MicroRNA-296-5p inhibits cell proliferation by targeting HMGA1 in colorectal cancer

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Abstract. An increasing body of evidence indicates the involvement of microRNAs (miRNAs/miRs) in the initiation and progression of colorectal cancer (CRC). miR-296-5p was recently identified as a tumor suppressor in a variety of human cancer types; however, its function in CRC remains largely unknown. The present study demonstrated that the expression of miR-296-5p was significantly downregulated in CRC tissues and cell lines. The overexpression of miR-296-5p markedly inhibited proliferation, and induced cell cycle arrest and apoptosis in CRC cells. Bioinformatics analysis suggested that high mobility group AT-hook 1 (HMGA1) may be a target of miR-296-5p in CRC cells. Further experiments showed that miR-296-5p bound the 3'-untranslated region of HMGA1 and decreased its expression in CRC cells. HMGA1 was over-expressed in CRC tissues and was inversely correlated with the expression of miR-296-5p. The restoration of HMGA1 significantly reversed the inhibitory effect of miR-296-5p on the proliferation of CRC cells. Overall, the findings of the present study indicate that miR-296-5p suppressed the progression of CRC, at least partially via targeting HMGA1. Thus, miR-296-5p is a potential target for novel therapies in CRC.

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Introduction

Colorectal cancer (CRC) is considered to be one of the most common causes of cancer-associated mortality worldwide (1,2). Although therapeutic strategies, including surgical resection, chemotherapy and radiotherapy, have greatly improved the outcome of patients with CRC, the treatment of patients with CRC requires further improvement (3). Therefore, more efforts should be made in investigating the molecular mechanisms involved in the development of CRC, which might provide novel targets and therapeutic methods for the treatment of CRC.

MicroRNAs (miRNAs/miRs) are defined as a category of endogenous single-stranded, non-coding RNAs that are 21-23 nucleotides in length (4-6). miRNAs act as key negative regulators of gene expression, via binding to the 3'-untranslated regions (3'-UTRs) of target mRNAs, which consequently inhibit the translation or induce the degradation of mRNAs (7). Increasing evidence has indicated that miRNAs play essential roles in diverse cellular processes, including cell proliferation, differentiation and apoptosis (8). The vital function of miRNAs in the progression of human cancer was also demonstrated by recent studies (9-12). miRNAs modulate the progression of cancer by acting as oncogenes or tumor suppressors (9,12-16). Recently, miR-26b was found to improve the sensitivity of CRC cells to 5-flurouracil and inhibited the growth of CRC cells (17). Wang *et al* (18) reported that miR-410 promoted the malignancy of CRC and could be used as a potential biomarker in the progression of CRC. Inhibiting the expression of miR-30d promoted the cell proliferation and tumor growth of CRC by targeting G protein subunit α13 (19). miR-296-5p was recently reported as a tumor suppressor in non-small cell lung cancer by directly targeting polo-like kinase 1 (PLK1) (20). Similarly, miR-296-5p suppressed the proliferation of prostate cancer cells, implicating the potential application of miR-296-5p in the prognosis of prostate cancer (21). Although previous reports have demonstrated the tumor suppressive role of miR-296-5p in cancer, the function and molecular mechanism of miR-296-5p in CRC remains largely unknown.

The present study aimed to investigate the possible role of miR-296-5p in the progression of CRC and characterize the potential underlying molecular mechanism.

Materials and methods

Tissue samples and cell lines. A cohort of 40 CRC tissues and corresponding adjacent normal tissues (5 cm from the tumor margin and histologically confirmed) were collected from CRC patients (female: male=1.86:1; age range, 39-72 years; mean age, 61.4 years) at Zhongshan Hospital (Xiamen, China), via surgical resection between May 2012 and September 2014. None of these patients received chemotherapy, radiotherapy and immunotherapy prior to the tissue collection. Tissues were stored at -80˚C before further experiments. Tumors sample were staged according to the National Comprehensive Cancer Network guidelines (22). Informed consent was obtained from all patients. This study was approved by the Institutional Ethics Committee of Zhongshan Hospital, Xiamen University.

The CRC cell lines (HCT116, HT-29, LOVO) and human normal colorectal epithelial cell (FHC) were purchased from the American Type Culture Collection. The cells were cultured in DMEM (Thermo Fisher Scientific, Inc.), supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 μg/ml streptomycin, at 37˚C with 5% CO_2.

Reagents. The antibodies used in this study included: Anti-HMGA1 (cat. no. 7777; Cell Signaling Technology, Inc.), anti-GAPDH (cat. no. G8795; Sigma-Aldrich; Merck KGaA) and HRP-conjugated secondary antibody (cat. no. 7047; Cell Signaling Technology, Inc.).

