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MicroRNA-623 Targets Cyclin D1 to Inhibit Cell Proliferation and Enhance the Chemosensitivity of Cells to 5-Fluorouracil in Gastric Cancer

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The dysregulation of microRNAs (miRNAs) plays an important function in the onset and progression of gastric cancer (GC). In addition, aberrantly expressed miRNAs affect the chemosensitivity of GC cells to chemotherapeutic drugs. Hence, miRNA-based targeted therapy might be applied to treat patients with GC exhibiting chemotherapeutic resistance. In this study, miRNA-623 (miR-623) expression was downregulated in GC tissues and cell lines. Functional analysis showed that the restored miR-623 expression could inhibit the proliferation of GC cells and enhance their chemosensitivity to 5-FU via the cell apoptosis pathway. Cyclin D1 (CCND1) was identified as a direct target gene of miR-623 in GC. The overexpressed CCND1 in GC tissues was negatively correlated with miR-623 level. The recovered CCND1 expression counteracted the effects of miR-623 on GC cell proliferation, chemosensitivity, and 5-FU-induced apoptosis. Thus, our results suggest that miR-623 might function as a tumor suppressor in GC and could be a promising therapeutic target for patients with GC, especially those with chemotherapeutic resistance.

Key words: MicroRNA-623; Gastric cancer (GC); Chemosensitivity; 5-Fluorouracil (5-FU); Cyclin D1 (CCND1)

INTRODUCTION

Gastric cancer (GC), the most common cancer affecting the digestive tract, ranks as the fifth most frequently occurring cancer and the third leading cause of cancer-associated mortalities globally[1]. Approximately 1,000,000 novel GC cases and over 700,000 GC-related deaths are annually recorded worldwide[2]. Several risk factors, such as dietary habits, *Helicobacter pylori* infection, pernicious anemia, and chronic atrophic gastritis, have been validated to be involved in the occurrence and development of GC[3,4]. Despite advancements in treatments for GC, the prognosis of patients with advanced GC remains unsatisfactory[5]. The 5-year overall survival rate of patients with GC diagnosed in advanced stages is 4%[6]. Clinical studies have emphasized that the poor therapeutic outcomes of patients with GC is associated with late diagnosis, metastasis, and recurrence[7,8]. Moreover, chemoresistance impedes successful cancer treatment[9]. Therefore, the molecular mechanism associated with GC pathogenesis should be investigated to develop novel treatment options and improve the clinical outcomes of patients with this disease.

A previous study on microRNAs (miRNAs) has helped enhance our understanding of chemotherapy resistance[10]. miRNAs are a large family of short noncoding single-stranded RNA molecules that can play major regulatory roles[11]. miRNAs participate in gene regulation by directly interacting with the 3′-untranslated regions (3′-UTRs) of their target genes in a base-pairing manner, resulting in the degradation of mRNA or inhibition of translation[12]. Aberrant miRNA expression has been widely reported in almost all types of human malignancies, such as GC[13], renal cell carcinoma[14], colorectal cancer[15], and ovarian cancer[16]. Such expression is also implicated in tumorigenesis and tumor development through the regulation of cell proliferation, cell cycle, apoptosis, and metastasis[17,18]. miRNAs are also involved in the chemosensitivity and chemoresistance of human cancer types[19]. For example, miR-939 upregulation increases the chemosensitivity of drug-resistant GC cells to vincristine and adriamycin by directly targeting EZH2[20]. Therefore, targeting miRNAs may be a new therapeutic strategy for the treatment of chemoresistant GC.

miR-623 plays important roles in lung adenocarcinoma[21]. However, its expression pattern, biological functions, and biological mechanism in GC remain unclear. In this study, we confirmed that miR-623 expression

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was reduced in GC tissues and cell lines. Upregulation of miR-623 suppressed the proliferation of GC cells and increased their chemosensitivity to 5-fluorouracil (5-FU). Cyclin D1 (CCND1) was also identified as a direct target of miR-623 in GC.

MATERIALS AND METHODS

Acquisition of Tissue Specimens

This research was approved by the Ethics Committee of Linyi Third People's Hospital (Shandong, P.R. China). Full written informed consent was provided by all GC patients who participated in the current study. A total of 31 paired GC tissues and corresponding adjacent normal tissues were obtained from patients diagnosed with GC at the Linyi Third People's Hospital. All the patients were treated with surgical resection between August 2010 and January 2016. No patients had been treated with chemotherapy or radiotherapy before surgery. Both GC tissues and corresponding adjacent normal tissues were quickly frozen in liquid nitrogen and kept in the cryopreservation refrigerator at −80°C.

