were seeded in the upper chamber that pre-coated with Matrigel (BD Biosciences, San Jose, CA, USA). Lower chamber was filled with serum-contained medium. After incubation for 48 h, the non-invasive cells were removed using a cotton swab. Invasive cells were fixed with methanol, stained with crystal violet, and counted under microscope (Olympus, Tokyo, Japan).

Western Blot

The protein from cultured cells was isolated using radioimmunoprecipitation assay lysis buffer (Beyotime, Haimen, Jiangsu, China) and quantified using bicinchoninic acid kit (Beyotime, Haimen, Jiangsu, China). An equal amount of protein sample (50 µg) was isolated at 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride membrane (PVDF) at room temperature. After incubation with suitable primary antibodies (anti-YPEL1: ab174736, anti- glyceraldehyde-3-phos-

phate dehydrogenase (GAPDH): ab181602; Abcam, Cambridge, MA, USA) at 4°C for overnight, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (ab6721; Abcam, Cambridge, MA, USA) at room temperature for 4 h. The band signals were visualized using BeyoECL kit (Beyotime, Haimen, Jiangsu, China) according to the manufacturer's instruction. Band density was analyzed with Image 1.42 software (NIH, Bethesda, MD, USA).

Bioinformatic Analysis

The targets for miR-885-5p was analyzed at TargetScan V_7.2 (http://www.targetscan.org/vert_72/).

Luciferase Reporter Assay

YPEL1 wild-type (wt) with miR-885-5p binding sites or mutant (mt) sequence was inserted into psi-CHECK-2 (Promega, Madison, WI, USA) to generate wt-YPEL1 or mt-YPEL1, respectively. The binding site of miR-885-5p on YPEL1 was mutated into CAUUACC. The cells were transfected with Luciferase vectors or miRNAs using Lipofectamine 2000. After 48 h of transfection, the Luciferase activities were measured with the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Kaplan-Meier Analysis

The Kaplan-Meier plotter (www.kmplot.com), an open public access database, was used to analyze the effect of miR-885-5p on overall survival of GC patients. The cut-off value was autoselected in the algorithm.

Statistical Analysis

Statistical Package for the Social Sciences version 19.0 software (IBM Corp, Armonk, NY, USA) was used to analyze the data. Data were presented as mean ± standard deviation. The differences in the groups were analyzed by Student's *t*-test or One-way analysis of variance and Tukey post-hoc test. $p < 0.05$ was considered as the statistical significance.

Results

Upregulation of MiR-885-5p in GC Cell Lines

qRT-PCR results showed that miR-885-5p expression level was significantly increased in GC cell lines compared with normal cell line (Figure 1A). By utilizing KM plotter analysis, we found that high miR-885-5p was a predictor for poorer overall survival of GC patients (Figure 1B).

Knockdown of MiR-885-5p Inhibits GC Cell Growth and Invasion

As miR-885-5p was found expressed at high levels in GC, we supposed that miR-885-5p may exert an oncogenic role in GC. To this end, miR-885-5p inhibitor was introduced into GC cells to decrease miR-885-5p expression level (Figure 2A). The CCK-8 assay was conducted to examine cell proliferation rate and the results indicated that miR-885-5p downregulation inhibits cell proliferation (Figure 2B). The colony formation assay demonstrated that the knockdown of miR-885-5p inhibits GC cell colony formation ability (Figure 2C). In addition, we examined GC cell invasion ability using the transwell