Cell transfection. The miR-296-5p mimics (5'-AGGGCCCCCCCUCAAUCCUGU), control miRNA (5'-GGUUCGUACGUACACUGUUCA), miR-296-5p antagomir (5'-UCCCGGGGGGGAGUUAGGACA-3') and the negative control miRNA (5'-CGGUACGAUCGCGGC GGGAUAUC-3') were synthesized by Shanghai GenePharma Co., Ltd. Both the negative control miRNAs were non-targeting sequences. To construct Flag-tagged HMGA1, the full-length of HMGA1 cDNA was obtained by reverse transcription from RNA samples extracted form HCT116 cells and ligated into the c-Flag pcDNA3 vector (cat. no. 20011; Addgene, Inc.) at the EcoRI and XhoI sites. A total of 20 nM miRNAs and/or 0.5 μg of Flag-HMGA1 or Flag-vector were transfected into HCT116 and HT-29 cells, seeded at the density of 10,000 cells/well using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Following transfection for 48 h, the cells were harvested for the subsequent experiments.

Reverse transcription-quantitative (RT-q)PCR. The extraction of RNA from CRC tissues and cell lines was performed using the TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). RNA was reverse transcribed into cDNA using the miScript RT kit (Takara Biotechnology Co., Ltd.), according to the manufacturer's protocols using the temperature protocol of 25˚C for 5 min, 46˚C for 20 min and 95˚C for 1 min. qPCR was performed to determine the expression of miR-296-5p or HMGA1, using SYBR Green mix (Bio-Rad Laboratories, Inc.) on the Applied Biosystems 7500 Sequence Detection System (Applied Biosystems; Thermo Fisher Scientific, Inc). The primers used in this study were as follows: miR-296-5p forward, 5'-GTATCCAGTGCAGGGTCCGA-3'; miR-296-5p reverse, 5'-CGACGAGGGCCCCCCCT-3'; U6 RNA forward, 5'-CGAGCACAGAATCGCTTCA-3'; U6 RNA reverse, 5'-CTCGCTTCGGCAGCACATAT-3'; HMGA1 forward, 5'-CAACTCCGGGGAGGAAACCA-3'; HMGA1 reverse, 5'-AGGACTCCTGGGAGATGC-3'; GAPDH forward, 5'-GCCTTCTCCATGGTGGTGAA-3'; and GAPDH reverse, 5'-GGTCGGTGTGAACGGATTTG-3'. The PCR conditions were as follows: 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 sec and 58˚C for 60 sec. The relative expression of miR-296-5p and HMGA1 was calculated using the $2^{-\Delta\Delta Cq}$ method (23) and was normalized to the expression of U6 RNA or GAPDH, respectively.

Targets prediction. The potential targets of miR-296-5p were predicted using the miRDB (http://mirdb.org/) (24) and TargetScan databases (release 7.2; http://www.targetscan.org/vert_72/).

Western blot analysis. Protein was extracted by lysing the CRC cells using NP-40 buffer (Beyotime Institute of Biotechnology) containing protease inhibitors (Roche Diagnostics). Protein concentration was quantified using the bicinchoninic acid method (Beyotime Institute of Biotechnology). A total of 20 μg protein was separated by SDS-PAGE on a 15% gel, and transferred onto a PVDF membrane (EMD Millipore). After blocking with 5% non-fat milk at room temperature (RT) for 1 h, the membrane was incubated with anti-HMGA1 (1:1,000), anti-GAPDH (1:3,000) or anti-Flag (1:3,000; cat. no. F7425; Sigma-Aldrich; Merck KGaA) primary antibodies overnight at 4˚C. After washing twice with PBS, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:10,000) for 1 h at RT. The blot signals were visualized with enhanced chemiluminescence chromogenic substrate (EMD Millipore), according to the manufacturer's instructions. The expression of GAPDH was determined as the loading control. The protein bands were analyzed with Image J analysis software (v.1.52n; National Institutes of Health). Western blot analysis was performed three times independently.

Ultrasound-mediated microbubble destruction. The doxorubicin (Dox)-liposome-microbubble complex (DLMC) was prepared as previously described (19). To induce the uptake of Dox, CRC cells were seeded in a 24-well plate and incubated overnight at 37˚C. Cells were subsequently incubated with DLMC containing 10 μg/ml Dox, and ultrasound (US) radiation was applied for 15 sec by moving a 20 mm US probe E1609 (Valpey Fisher, Inc.) over the cell culture plate with the following parameters: 1 MHz, 20% duty cycle, US intensity of 1.65 W/cm^2 and US peak intensity of 0.35 MPa, as described previously (25). The cells were then rinsed with serum-free DMEM to remove the uninternalized Dox. Cells with DLMC containing 10 μg/ml of Dox without US treatment were investigated as the mock. Cells were then cultured with fresh medium overnight at 37˚C and subjected to subsequent experiments.

Cell Counting Kit-8 (CCK-8) assay. Both HCT116 and HT-29 cells were plated in the 96-well plate at the density of 1,000 cells per well. Following culture for 24 h, the cells were transfected with miR-296-5p mimics or control miRNA using

Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). After 24 h, 10 μl CCK-8 solution (Dojindo Molecular Technologies, Inc.) was added into the medium and incubated at 37˚C for an additional 3 h, according to the manufacturer's protocol. The absorbance of each well at 450 nm was measured using a microplate reader (Bio-Rad Laboratories, Inc.). The experiments were performed in triplicate.

