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Overexpression of miR-215-3p sensitizes colorectal cancer to 5-fluorouracil induced apoptosis through regulating CXCR1

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Abstract. – OBJECTIVE: Chemo-resistance of colon cancer remains a major problem in therapy. The role of miR-215-3p in the chemo-sensitivity of colon cancer remains unidentified.

PATIENTS AND METHODS: Here, we constructed a 5-Fluoracil (5-Fu) resistant HCT116 cell line (HCT116/5-Fu) and miR-215-3p expression levels were measured in 56 cases of colon cancer tissues and 23 cases of normal tissues by quantitative real-time polymerase chain reaction (qRT-PCR). The effects of miR-215-3p on colon cancer cell growth and apoptosis were investigated using cell counting kit-8 (CCK-8) and apoptosis assay, respectively. In addition, CXC-chemokine receptor type1 (CXCR1) was identified as a target of miR-215-3p by using luciferase reporter assay.

RESULTS: miR-215-3p was down-expressed in the 5-FU resistant cell compared to the parent cell. The level of miR-215-3p was correlated with the 5-Fu sensibility of colorectal cancer cell and the alteration of miR-215-3p affected the sensibility of colorectal cancer cells toward 5-Fu. Furthermore, miR-215-3p accelerated the apoptosis of colorectal cancer cell which was treated with 5-Fu. Mechanically, miR-215-3p regulated the level of endogenous CXCR1 in HCT116 cell and alternation of CXCR1 affected the 5-Fu sensibility mediated by miR-215-3p. Finally, overexpression of miR-215-3p restrained the growth of HCT116/5-Fu cells in the xenograft model.

CONCLUSIONS: MiR-215-3p improved the 5-Fu sensibility via regulating the expression of CXCR1 in the colorectal cancer cell.

Key Words:
MiR-215-3p, Colorectal cancer, 5-FU, CXCR1.

Abbreviations

GEO = gene expression omnibus; qRT-PCR, quantitative real-time polymerase chain reaction; 5-Fu = 5-Fluoracil; CXCR1 = CXC-chemokine receptor type1; CCK-8 = cell counting kit-8; 3'-UTR = 3'-untranslated region; TFAM = mitochondrial transcription factor A; HMGA2 = high-mobility group AT-hook 2; GPCR = G-protein-coupled receptors; FBS = fetal bovine serum; DMEM = Dulbecco's Modified Eagle's Medium; miR-NC = mimic scrambled control; shRNA, short hairpin RNA; cDNA = complementary DNA; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; WT, wild-type; MUT = mutant type; ECL = enhanced chemiluminescence.

Introduction

Colorectal cancer remains the third most frequent malignant cancer with high morbidity in the world[1]. The 1-year and 5-year survival rates are 83.2% and 64.3%, respectively. For patients with unresectable cancers, systemic chemotherapeutic is the typical first-line therapeutic approach[2]. Despite the strategies of initial diagnosis, surgery, chemotherapy, and adjuvant treatment in colorectal cancer remarkably improve in recent years, the prognosis of patients with advanced colorectal cancer remains poor owing to the chemo-resistance[3]. Therefore, it is vital to elucidate the underlining mechanisms of chemo-resistance and explore more effective therapeutic options of colorectal cancer. Fluorouracil (also known as 5-Fu) is a typical chemotherapy agent that has a profound influence on the survival for the patients with colorectal cancer[4]. But, chemo-resistance of colorectal cancer to 5-Fu is a major problem in the treatment of this malignant neoplasm[5]. MicroRNA (miRNA) is a family belonging to small non-coding RNA, which composes of about 22 nucleotides. MiRNAs function as vital regulators by binding to the 3'-untranslated regions (3'-

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UTR) of their target genes[6]. Scholars[6,7] suggest that aberrantly expressed miRNAs are connected with the proliferation, apoptosis, and neoplastic transplantation of several cancers, including pancreatic cancer, gastric cancer, and hepatocellular carcinoma. Recently, substantive studies demonstrate that several miRNAs regulate cancer cells chemo-sensitivity. In gastric cancer, miR-33b-5p acts a therapeutical target for gastric cancer and functions as tumor-suppressive miRNA through regulating high-mobility group AT-hook 2 (HMGA2)[8]. Moreover, miR-199a-3p enhances breast cancer cells sensitivity to cisplatin by down-regulating mitochondrial transcription factor A (TFAM)[9]. Chen et al[10] demonstrate that miRNA-215 could restrain cell proliferation, migration and invasion of colon cancer by suppressing Yin-Yang 1. On the other hand, the function of miR-215 in colon cancer chemo-resistance is not well explored. Chemokines are a family of small proteins, which are divided into four kinds (C, CC, CXC and CX3C)[11]. CXC chemokines bind to the G-protein-coupled receptors (GPCR) of CXCR1 and CXCR2, which play vital roles in tumor development and metastases[12]. A lot of researches have indicated that chemokine receptor CXCR1/2 acts key parts in the invasion, metastasis, angiogenesis and drug resistance of many cancers, including malignant melanoma, pancreatic carcinoma and ovarian cancer[13,14]. In this work, miR-215-3p was found down-regulated in colon cancer and we identified that miR-215-3p regulated the expression of CXCR1. MiR-215-3p strengthened the chemotherapy sensitivity of colorectal cancer cell toward 5-Fu by inhibiting CXCR1 *in vitro* and *in vivo*. All these findings suggested the function of the miRNA215-3p/CXCR1 axis in regulating chemotherapy resistance in colon cancer