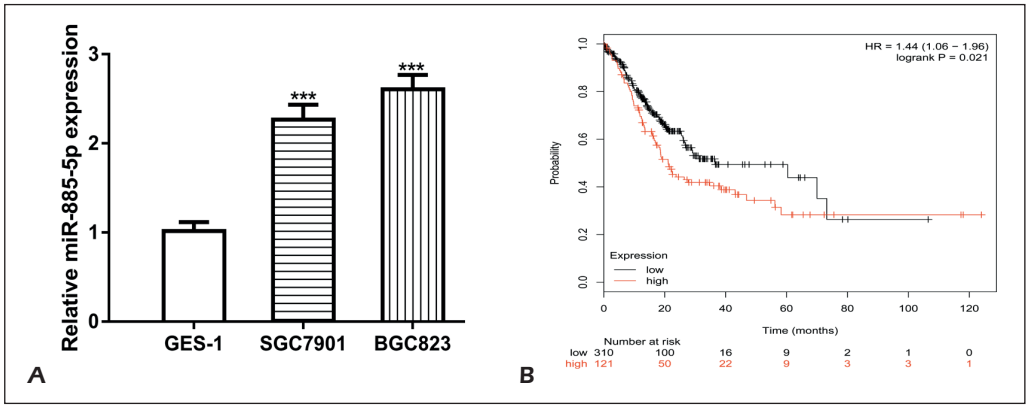

Figure 1. MiR-885-5p expression was elevated and correlated with poor overall survival of GC. **A**, MiR-885-5p expression in GC cell lines and normal cell line. **B**, High miR-885-5p was a predictor for poor overall survival of GC patients. MiR-885-5p: microRNA-885-5p; GC: gastric cancer.

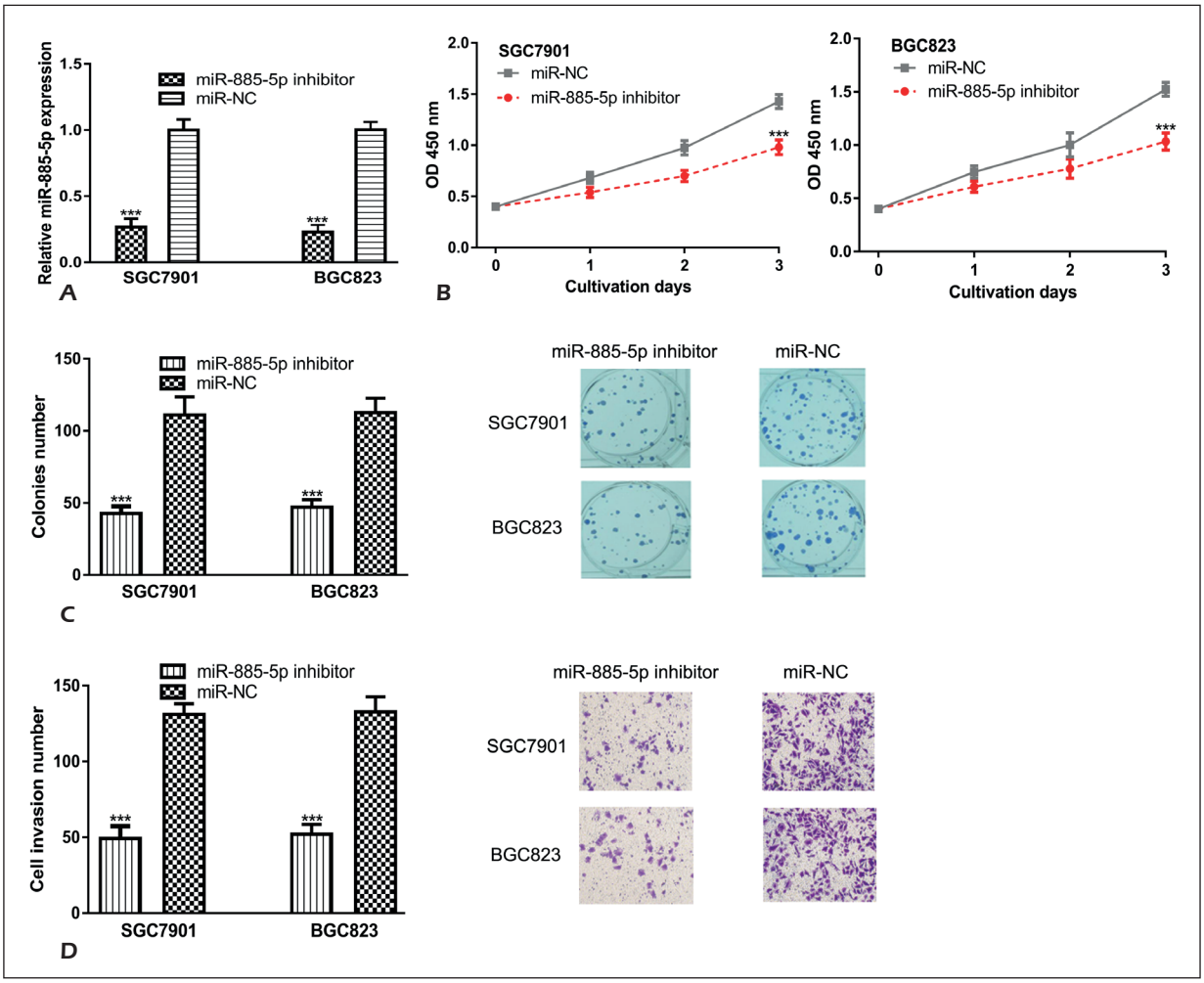

Figure 2. Knockdown of miR-885-5p inhibits GC cell proliferation, colony formation, and cell invasion *in vitro*. (**A**) Expression of miR-885-5p, (**B**) cell proliferation, (**C**) colony formation (magnification: x200), and (**D**) cell invasion (magnification: x200) in GC cells with miR-885-5p inhibitor or miR-NC transfection. MiR-885-5p: microRNA-885-5p; GC: gastric cancer; miR-NC: negative control miRNA.