Luciferase reporter assay. The 3'-UTR of HMGA1 containing the putative binding sites of miR-296-5p was amplified from human genomic DNA and constructed into the pGL3 luciferase reporter vector (Promega Corporation). A total of 0.5 μg luciferase vector encoding the wild-type or mutant 3'-UTR, and miR-296 mimics or control miRNA were co-transfected into the CRC cells (density, ~10,000 cells/well) with Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). After transfection for 48 h, the cells were harvested and the luciferase activity was determined using the Dual-Luciferase Reporter Assay System (Promega Corporation). The activity of Renilla luciferase was detected for normalization.

Cell apoptosis analysis. Both HCT116 and HT-20 cells were seeded on a 6-well plate and transfected with miR-296-5p mimics or control miRNA. After transfection for 48 h, the percentage of cell apoptosis was evaluated using the Annexin-V FITC Apoptosis Detection kit (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Cell apoptosis was determined using a flow cytometer (BD Biosciences). Data analysis was performed using ModFit software (v.3.3; BD Biosciences).

Cell cycle analysis. Cells were cultured with serum-free medium for 24 h and then transfected with miR-296-5p mimics or control miRNA for 48 h with medium containing 10% FBS. The transfected cells were harvested and washed twice with pre-cooled PBS and fixed with 70% ethanol overnight at 4˚C. After washing with PBS, the cells were stained with 100 μg/ml propidium iodide and 50 μg/ml RNase for 30 min at RT in the dark. The cell cycle was detected with a flow cytometer (BD Biosciences). The profile was analyzed using ModFit software (v.3.3; BD Biosciences).

Statistical analysis. Data are presented as the mean ± standard deviation and were analyzed with the GraphPad Prism software (v.5.0; GraphPad Software, Inc.). The differences between two groups were analyzed using the Student's t-test; the differences between normal colon and colon cancer tissues were analyzed by paired t-test. The comparisons between three or more groups were assessed using the one-way analysis of variance, followed by Tukey's test. The correlation between the expression of miR-296-5p and HMGA1 was determined by the Spearman test. The association between the level of miR-296-5p and the clinical features of patients with CRC was analyzed using the χ^2 test. $P<0.05$ was considered to indicate a statistically significance difference.

Results

Expression of miR-296-5p is downregulated in CRC. To evaluate the potential involvement of miR-296-5p in CRC, the expression of miR-296-5p in CRC tissues and matched adjacent normal tissues was detected by RT-qPCR. The results showed that the expression of miR-296-5p was significantly decreased in CRC tissues compared with adjacent non-tumor tissues (Fig. 1A). Consistently, the downregulation of miR-296-5p was also observed in CRC cell lines, including HCT116, HT-29 and LOVO, compared with the expression of miR-296-5p in normal FHC cells (Fig. 1B). To further characterize the association between the expression of miR-296-5p and the prognosis of patients with CRC, all 40 patients enrolled in the present study were divided into a miR-296-5p high and low group, according to the mean value (3.85) of miR-296-5p expression. The results showed that low expression of miR-296-5p was significantly associated with a higher Tumor-Node-Metastasis stage, lymph node metastasis and tumor size, suggesting the potential clinical significance of miR-296-5p in CRC (Table I). The decreased expression of miR-296-5p in CRC cells compared with non-cancerous colon tissues and cell lines suggested the potential role of miR-296-5p in CRC.

Table I. Association between the expression of miR-296-5p and the clinicopathological characteristics of patients with colorectal carcinoma.

Clinical characteristics	Cases, n	Low, n	High, n	P-value
Age, years				0.345
≤60	14	10	4	
>60	26	18	8	
Sex				0.176
Male	19	15	4	
Female	21	13	8	
Tumor size, cm				0.004
≥5	24	19	5	
<5	16	9	7	
TNM stage				<0.001
I + II	15	7	8	
III + IV	25	21	4	
Metastasis				<0.001
Yes	27	22	5	
No	13	6	7	

TNM, tumor-node-metastasis.

Overexpression of miR-296-5p inhibits proliferation and induces apoptosis in CRC cells. In order to explore the regulatory role of miR-296-5p in CRC progression, HCT116 and HT-29 cells were transfected with miR-296-5p mimics or control miRNA. As presented in Fig. 2A, compared with the control cells, the expression of miR-296-5p was significantly increased in both HCT116 and HT-29 cells following the transfection with miR-296-5p mimics. The CCK-8 assay was performed to determine the effect of miR-296-5p on the proliferation of CRC cells. The data showed that the overexpression of miR-296-5p significantly inhibited the proliferation of both HCT116 and HT-29