Cell Culture and Transfection Condition

Four human GC cell lines (MKN-45, SGC-7901, BGC-823, and MGC-803) and the human normal gastric epithelial cell line GES-1 were ordered from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, P.R. China). All these cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin and streptomycin (all Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), and grown at 37°C in a humidified chamber containing 5% CO_2 and 95% air.

miR-623 mimic and negative control miRNA mimic (miR-NC) were obtained from Guangzhou RiboBio Co., Ltd. (Guangzhou, P.R. China). Empty pcDNA3.1 vector and constructed CCND1 overexpression vector pcDNA3.1-CCND1 were designed and synthesized by Integrated Biotech Solutions (Shanghai, P.R. China). For cell transfection, cells were inoculated into six-well plates with a density of 5×10^5 cells/well. Cell transfection was conducted using Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), in accordance with the manufacturer's protocol, when the cells reached about 60% confluence. Eight hours posttransfection, cell culture medium was replaced with fresh DMEM containing 10% FBS. Transfection efficiency was evaluated using reverse transcription quantitative polymerase chain reaction (RT-qPCR) or Western blot analysis.

RNA Isolation and RT-qPCR

Total RNA was prepared from tissue samples or cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. For the quantification of miRNA expression, total RNA was reverse transcribed into cDNA using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). Subsequently, qPCR was carried out on the Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Inc.) using a TaqMan MicroRNA PCR Kit (Applied Biosystems). For the detection of mRNA expression, reverse transcription was performed with M-MLV Reverse Transcription system (Promega Corporation, Madison, WI, USA). Afterward, SYBR Green I Mix (Takara Biotechnology Co., Ltd., Dalian, P.R. China) was utilized to carry out qPCR. U6 snRNA and GAPDH were applied as internal control for miR-623 and CCND1 mRNA, respectively. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method[22].

Cell Counting Kit-8 (CCK-8) and Cell Chemosensitivity Assays

Transfected cells were collected and inoculated into 96-well plates at a density of 3×10^3 cells/well in triplicate. The extent of cell proliferation was evaluated at 0, 24, 48, and 72 h after incubation at 37°C in a humidified chamber containing 5% CO_2 and 95% air. CCK-8 solution (10 μl; Beyotime Institute of Biotechnology, Haimen, P.R. China) was added to each well. After the cells were incubated at 37°C for another 2 h, absorbance was determined at a wavelength of 450 nm with a SpectraMax M5 microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA).

Cell chemosensitivity was determined using the CCK-8 method. At 24 h after transfection, the cells were seeded into 96-well plates with a density of 3×10^3 cells/well. Afterward, the cells were incubated overnight and treated with 5-FU (Sigma-Aldrich, St. Louis, MO, USA) at different concentrations (0–32 μM). Cell chemosensitivity assay was conducted at 48 h after 5-FU was administered. The dose–response curve was plotted at various concentrations.

Flow Cytometry Cell Apoptosis Analysis

A total of 5×10^5 cells were plated into six-well plates and cultured at 37°C with 5% CO_2. At 48 h after transfection, the cells were probed with 8 μM 5-FU and further incubated at 37°C for another 48 h. The cells were harvested, washed with ice-cold phosphate-buffered saline, and collected into a 1.5-ml tube. Cell apoptosis was then detected using an Annexin-V-FITC apoptosis detection kit (Invitrogen Corporation). In brief, 300 μl of 1× binding buffer was added to a 1.5-ml tube, gently mixed, and added with 5 μl of annexin V and 5 μl of PI. The cells were further incubated at room temperature in the dark for 15 min, and cell apoptosis was analyzed using a flow cytometer (Beckman Coulter, Inc., Brea, CA, USA). Each assay was performed in triplicate.

Bioinformatics Analysis

TargetScan (www.targetscan.org/) and miRanda (www.microrna.org) were utilized to predict the potential targets of miR-623. Among these candidates, CCND1 was selected for further confirmation, as it has previously been demonstrated to contribute to the chemosensitivity of GC cells.

Dual-Luciferase Reporter Assay

A wild-type (WT) CCND1 3′-UTR containing the predicted binding sequences of miR-623 and a mutant (MUT)-type CCND1 3′-UTR lacking the binding sequences of miR-623 were synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, P.R. China), cloned into a psiCHECK-2 vector (Promega Corporation), and designated as psiCHECK-WT-CCND1-3′-UTR and psi CHECK-MUT-CCND1-3′-UTR, respectively. The cells were seeded into 24-well plates (2.0×10^3/well) 24 h prior to transfection and then transfected with miR-623 mimic or miR-NC and psiCHECK-WT-CCND1-3′-UTR or psiCHECK-MUT-CCND1-3′-UTR using Lipofectamine™ 2000 according to the manufacturer's protocol. Luciferase activities were detected using a Dual-Luciferase Reporter Assay System (Promega Corporation) at 48 h posttransfection. *Renilla* luciferase activity was used as an internal reference.