Materials and Methods

Cell Lines and Clinical Samples

Colon cancer cell lines HCT116 and LOVO were maintained in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA). HCT116 and HT-29 colon cancer cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% FBS. The 5-Fu-resistant HCT116 cells (named as HCT116/5-Fu) were cultured with gradually increased concentration solutions of 5-Fu (Sigma-Aldrich, Shanghai, China) at 0.5, 1, 1.5, 2, 3, and 5 μg/ml. After HCT116 cells survived in media contain 5-Fu (5 μg/ml) for three passages, the cells were considered as 5-Fu-resistant HCT116 cell line. Totally 56 cases of colon cancer tissues and 23 cases of normal tissues were collected from the Southeast University Affiliated Zhongda Hospital from 2001 to 2013. This investigation was approved by the Institutional Research Ethics Committee of Southeast University Affiliated Zhongda Hospital. Colon cancer tissues and paired adjacent cancerous tissues are frozen and stored in liquid nitrogen for further analysis.

Cell Transfection

MiR-215-3p mimics, mimic scrambled control (miR-NC), miR-215-5p inhibitor (miR-215-5p^{inhi}), pcDNA3.1(+) CXCR1, short hairpin RNA (shRNA) specifically targeting CXCR1 (shCXCR1), scrambled negative control shRNA (shCon) were chemically synthesized by GenePharma Co., Ltd. (Shanghai, China). Cells in the logarithmic phase were transfected with miRNAs, shRNAs or controls using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Cell Survivability Assay

Cell viability was detected by a Cell Counting kit (CCK-8, Beyotime Institute of Biotechnology, Nanjing, Jiangsu, China). In brief, the transfected cells were seeded into a 96-well plate and the cells were cultured for 24 h, 48 h, and 72 h. Then, 10 μl of CCK-8 solution was added into each well and the absorbance was determined at 450 nm. The 50% inhibition of the growth (IC50) was calculated by SPSS software.

Flow Cytometry Assay

Cells were cultured in 6-well plates and the single cell suspension was prepared. After being washed with PBS, cells in 500 μl of binding buffer solution were stained with fluorescein isothiocyanate (FITC)-conjugated Annexin V and PI (Beyotime Institute of Biotechnology, Nanjing, Jiangsu, China). After incubated for 15 min at room temperature, cells apoptosis were examined with flow cytometry method.

Quantitative Real Time-PCR (qRT-PCR)

Total RNA was abstracted from cells by the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and complementary DNA (cDNA) was synthesized from total RNA using PrimerScript RT Reagent kit (Takara, Tokyo, Japan). MiRNA from

total RNA was reverse transcribed using the Prime-Script miRNA cDNA Synthesis Kit (TaKaRa, Otsu, Shiga, Japan). qRT-PCR was conducted using the SYBR green Premix Ex Taq II (Takara, Otsu, Shiga, Japan) on Applied Biosystems Step One Plus Real Time-PCR System (Applied Biosystems, Carlsbad, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as endogenous control for analysis the expression of mRNA, while U6 was used as endogenous control for analysis the level of miRNA. The primers used for PCR were as follows: GAPDH: 5′-AGGTCGGTGTGAACGGATTTG-3′ and 5′-TGTAGACCATGTAGTTGAGGTCA-3′; U6: 5′-CCCCTGGATCTTATCAGGCTC-3′ and 5′-GCCATCTCCCCGGACAAAG-3′; miR-215-3p: 5′-GCATGACCTATGAATTGACAGAC-3′; CXCR1: 5′-CTGACCCAGAAGCGTCACTTG-3′ and 5′-CCAGGACCTCATAGCAAACTG-3′.