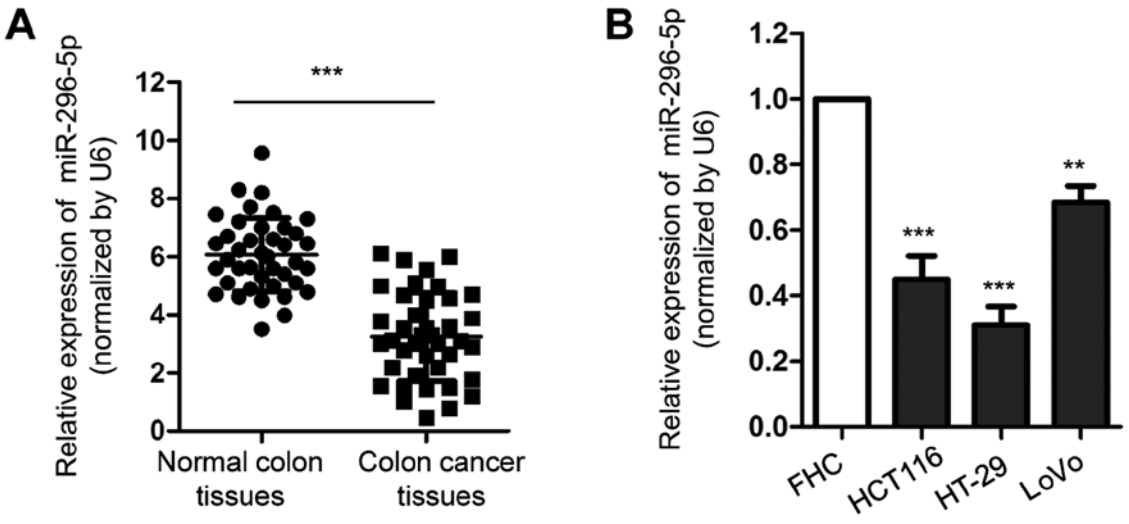

Figure 1. miR-296-5p is downregulated in CRC. (A) The expression of miR-296-5p in paired CRC tissues and corresponding normal tissues was detected by reverse transcription-quantitative PCR. ***P<0.001. (B) The expression level of miR-296-5p was significantly decreased in CRC cell lines compared with normal FHC cells. **P<0.01, ***P<0.001 vs. FHC. miR, microRNA; CRC, colorectal cancer.

cells (Fig. 2B and C). Additionally, the flow cytometry indicated that the overexpression of miR-296-5p led to G1-phase cell cycle arrest in HCT116 cells (Fig. 2D). In accordance, the apoptosis of CRC cells was also significantly upregulated following the overexpression of miR-296-5p (Fig. 2E).

To further validate the suppressive function of miR-296-5p in CRC, HCT116 and HT-29 cells were treated with Dox via US-mediated microbubble destruction. The results showed that high expression of miR-296-5p and treatment with Dox synergistically inhibited the proliferation of CRC cells (Fig. 2F). These findings suggested the potential tumor suppressive function of miR-296-5p in modulating the growth of CRC cells.

HMGA1 is a target of miR-296-5p in CRC cells. To further investigate the functional mechanism of miR-296-5p in CRC, the potential targets of miR-296-5p were predicted using the miRDB and TargetScan databases. Following the search, HMGA1 was identified as a possible target of miR-296-5p. The putative complementary sequence of miR-296-5p at the 3'-UTR of HMGA1 is presented in Fig. 3A. To further confirm this, the luciferase reporter assay was performed by co-transfecting luciferase vectors harboring the wild-type or mutant 3'-UTR of HMGA1, and miR-296-5p mimics or control miRNA, into both HCT116 and HT-29 cells. The overexpression of miR-296-5p significantly decreased the luciferase activity of the wild-type, but not the mutant, 3'-UTR of HMGA1 (Fig. 3B and C). To determine whether the binding of miR-296-5p with the 3'-UTR of HMGA1 affects the mRNA stability of HMGA1, RT-qPCR was performed following the transfection of HCT116 and HT-29 cells with miR-296-5p mimics or control miRNA. The results showed that the overexpression of miR-296-5p significantly decreased the mRNA levels of HMGA1 in CRC cells (Fig. 3D). Consistently, the protein abundance of HMGA1 was also decreased in both HCT116 and HT-29 cells overexpressing miR-296-5p (Fig. 3E). To further validate the suppressive effect of miR-296-5p on the expression of HMGA1, miR-296-5p was downregulated by transfecting CRC cells with miR-296-5p antagomir. The transfection efficiency of miR-29605p was validated via RT-qPCR, with the GFP as the transfection control (Fig. 3F). In accordance, the downregulation of miR-296-5p resulted in significantly increased mRNA and protein levels of HMGA1 (Fig. 3G and H). Overall, these results demonstrated that miR-296-5p targeted HMGA1 and negatively modulated the expression levels of HMGA1 in CRC cells.

Overexpression of HMGA1 attenuates the suppressive role of miR-296-5p on the proliferation of CRC cells. The expression of HMGA1 in CRC tissues and matched adjacent normal tissues was detected via RT-qPCR. The data showed that the mRNA level of HMGA1 was significantly overexpressed in CRC tissues compared with the adjacent normal tissues (Fig. 4A). As HMGA1 was identified as a target of miR-296-5p, the correlation between the expression of miR-296-5p and HMGA1 in CRC tissues was determined by Spearman analysis. As presented in Fig. 4B, a significantly negative correlation was observed between the level of miR-296-5p and HMGA1 in CRCC tissues.