Western Blot Analysis

In this study, mouse anti-human monoclonal CCND1 (1:1,000 dilution; Catalog No. sc-8396) and mouse anti-human monoclonal β-actin (1:1,000 dilution; Catalog No. sc-81178) primary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). β-Actin was utilized as a loading control for protein level normalization. Total protein was isolated from tissues or cells using a total protein extraction kit (Nanjing KeyGen Biotech Co., Ltd., Nanjing, P.R. China). The total protein concentration was assessed with a BCA protein assay kit (Pierce; Thermo Fisher Scientific, Inc.). Equal quantities of protein sample were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were subsequently blocked with 5% fat-free milk in TBS containing 0.1% Tween 20 (TBST), incubated with primary antibodies at 4°C overnight, washed with TBST, and probed with the corresponding horseradish peroxidase-conjugated secondary antibodies (1:5,000 dilution; Catalog No. sc-2005; Santa Cruz Biotechnology, Inc.) at room temperature for 2 h. The blots were visualized using an enhanced chemiluminescence solution (ECL; Pierce; Thermo Fisher Scientific, Inc.).

Statistical Analysis

Data were expressed as mean ± standard deviation and statistically analyzed using SPSS 15.0 (SPSS, Inc., Chicago, IL, USA). Differences between two groups were examined using Student's *t*-test, and one-way ANOVA was performed when there were more than two groups. Student–Newman–Keuls test was conducted as a post hoc test following ANOVA. A two-tailed value of $p<0.05$ was considered significant.

RESULTS

miR-623 Expression Is Downregulated in GC Tissues and Cell Lines

To explore the expression pattern of miR-623 in GC, we initially detected its expression in 31 paired GC tissues and the corresponding adjacent normal tissues. RT-qPCR analysis revealed that miR-623 expression was downregulated in GC tissues compared with those in adjacent normal tissues ($p<0.05$) (Fig. 1A). We then measured the miR-623 expression levels in four human GC

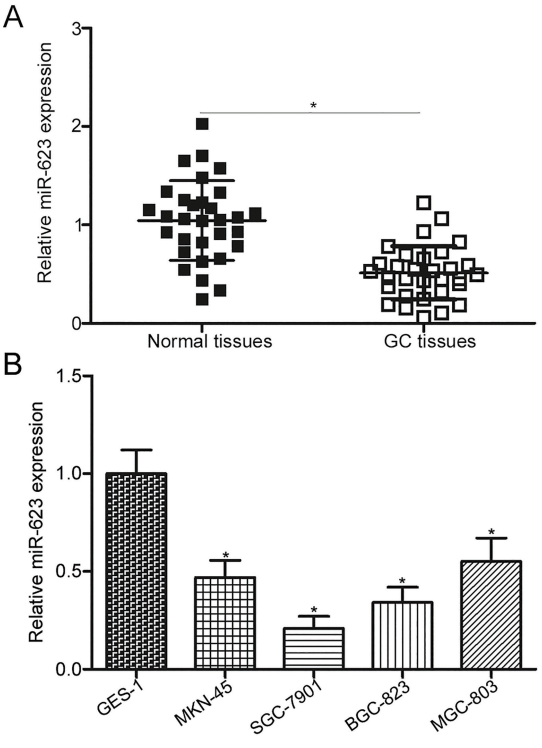

Figure 1. MicroRNA-623 (miR-623) is downregulated in gastric cancer (GC) tissues and cell lines. (A) Relative miR-623 expression levels in 31 paired GC tissues and their corresponding adjacent normal tissues were detected through RT-qPCR. *$p<0.05$ compared with normal tissues. (B) Relative miR-623 expression was determined in four human GC cell lines (MKN-45, SGC-7901, BGC-823, and MGC-803) and a human normal gastric epithelial cell line (GES-1). *$p<0.05$ compared with GES-1.

cell lines and a human normal gastric epithelial cell line GES-1. miR-623 was underexpressed in all of the tested GC cell lines compared with that of GES-1 ($p<0.05$) (Fig. 1B). SGC-7901 and BGC-823 cell lines, which exhibited a relatively lower miR-623 expression out of the four examined cell lines, were selected for subsequent experiments. These results suggested that the downregulation of miR-623 might be associated with GC progression.

miR-623 Overexpression Represses Cell Proliferation and Increases the Chemosensitivity of Cells to 5-FU in GC

miR-623 was markedly overexpressed in SGC-7901 and BGC-823 cells transfected with miR-623 mimic to explore the biological function of miR-623 in GC ($p<0.05$) (Fig. 2A). CCK-8 assay was then performed to investigate the effect of miR-623 overexpression on GC cell proliferation. The results revealed that the restored miR-623 expression significantly repressed the proliferation of SGC-7901 and BGC-823 cells ($p<0.05$) (Fig. 2B). Therefore, decreased miR-623 expression might promote GC progression by inducing the proliferation of GC cells.