Luciferase Reporter Assay

The wild-type (WT) 3'-UTR CXCR1 or mutant type (MUT) 3'-UTR CXCR1 was inserted into the pmiR-GLO vector. HCT116 cells were co-transfected with miR-215-3p mimic or miR-NC as well as WT 3'-UTR CXCR1 or MUT 3'-UTR CXCR1 using lipofectamine 2000 reagent. Luciferase reactivity was examined by luciferase reporter system (Promega, Madison, WI, USA).

Western Blot

Total proteins were collected using radioimmunoprecipitation assay (RIPA) buffer containing protease and phosphatase inhibitors. 25 μg cell lysates were separated on 8% SDS-PAGE and transferred onto PVDF membranes. Membranes were incubated with antibodies to CXCR1 (1:1000, Cell Signaling Technology, Danvers, MA, USA), Bcl-2 (1:1000, Cell Signaling Technology, Danvers, MA, USA), Bax (1:1000, Cell Signaling Technology, Danvers, MA, USA), or GAPDH (1:1000, Cell Signaling Technology, Danvers, MA, USA) for 24 h. The membranes were washed with TBST and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody. Proteins were assessed by the enhaned chemiluminescence (ECL) system (Millipore, Braunschweig, Germany) and imagined with the ChemiDoc XRS system (Bio-Rad, Hercules, CA, USA).

Tumor Xenograft

HCT116/5-Fu cells that transfected with miR-215-3p or miR-NC were subcutaneously injected into female athymic nude mice. All the mice were then treated with 15 mg/kg 5-Fu by intragastric administration every week. The tumor mass was checked every week and volumes were calculated with the formula: $(L \times W^2) \times 0.5$, where L is the length and W is the width of the tumor. After 35 days, the tumors were collected and tumor weights were measured. The CXCR1, Bcl-2 and Bax were detected by western blotting method. The animal experiment was approved by the Institutional Animal Care and Use Committee of Southeast University affiliated Zhongda hospital.

Statistical Analysis

Statistical analysis was conducted by the SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Multi-group comparisons of the means were carried out by the one-way analysis of variance (ANOVA) test with post-hoc contrasts by the two-tailed Student's *t*-test. Statistical significance was defined a value of $p < 0.05$.

Results

MiR-215-3p is Down-Regulated in 5-Fu Resistant HCT116 Cells

To identify the potential miRNAs that were aberrantly expressed in colon cancer, we compared the expressed pattern of miRNAs between control normal *vs.* colon cancer tissues using GEO dataset GSE101502. The heat map generated using differential miRNAs showed that miR-215-3p was remarkably down-regulated in colon cancer (Figure 1A). To explore the functions of miR-215-3p in 5-Fu chemo-resistance of colorectal cancer cells, we constructed the 5-Fu-resistant HCT-116 cell line (HCT-116/5-Fu). While cells cultured in the normal condition without 5-Fu, the parental and 5-Fu-resistant HCT116 cells exhibited no discrepancy in cell viability (Figure 1B). Nevertheless, after treated with different concentrations of 5-Fu, the HCT116/5-Fu cells showed enhanced viability in comparison with the control HCT116 (Figure 1C), which suggested that HCT116/5-Fu cells had tolerance toward 5-Fu. Utilizing the qRT-PCR assay, we found that miR-215-3p was down-expressed in the HCT116/5-Fu cell as compared to the control cell (Figure 1D). We then analyzed the expression of miR-215-3p in colon cancer tissues and the adjacent normal tissues. As shown in Figure 1E, we found that miR-215-3p was down-regulated in colon cancer samples compared to the adjacent normal samples. Moreover, the lower level of miR-215-3p was closely