To further confirm the function of HMGA1 in the tumor suppressive role of miR-296-5, the expression of HMGA1 was restored through transfecting Flag-tagged HMGA1 into both HCT116 and HT-29 cells (Fig. 4C). The CCK-8 assay showed that the transfection of HMGA1 significantly reversed the inhibitory effect of miR-296-5p on the proliferation of both HCT116 and HT-29 cells (Fig. 4D and E). Moreover, the overexpression of HMGA1 eliminated the miR-296-5p-induced apoptosis of CRC cells (Fig. 4F). These results indicated the essential role of HMGA1 in mediating the suppressive function of miR-296-5p in CRC.

Discussion

Accumulating evidence suggests the critical roles of miRNAs in the initiation and progression of cancer by modulating the expression of target genes (13-15). The aberrant expression

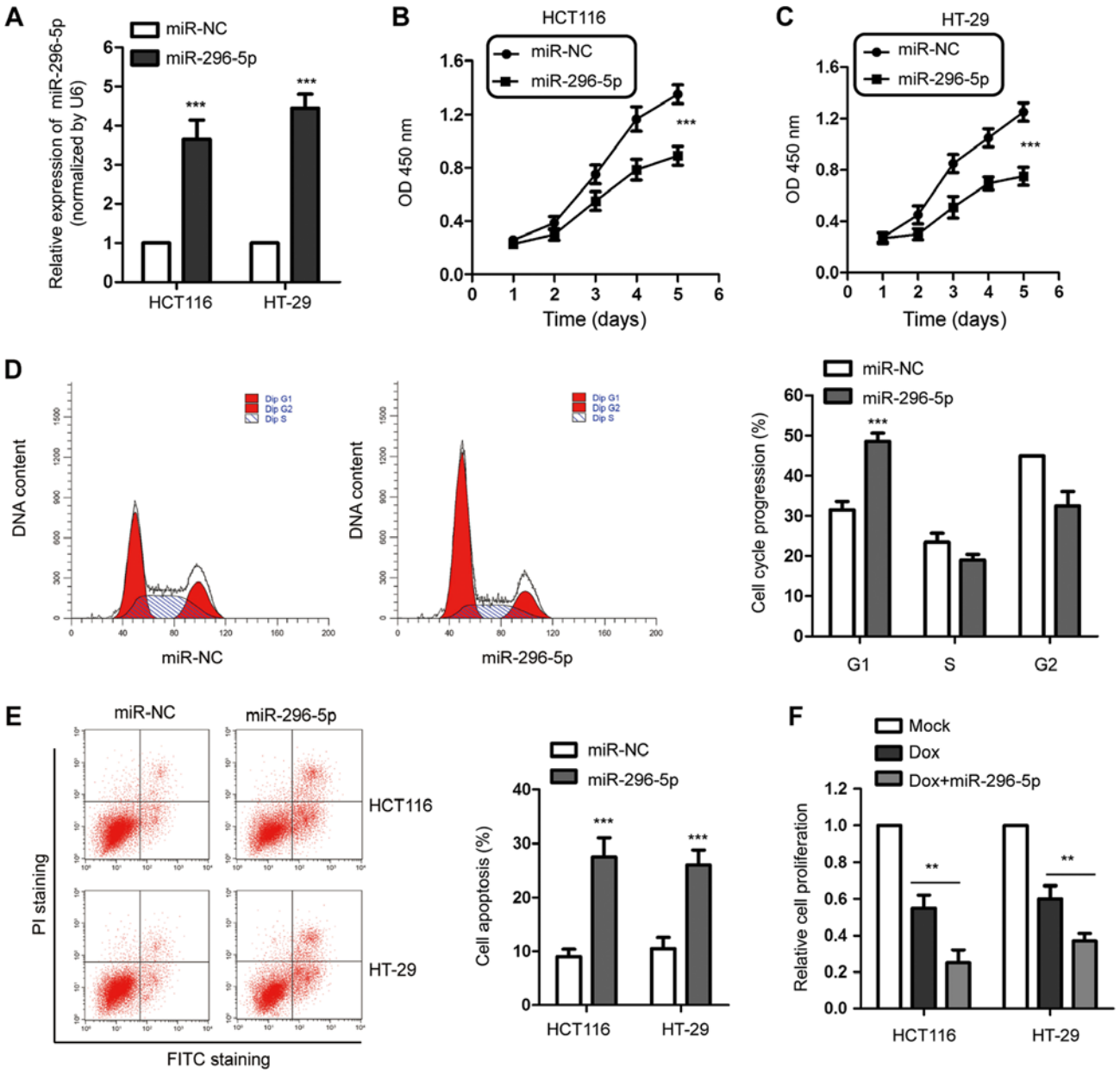

Figure 2. Overexpression of miR-296-5p inhibits the proliferation of CRC cells. (A) Validation of the miR-296-5p expression level after transfection with miR-296-5p mimics or control miRNA for 48 h in both HCT116 and HT-29 cells. ***P<0.001 vs. respective miR-NC group. The proliferation of (B) HCT116 and (C) HT-29 cells transfected with miR-296-5p was significantly decreased compared with those transfected with control miRNA. ***P<0.001 vs. respective miR-NC group. (D) The cell cycle progression of colorectal cancer cells was determined by flow cytometry, following the overexpression of miR-296-5p. ***P<0.001 vs. respective miR-NC group. (E) The apoptosis of HCT116 and HT-29 cells was measured following transfection with miR-296-5p for 48 h. ***P<0.001 vs. respective miR-NC group. (F) The overexpression of miR-296-5p significantly promoted the inhibitory effect of Dox on the growth of HCT116 and HT-29 cells. **P<0.01. miR/miRNA, microRNA; CRC, colorectal cancer; Dox, doxorubicin; NC, negative control; PI, propidium iodide.