Cell chemosensitivity assays were conducted to evaluate whether miR-623 is involved in the development of chemoresistance in GC cells. In Figure 2C, SGC-7901 and BGC-823 cells transfected with miR-623 mimic exhibited a significantly decreased survival rate after they were treated with 5-FU ($p<0.05$), suggesting that miR-623 overexpression might reverse the chemoresistance of GC cells to 5-FU. The antitumor activity of 5-FU was mainly caused by promoting cell apoptosis. Hence,

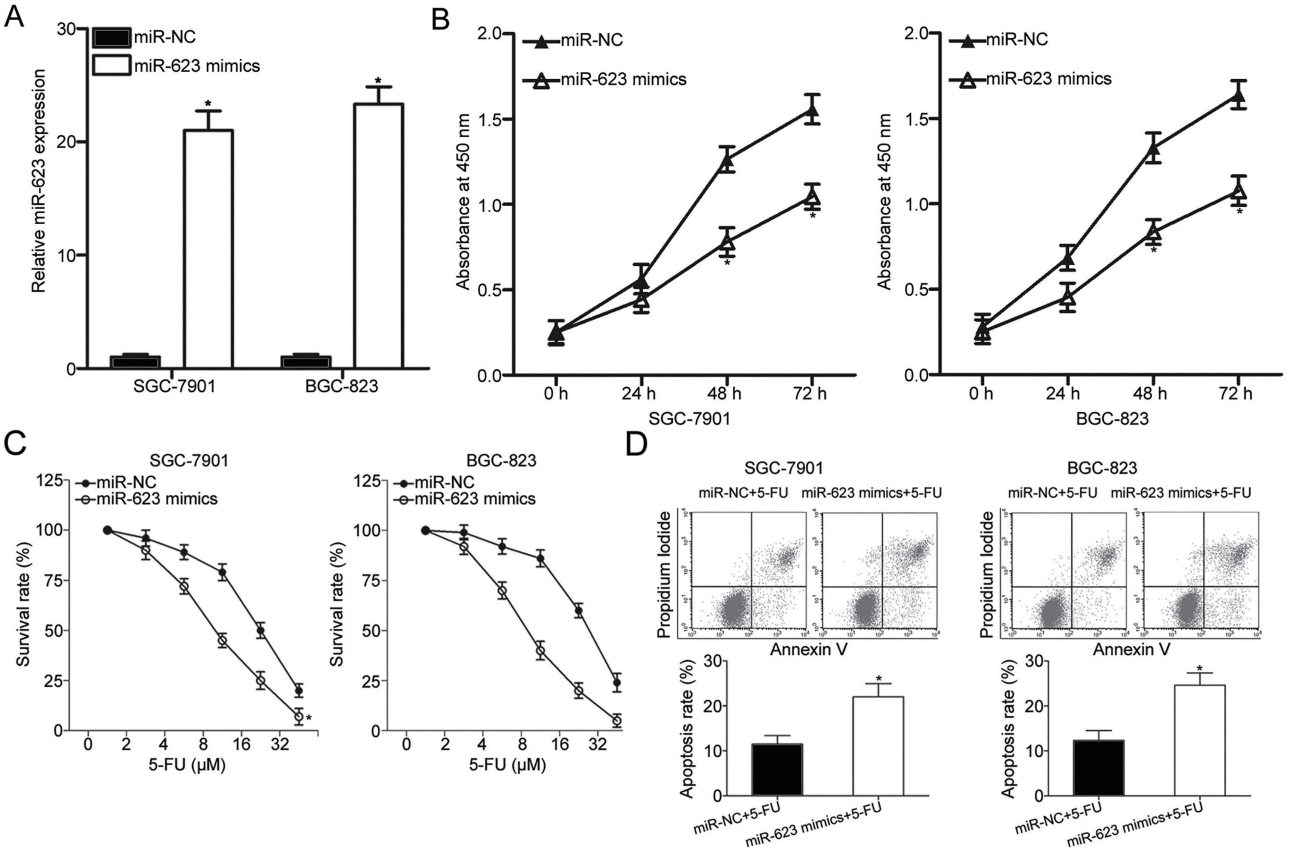

Figure 2. miR-623 inhibits the proliferation and enhances the chemosensitivity of SGC-7901 and BGC-823 cells to 5-fluorouracil (5-FU). (A) SGC-7901 and BGC-823 cells were transfected with miR-623 mimic or miR-negative control (NC). At 48 h posttransfection, relative miR-623 expression was evaluated through reverse transcription quantitative polymerase chain reaction (RT-qPCR). *$p<0.05$ compared with miR-NC. (B) The proliferation of SGC-7901 and BGC-823 cells was determined through cell counting kit-8 (CCK-8) assay following transfection with miR-623 mimic or miR-NC. *$p<0.05$ compared with miR-NC. (C) miR-623 mimic or miR-NC was introduced to SGC-7901 and BGC-823 cells. After 24 h of transfection, different concentrations of 5-FU were added and incubated for another 48 h. Cell chemosensitivity assay was performed to detect the chemosensitivity of SGC-7901 and BGC-823 cells to 5-FU. *$p<0.05$ compared with miR-NC. (D) SGC-7901 and BGC-823 cells were transfected with miR-623 mimic or miR-NC and incubated with 8 μM 5-FU 48 h after transfection. Cell apoptosis was analyzed through flow cytometry to examine the cell apoptosis rate. *$p<0.05$ compared with miR-NC + 5-FU.

cell apoptosis was analyzed through flow cytometry to elucidate whether miR-623 mediated the chemosensitivity of GC cells to 5-FU by promoting 5-FU-induced cell apoptosis. In Figure 2D, enforced expression of miR-623 expedited the 5-FU-stimulated apoptosis of SGC-7901 and BGC-823 cells ($p<0.05$). These results implied that miR-623 was involved in the regulation of the chemosensitivity of GC cells to 5-FU via the cell apoptosis pathway.

CCND1 Is a Direct Target Gene of miR-623 in GC

Bioinformatic analysis was conducted to search for the potential targets of miR-623 and to explore the mechanisms that might be responsible for the action of miR-623 in GC. Among these candidates, CCND1 (Fig. 3A) was selected for further confirmation because it contributes to the chemosensitivity of GC cells[23–26]. RT-qPCR and Western blot analysis were applied to detect the mRNA and protein expression of CCND1 in SGC-7901 and BGC-823 cells transfected with miR-623 mimic or miR-NC. Our data showed that the ectopic miR-623 expression decreased the CCND1 expression in SGC-7901 and BGC-823 cells at the mRNA ($p<0.05$) (Fig. 3B) and protein ($p<0.05$) (Fig. 3C) levels, suggesting that miR-623 negatively regulated the endogenous CCND1 expression in GC. To further confirm that CCND1 is a direct target of miR-623 and to determine whether miR-623 can directly target the 3′-UTR of CCND1, we performed a dual-luciferase reporter assay. SGC-7901 and BGC-823 cells were transfected with miR-623 mimic or miR-NC in combination with psiCHECK-WT-CCND1-3′-UTR or psiCHECK-MUT-CCND1-3′-UTR. The transfection