invasion assay. We found the miR-885-5p inhibitor transfection significantly decreased the invasion ability of GC cells (Figure 2D).

YPEL1 Was a Direct Target of MiR-885-5p in GC

To reveal the mechanisms of miR-885-5p in GC, TargetScan was used to predict targets of miR-885-5p. We found that YPEL1 contains a putative binding site for miR-885-5p in its 3'-UTR (Figure 3A). The Dual-Luciferase reporter assay showed that miR-885-5p inhibitor significantly increased Luciferase activity in cells with wt-YPEL1 transfection but did not affect those with mt-YPEL1 transfection (Figure 3B). To further observe whether miR-885-5p could regulate YPEL1 expression, Western blot was conducted. It was found that miR-885-5p inhibitor transfection increased YPEL1 levels in GC cells (Figure 3C).

Silencing of YPEL1 Promotes GC Cell Growth and Invasion

To explore whether YPEL1 was involved in the function of miR-885-5p, rescue experiments were performed by co-transferring si-YPEL1 and miR-885-5p inhibitor into GC cells. Si-YPEL1 transfection significantly decreased YPEL1 expression in GC cells compared with siR-NC (Figure 4A). Silencing YPEL1 expression promotes GC cell proliferation, colony formation, and cell invasion (Figures 4B-4D). In addition, YPEL1 knockdown partially abrogated the effects of miR-885-5p on GC cell behaviors (Figures 4B-4D).