of miR-296-5p has been found in a number of different types of cancer in humans, including non-small cell lung cancer, prostate cancer and hepatocellular carcinoma (20,21,26). The downregulation of miR-296-5p is associated with a poor prognosis in patients with cancer (27). In the present study, it was found that the expression of miR-296-5p was significantly decreased in CRC tissues and cell lines, suggesting the potential involvement of miR-296-5p in CRC.

The tumor suppressive function of miR-296-5p has been established in several types of cancer. For example, miR-296-5p is prominently downregulated in hepatocellular carcinoma (HCC) and inhibits the epithelial-to-mesenchymal transition of HCC (26). The decreased expression of miR-296-5p is significantly associated with a favorable prognosis in patients with HCC (26). The inhibitory effect of miR-296-5p on the growth of cancer cells has also been observed in non-small cell lung cancer, by targeting PLK1 (20). Additionally, miR-296-5p plays a tumor-suppressive role in prostate cancer by downregulating peptidyl-prolyl cis-trans isomerase NIMA-interacting 1, indicating its potential application in the prognosis of prostate cancer (21). Studies have also demonstrated the promoting effect of miR-296-5p on the development of cancer (28,29). For example, miR-296-5p enhances the invasiveness of glioblastoma by suppressing the

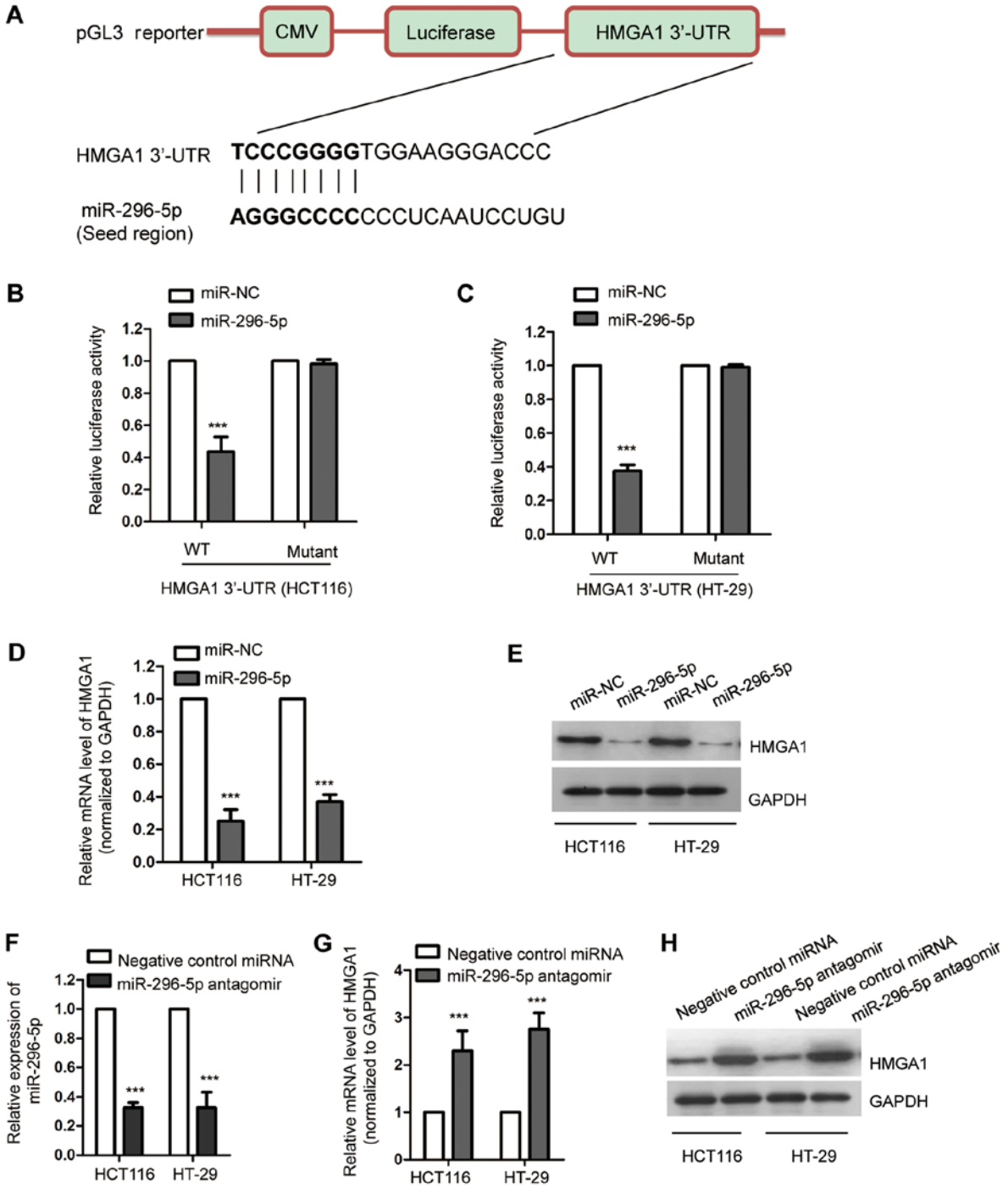

Figure 3. HMGA1 is a target of miR-296-5p in CRC cells. (A) Schematic representation of potential binding sites of miR-296-5p in the 3'-UTR of HMGA1. Dual luciferase activity of CRC cells following transfection with miR-296-5p mimics or control miRNA in (B) HCT116 and (C) HT-29 cells. The (D) mRNA and (E) protein expression of HMGA1 in HCT116 and HT-29 cells, following transfection with miR-296-5p. (F) Transfection with miR-296-5p mimic significantly decreased its expression in CRC cells. The (G) mRNA and (H) protein expression level of HMGA1 in CRC cells, following the overexpression of miR-296-5p by the antagomir. ***P<0.001 vs. respective miR-NC group. HMGA1, high mobility group AT-hook 1; miR/miRNA, microRNA; CRC, colorectal cancer; UTR, untranslated region; NC, negative control; WT, wild-type.

expression of nerve growth factor receptor and caspase-8 (29). Similarly, miR-296-5p enhances the cell proliferation of gastric cancer (28). In the present study, miR-296-5p was downregulated in CRC tissues compared with matched normal tissues. The overexpression of miR-296-5p significantly inhibited the proliferation and induced apoptosis of CRC cells. These results indicated the tumor suppressive function of miR-296-5p in the progression of CRC.

As an important transcription factor, HMGA1 is involved in regulating autophagy and cell invasion, which contributes to cancer progression (30,31). Moreover, HMGA1 was found to enhance tumorigenesis by conferring resistance to chemotherapies, including trabectedin, temozolomide, paclitaxel, doxorubicin and antineoplastic drugs (32-35). These findings suggested the potential oncogenic function of HMGA1 in cancer progression. Notably, HMGA1 has been identified as the target