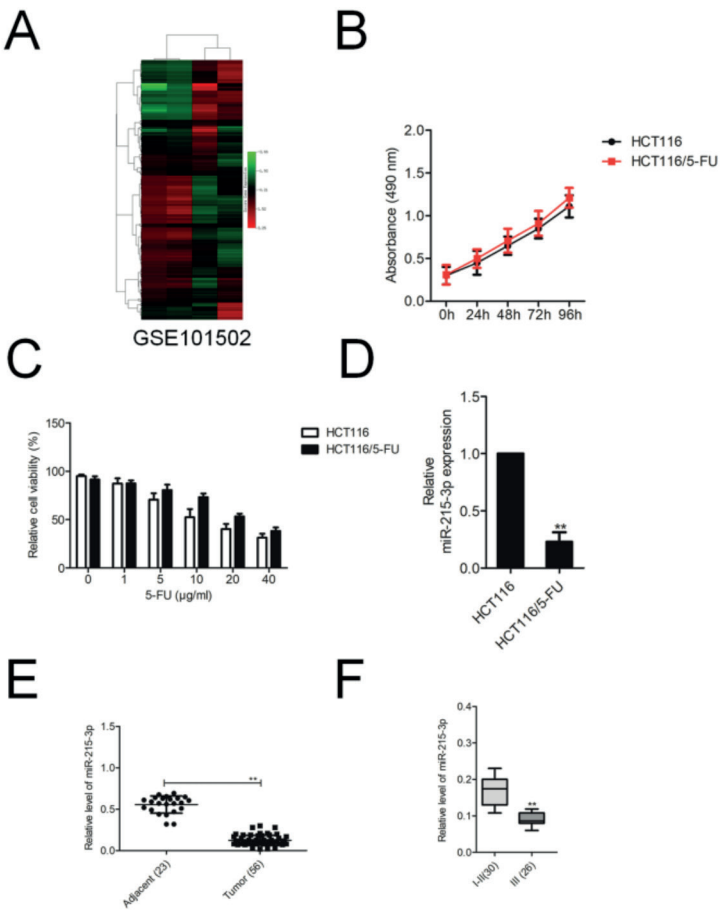

Figure 1. MiR-215-3p is in down-regulation of 5-Fu resistant HCT116/5-Fu colorectal cancer cells. ***A,*** Microarray analysis of miRNA expression in colon cancer tissues and corresponding the normal tissues; ***B,*** Cellular viability of parental HCT116 and HCT116/5-Fu cells treated with 5-FU were determined by CCK-8; ***C,*** HCT116 and HCT116/5-Fu cells were treated with various concentrations of 5-Fu. Cellular viability was assessed by CCK-8; ***D,*** The level of miR-215-3p was detected by qRT-PCR assay. $^{**}p < 0.01$ as compared to HCT116 cells; ***E,*** Relative levels of miR-215-3p in colon cancer tissues and adjacent normal tissues were determined by qRT-PCR; ***F,*** The connection between miR-215-3p and stage I-II or III was shown. $^{**}p < 0.01$ as compared to stage of I-II.

related to the advanced clinical stage (III stage, n=26) (Figure 1F). Totally, all the above data indicated that miR-215-3p was down-expressed in colon cancer.

MiR-215-3p Enhances Chemo-Sensitivity of Colorectal Cancer Cells to 5-Fu

To validate the roles of miR-215-3p in 5-Fu chemo-resistance of colon cancer, we examined the IC50 value and the level of miR-215-3p in a panel of colon cancer cell lines (HCT116, HT-29, LOVO, and SW480) and HCT116/5-Fu. As shown in Figure 2A, HCT116/5-Fu cells had the topmost tolerance ability to 5-Fu, HT-29, and LOVO were the relatively sensitive cell lines. The qRT-PCR experiment suggested that HCT116/5-Fu and SW480 cells had an inferior level of miR-215-3p, and LOVO and HT- 29 cells had a relatively superior level of miR-215-3p (Figure 2B). To furthermore assess the function of miR-215-3p in 5-Fu chemo-sensitivity, colon cancer cells were transfected with miR-215-3p inhibitor (miR-215-3p^{inhi}) or miR-215-3p mimics to decrease or increase the level of miR-215-3p, respectively (Figure 2C). The down-regulation of miR-215-3p increased the tolerance of 5-Fu in HCT116 cells however overexpression of miR-215-3p lessened 5-Fu resistance of HCT116/5-Fu cells (Figure 2D). Furthermore, in SW480 cells which had comparative stronger chemo-resistance, miR-215-3p transfected decreased the resistance ability to 5-Fu (Figure 2E), and the

down-expression of miR-215-3p caused SW480 cells less sensitive to 5-Fu (Figure 2F). All those findings suggested that miR-215-3p decreased chemo-resistance of colorectal cancer cells to 5-Fu.

MiR-215-3p Promotes HCT116 Cell Apoptosis in the Presence of 5-Fu

After that, the mechanism of miR-215-3p which regulated the 5-Fu chemo-resistance of colon cancer cells was investigated. Annexin V-FITC/PI apoptosis detection was used for the apoptosis analysis of colon cancer cells. While cultured without 5-Fu, the apoptosis of the HCT116 and HCT116/5-Fu cells were not changed by miR-215-3p (Figure 3A). On the other hand, in the presence of 5-Fu, the down-expression of miR-215-3p moderated the apoptosis of HCT116 cells, whereas the overexpression of miR-215-3p increased the apoptosis of HCT116/5-Fu cells (Figure 3B-3C). Furthermore, the levels of Bcl-2 and Bax in those cells were determined. The expression of Bax