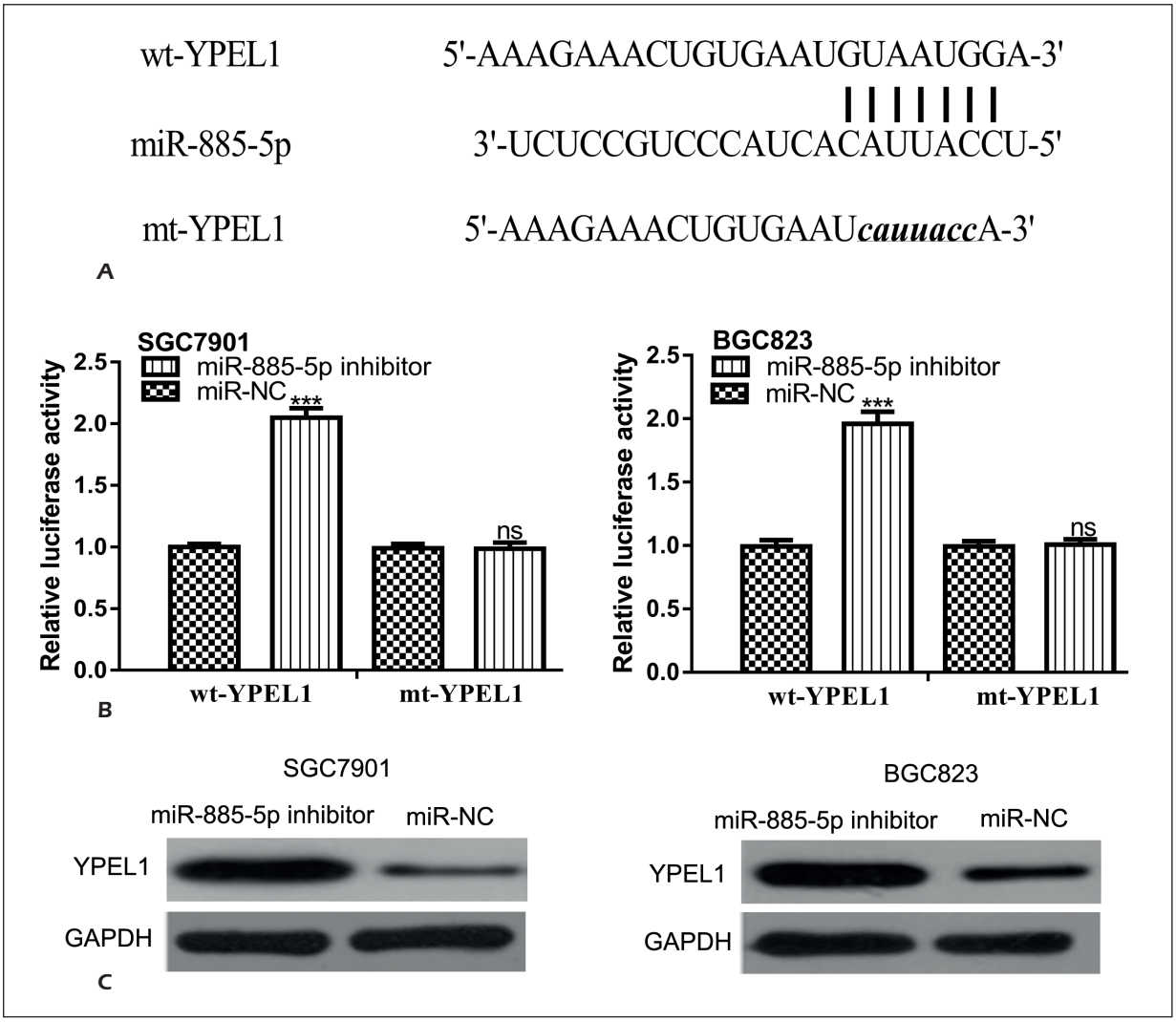

Figure 3. YPEL1 was a target of miR-885-5p in GC. **A**, The predicted miR-885-5p binding site in the 3'-UTR of YPEL1. **B**, Relative Luciferase activity in GC cells with Luciferase reporter vectors or synthetic miRNAs transfection. **C**, Expression of YPEL1 in GC cells with miR-885-5p inhibitor or miR-NC transfection. MiR-885-5p: microRNA-885-5p; GC: gastric cancer; miR-NC: negative control miRNA; UTR: untranslated region; wt: wild-type; mt: mutant; YPEL1: Yippee-like-1.

Discussion

GC is a heavy health burden in China, as the pathogenesis process is very complex, which results in the understanding of GC progression remains limited[13]. In the past years, extensive studies[6,7,14] have revealed miRNAs are crucial regulators in GC progression. Aberrant expression of miRNAs may be used as prognosis prediction markers or therapeutic targets for GC[6,7,14].

In this work, we profiled the expression level of miR-885-5p in GC cell lines and normal cell line. We found that miR-885-5p expression was significantly upregulated in GC cell lines compared with in normal cell line. Moreover, we found that high miR-885-5p was a predictor for poor overall survival of GC patients through Kaplan-Meier curve analysis. Here, we showed the knockdown of miR-885-5p inhibits GC cell proliferation, colony formation, and cell invasion *in vitro*. These results indicated that miR-885-5p may function an oncogenic role in GC progression, which is similar to its role in colorectal cancer[9].

It is well known that miRNA can regulate gene expression by 3'-UTR binding[4]. Hence, like many other miRNAs, miR-885-5p was found to exert either oncogenic or tumor suppressive roles in cancers[8-10]. Genes including Wnt/β-catenin signaling pathway, targeting suppressor of cytokine signaling, and Rac family small GTPase 1 have