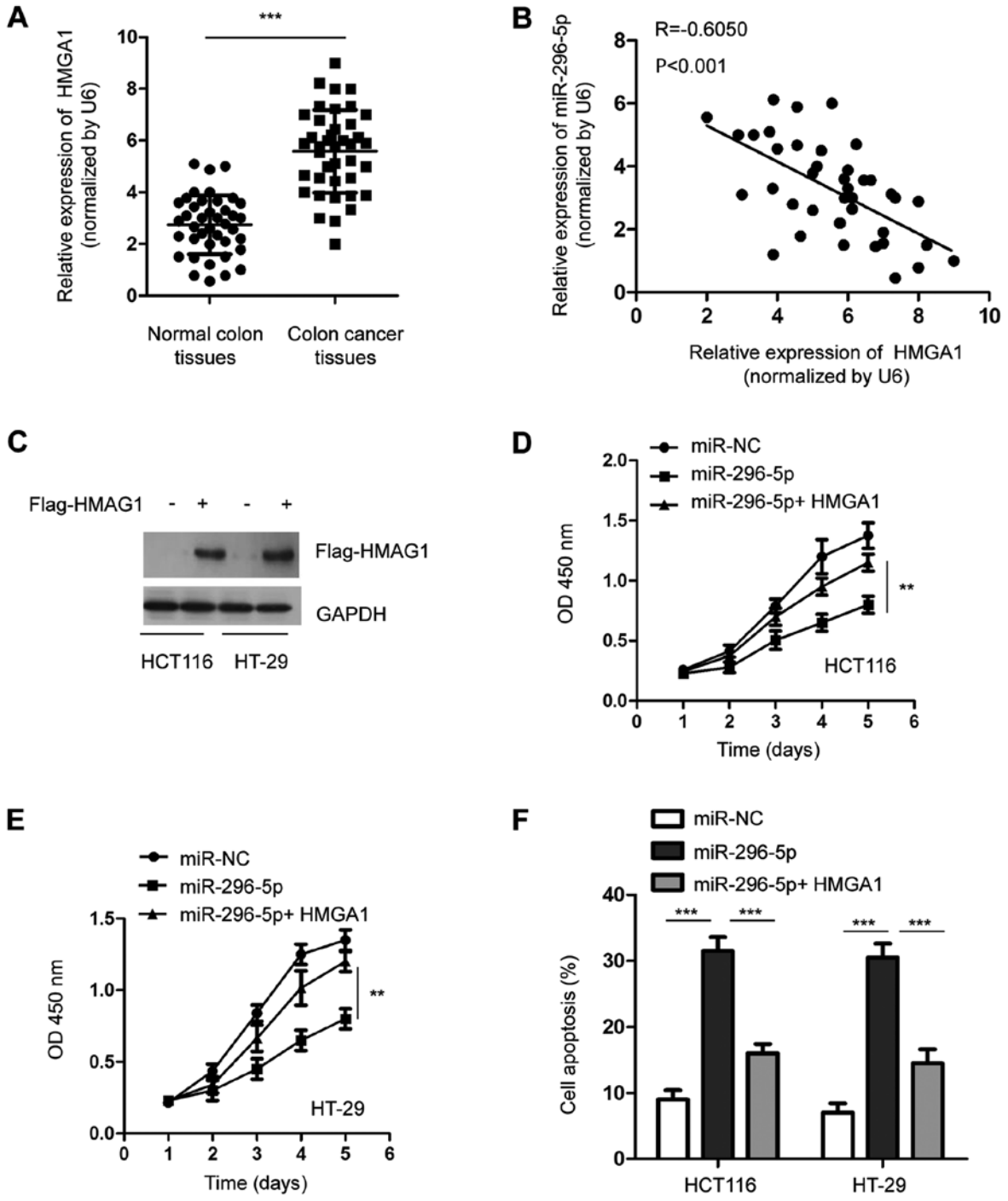

Figure 4. Restoration of HMGA1 reverses the inhibitory effect of miR-296-5p on the growth of CRC cells. (A) The expression level of HMGA1 in paired CRC tissues and adjacent normal tissues was detected by reverse transcription-quantitative PCR. ****P<0.001. (B) The correlation between the expression of miR-296-5p and HMGA1 in CRC tissues was analyzed with the Spearman test. (C) Validation of HMGA1 expression in HCT116 and HT-29 cells transfected with Flag-HMGA1. Overexpression of Flag-HMGA1 significantly reversed the inhibitory role of miR-296-5p in regulating the proliferation of (D) HCT116 and (E) HT-29 cells. **P<0.01. (F) Transfection with Flag-HMGA1 decreased the cell apoptosis induced by miR-296-5p. ***P<0.001. HMGA1, high mobility group AT-hook 1; miR/miRNA, microRNA; CRC, colorectal cancer; NC, negative control.

of several miRNAs, including miR-26a, miR-195, miR-625 and miR-142-3p (36-39). miR-26a targets and decreases the expression of HMGA1 and inhibits the migration of osteosarcoma cells (40). HMGA1 has also been identified as a target of miR-214, which suppresses the proliferation, migration and invasion of cervical and colorectal cancer cells (41). Additionally, decreased expression of miR-195 promotes the progression of prostate cancer cells by targeting HMGA1 (37). In the present study, HMGA1 was predicted as one of the targets of miR-296-5p. miR-296-5p bound the 3'-UTR of HMGA1 and inhibited the expression of HMGA1 in CRC cells. A higher abundance of HMGA1 was observed in CRC tissues, which was inversely correlated with the expression of miR-296-5p. The restoration of HMGA1 reversed the inhibitory effect of miR-296-5p on the proliferation of CRC cells. These results suggest the important role of miR-296-5p/HMGA1 axis in regulating the progression of CRC.