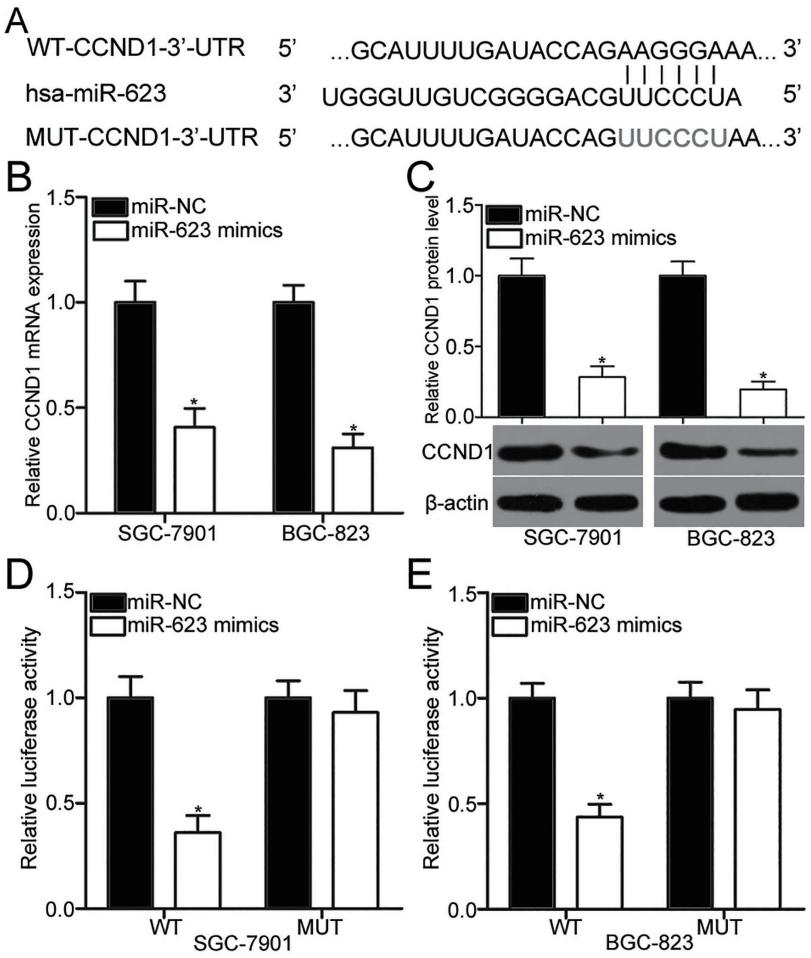

Figure 3. miR-623 directly targets cyclin D1 (CCND1) in GC. (A) Wild-type (WT) and mutated (MUT) miR-623 binding sequences in the 3′-untranslated region (3′-UTR) of CCND1. (B) RT-qPCR and (C) Western blot were performed to determine the mRNA and protein levels of CCND1 in SGC-7901 and BGC-823 cells transfected with miR-623 mimic or miR-NC. *$p<0.05$ compared with miR-NC. miR-623 or miR-NC was transfected in (D) SGC-7901 and (E) BGC-823 cells with psiCHECK-WT-CCND1-3′-UTR or psiCHECK-MUT-CCND1-3′-UTR. Relative luciferase activity levels were measured at 48 h posttransfection. *$p<0.05$ compared with miR-NC.

of SGC-7901 and BGC-823 cells with miR-623 mimic reduced the luciferase activities of the reporter containing the WT 3′-UTR of CCND1 ($p<0.05$) but not the activities of the reporter with the respective MUT 3′-UTR (Fig. 3D and E). These findings suggested that CCND1 is a direct target of miR-623 in GC.

CCND1 Expression Increases in GC Tissues and Shows a Negative Correlation With miR-623 Levels

The expression level of CCND1 in GC tissues and adjacent normal tissues was measured through RT-qPCR and Western blot analysis. The results indicated that the mRNA expression level of CCND1 increased in GC tissues compared with that in adjacent normal tissues ($p<0.05$) (Fig. 4A). In addition, CCND1 protein expression was upregulated in GC tissues compared with that in adjacent normal tissues (Fig. 4B). Spearman's correlation analysis revealed that miR-623 was remarkably associated with the mRNA of CCND1 in these GC tissues ($r=-0.5849$, $p=0.0005$) (Fig. 4C).

Reintroduction of CCND1 Counteracts the Effects of miR-623 on GC Cells

Considering that CCND1 served as a direct functional target of miR-623, we hypothesized that the recovered CCND1 expression in the miR-623-expressing cells might rescue the effects of miR-623 overexpression on GC. To confirm this hypothesis, we performed rescue experiments and introduced miR-623 mimic to SGC-7901 and BGC-823 cells in combination with pcDNA3.1 or pcDNA3.1-CCND1. We evaluated the transfection efficiency through Western blot analysis and observed that the protein level of CCND1 was restored in SGC-7901 and BGC-823 cells after they were cotransfected with pcDNA3.1-CCND1 ($p<0.05$) (Fig. 5A). Similarly, the subsequent experiments confirmed that the restored CCND1 expression counteracted the effects on SGC-7901 and BGC-823 cell proliferation ($p<0.05$) (Fig. 5B), chemosensitivity ($p<0.05$) (Fig. 5C), and 5-FU-induced apoptosis ($p<0.05$) (Fig. 5D) caused by miR-623 overexpression. These results demonstrated that miR-623 might play tumor-suppressive roles in GC partly by downregulating CCND1.