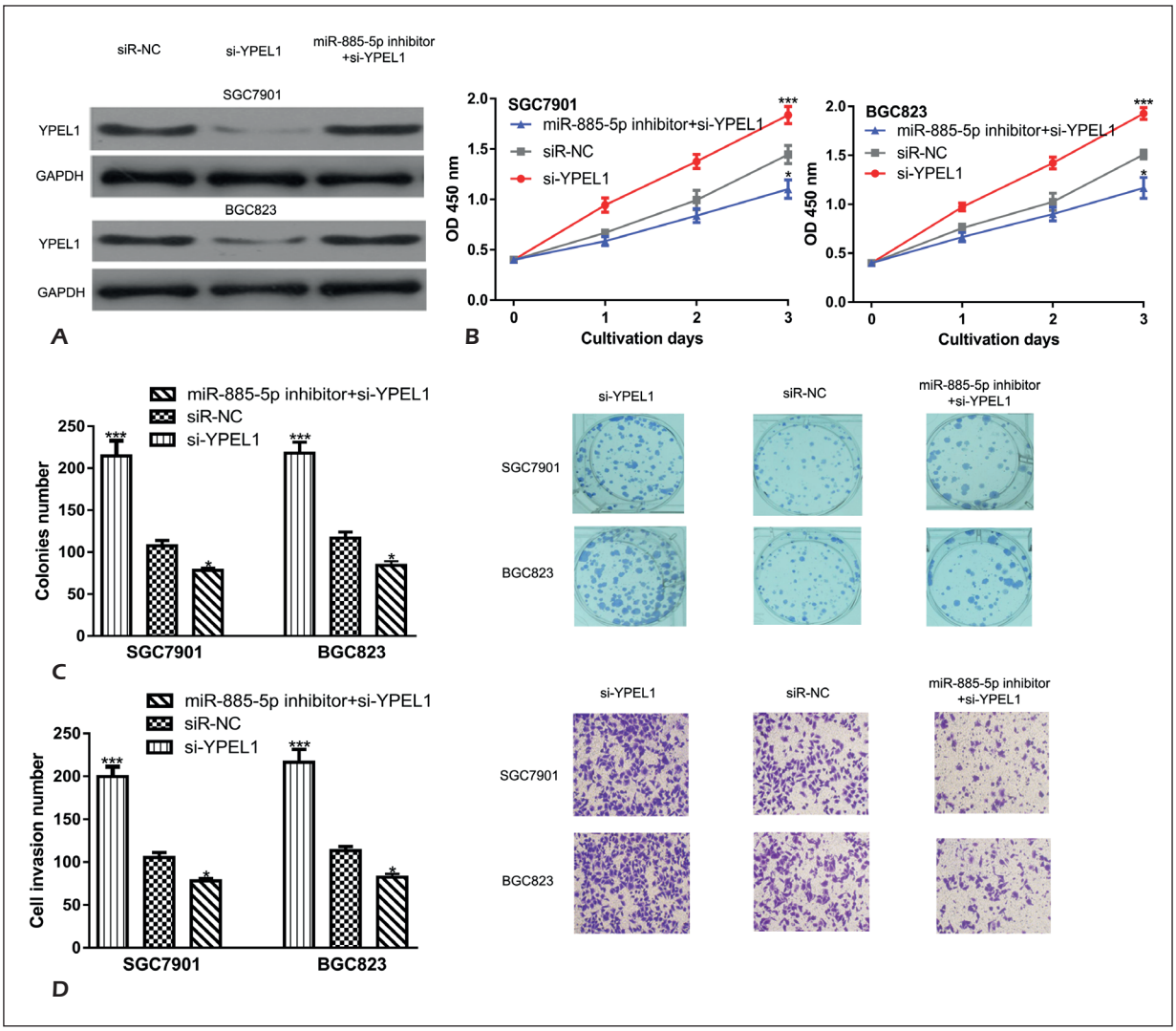

Figure 4. Knockdown of YPEL1 reversed the effects of miR-885-5p on GC cell behaviors. (**A**) Expression of YPEL1, (**B**) cell proliferation, (**C**) colony formation (magnification: x200), and (**D**) Cell invasion (magnification: x200) in GC cells with si-YPEL1, siR-NC, or si-YPEL1, and miR-885-5p inhibitor transfection. MicroRNA-885-5p; GC: gastric cancer; YPEL1: Yippee-like-1; si-YPEL1: small interfering RNA targeting YPEL1; siR-NC: negative control small interfering RNA.

been previously identified as the downstream targets for miR-885-5p. To the best of our knowledge, the mechanisms regarding miR-885-5p in GC remain to be elucidated. By utilizing the Luciferase activity reporter assay and Western blot assay, YPEL1 was identified as a potential target of miR-885-5p. We showed that knockdown YPEL1 promotes GC cell growth and invasion, indicating that YPEL1 may function as tumor suppressor in GC Abiatari et al[11]. Showed that YPEL1 was decreased in pancreatic cancer, which also indicated the tumor suppressive role of YPEL1. In addition, we demonstrated that the knockdown YPEL1 partially reversed the effects of miR-885-5p on GC cell behaviors.

Conclusions

Our study demonstrated that miR-885-5p expression was elevated in GC cell lines and indicated poorer overall survival of GC patients. Functionally, the knockdown of miR-885-5p inhibits GC cell proliferation, colony formation, and cell invasion *in vitro*. YPEL1 was regarded as novel target of miR-885-5p and involved in miR-885-5p mediated effects on GC. These observations suggested that miR-885-5p may be a potential target for GC treatment.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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