Notably, a recent study showed that miR-296-5p inhibits glioblastoma cell stemness by targeting HMGA1 and Sox2 (42). The overexpression of Sox2 has been found in a variety of cancer types and is associated with increased cancer aggressiveness, resistance to chemoradiation therapy and a decreased survival rate (43). Since HMGA1 was identified to be a target of miR-296-5p, it would be interesting to further detect the effect of miR-296-5p on the expression of Sox2 in CRC. Additionally, HMGA1 has been reported to bind the AT-rich regions of DNA and to regulate the expression of cyclin D/E, which modulates cell cycle progression (44). A recent study demonstrated that HMGA1 activates the STAT3/cyclo-oxygenase 2 pathway and promotes the malignant behaviors of cancer (45). All these findings suggest the possible mechanism of HMGA1 in regulating the survival of CRC and merit further investigation. Recently, a nano/technological platform was designed to evaluate the expression of HMGA1b in the peripheral blood of patients with cancer (46). Thus, the influence of miR-296-5p on the secretion of HMGA1 in CRC cells should be determined in future studies.

In conclusion, the present study demonstrated the tumor suppressive function of miR-296-5p in CRC, at least in part by negatively modulating the expression of HMGA1. These results indicated the potential clinical significance of miR-296-5p in the diagnosis and prognosis of CRC.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

GY, SY and YZ designed the study. GY and SY performed most of the experiments and analyzed the data. SL collected the clinical samples and cell lines. WT constructed the luciferase reporter vector. XY performed the bioinformatics analysis. JJW analyzed the data, finished the revision and proof of the manuscript and provided funding support. YZ wrote the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Zhongshan Hospital, Xiamen University (approval no. 2015032013; Xiamen, China). Consent for participation was obtained from all the patients.

Patient consent for publication

Not applicable.

Competing interests

These authors declare that they have no competing interests.

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