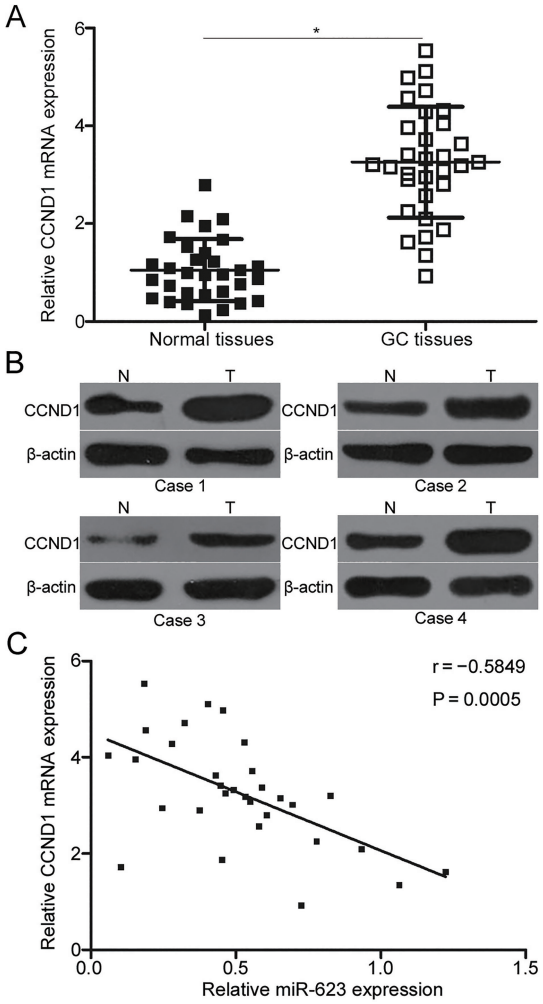

Figure 4. CCND1 overexpression in GC tissues is inversely correlated with miR-623 level. (A) RT-qPCR and (B) Western blot were applied to measure the mRNA and protein expression levels of CCND1 in GC tissues and adjacent normal tissues, respectively. *$p<0.05$ compared with normal tissues. (C) The association between CCND1 mRNA and miR-623 levels in GC tissues was assessed through Spearman's correlation analysis. $r=-0.5849$, $p=0.0005$.

DISCUSSION

The dysregulation of miRNAs plays an important function in the onset and progression of GC[27–29]. Aberrantly expressed miRNAs affect the chemosensitivity of cells to chemotherapeutic drugs in multiple types of human cancer. For example, the restored miR-16 expression enhances the chemosensitivity of breast cancer cells to paclitaxel by directly targeting inhibitor of nuclear factor κ B kinase subunit β (IKBKB)[30]. Xiao et al.[31] revealed that the chemosensitivity of acute myeloid leukemia cells to doxorubicin is increased by miR-217 overexpression. Cheng et al. found that ectopic expression of miR-451 sensitizes lung cancer cells to cisplatin by blocking Mcl-1[19]. Zhang et al.[32] demonstrated that resumption expression of miR-625 increases the chemosensitivity of glioma cells to temozolomide through regulation of AKT2. Xu et al.[33] reported that enforced expression of miR-330 targets thymidylate synthase (TYMS) to enhance the sensitivity of colorectal cancer cells to 5-FU by regulating TYMS. In GC, miR-147[34], miR-320a[35], miR-126[20], and miR-218[36] are implicated in the regulation of cell chemosensitivity. These findings suggested that miRNA-based

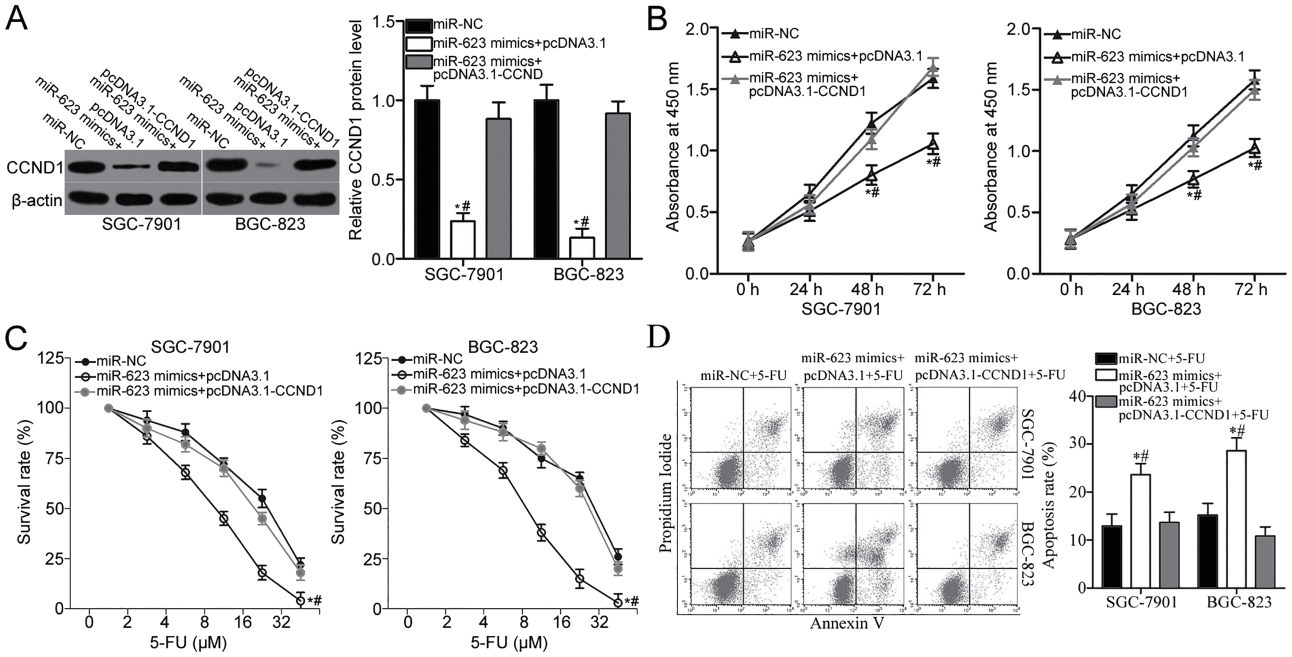

Figure 5. CCND1 overexpression reverses the effects of miR-623 on GC cells. (A) CCND1 protein expression was detected in SGC-7901 and BGC-823 cells cotransfected with miR-623 mimic and pcDNA3.1 or pcDNA3.1-CCND1 through Western blot. *$p<0.05$ compared with miR-NC. #$p<0.05$ compared with miR-623 mimics + pDNA3.1-CCND1. CCK-8 assay (B), cell chemosensitivity assay (C), and flow cytometry analysis of cell apoptosis (D) were performed to determine cell proliferation, chemosensitivity to 5-FU, and apoptosis induced by 5-FU in differently treated SGC-7901 and BGC-823 cells, respectively. *$p<0.05$ compared with miR-NC. #$p<0.05$ compared with miR-623 mimics + pcDNA3.1-CCND1.

targeted therapy might be applied to treat patients with tumors exhibiting chemotherapeutic resistance.

miR-623 is downregulated in lung adenocarcinoma tissues and cell lines. Decreased miR-623 level is significantly associated with tumor differentiation, lymphatic metastasis, and increased serum carcinoembryonic antigen level. The median overall survival of the patients with lung adenocarcinoma and low miR-623 expression is shorter than that of the patients with high miR-623 expression. Furthermore, miR-623 reexpression prevents the in vitro and in vivo growth and metastasis of lung adenocarcinoma cells by directly targeting Ku80[21]. In our study, miR-623 was underexpressed in GC tissues and cell lines. In the subsequent in vitro experiments, miR-623 overexpression attenuated the proliferation of GC cells and increased their chemosensitivity to 5-FU. CCND1 was validated as a direct target of miR-623 in GC. The upregulation of CCND1 in GC tissues was also associated with miR-623 level. Rescue experiments revealed that the recovered CCND1 expression counteracted the effects of miR-623 on GC cells. These results demonstrated that miR-623 directly targeted CCND1 to play tumor-suppressive roles in GC growth and chemoresistance, implying that miR-623 might be investigated as a therapeutic target to rapidly block the tumor growth and chemoresistance of patients with this malignancy.

Identifying miRNAs and their targets involved in gastric carcinogenesis and progression may help develop effective therapeutic strategies. In this study, CCND1 was validated to be a direct and functional target of miR-623 in GC. CCND1, located in chromosome 11q13, is a well-described oncogene overexpressed in numerous human cancer types, such as thyroid carcinoma[36], breast cancer[37], lung cancer[38], and bladder cancer[39]. In GC, CCND1 is also upregulated and strongly correlated with poor differentiation[40]. Survival analysis indicated that the CCND1 expression level was significantly associated with the disease-free survival and overall survival of patients with GC. The prognosis of these patients who exhibited a high CCND1 level was poorer than that of the patients with low CCND1. Multivariate analysis has identified CCND1 expression as an independent prognostic indicator of disease-free survival and overall survival in GC[23]. Functional assays have demonstrated that CCND1 contributes to the tumor initiation and progression of GC through the regulation of cell proliferation, cycle, migration, invasion, and apoptosis[23–25]. The inhibition of CCND1 enhances the chemosensitivity of GC cells to 5-FU[26]. These findings suggested that targeting CCND1 may provide advantages to treat patients with GC, especially those exhibiting chemotherapeutic resistance.

CCND1 has been determined to be regulated by miRNAs in multiple types of human cancer. For instance,

miR-193a-3p reexpression targets CCND1 to prohibit cell growth in prostate cancer[41]. In T-cell lymphoma, miR-373 overexpression represses cell proliferation by regulating CCND1[42]. In colon cancer, the miR-374a upregulation reduces cell growth, motility, and intrahepatic metastasis by inhibiting CCND1[43]. In non-small cell lung cancer, miR-326 attenuates cell proliferation and colony formation and promotes apoptosis by directly targeting CCND1[44]. In GC, miR-193b[24], miR-33a[45], and miR-449a[25] directly target CCND1 and inhibit GC progression. Combined with the present findings, the miRNA/CCND1 axis shows potential for the therapy of patients with GC.

In conclusion, miR-623 was significantly underexpressed in GC tissues and cell lines. In vitro studies demonstrated that miR-623 inhibited GC cell proliferation and increased cell chemosensitivity to 5-FU. CCND1 was mechanistically identified as a direct target gene of miR-623 in GC. However, further experiments should be conducted to evaluate whether miR-623 can enhance the chemosensitivity of GC cells to other chemotherapeutic drugs.

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