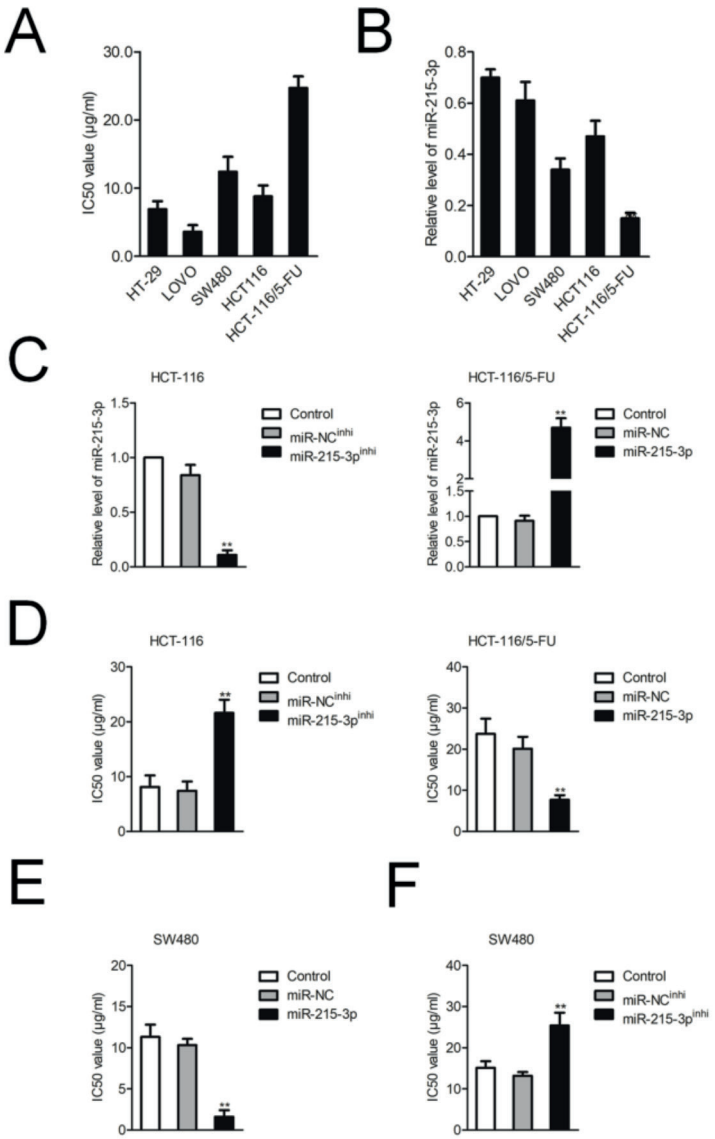

Figure 2. MiR-215-3p increases the chemo-resistance of colorectal cancer cells to 5-Fu. ***A,*** Cytotoxicity assay of 5-Fu was carried out in a panel of colorectal cancer cells by CCK-8 method and the IC50 figures were computed; ***B,*** The level of miR-215-3p in these cell lines were determined by qRT-PCR; ***C,*** The level of miR-215-3p was determined by qRT-PCR in indicated cells; ***D,*** HCT116/ 5-Fu cells were transfected with miR-215-3p and HCT116 cells were transfected with miR-215-3p^{inhi}, and cytotoxicity examination was achieved; ***E-F,*** miR-215-3p was improved or suppressed in SW480 cells, and cytotoxicity was analysis. $^{**}p < 0.01$ as compared to control.

was inhibited by miR-215-3p^{inhi} or was increased in HCT116/5-Fu cells transfected with miR-215-3p mimics whereas the level of Bcl-2 was raised in miR-215-3p^{inhi} transfected HCT116 cells and was inhibited after HCT116/5-Fu cells transfected with miR-215-3p mimic (Figure 3D).

MiR-215-3p Directly Binds to CXCR1 3'-UTR and Negatively Regulates CXCR1 Expression

Using the online analysis tool TargetScan (http://www.targetscan.org/vert_71/), we found that CXCR1 is a possible target of miR-215-3p, since the 3'-UTR of CXCR1 contains miR-215-3p binding sites. To prove whether CXCR1 was the target gene of miR-215-3p, CXCR1 3'-UTR-WT, and CXCR1 3'-UTR-MUT luciferase reporter plasmids were constructed (Figure 4A). After that, the luciferase reactivity experiments with HCT116 cells suggested that miR-215-3p mimic transfection restrained the luciferase activity; however, the inhibition of miR-215-3p increased the luciferase activity (Figure 4B). These influence relayed on miR-215-3p contained the binding sites within the WT 3'-UTR of CXCR1, since the inhibitory effect of miR-215-3p in the lucif-

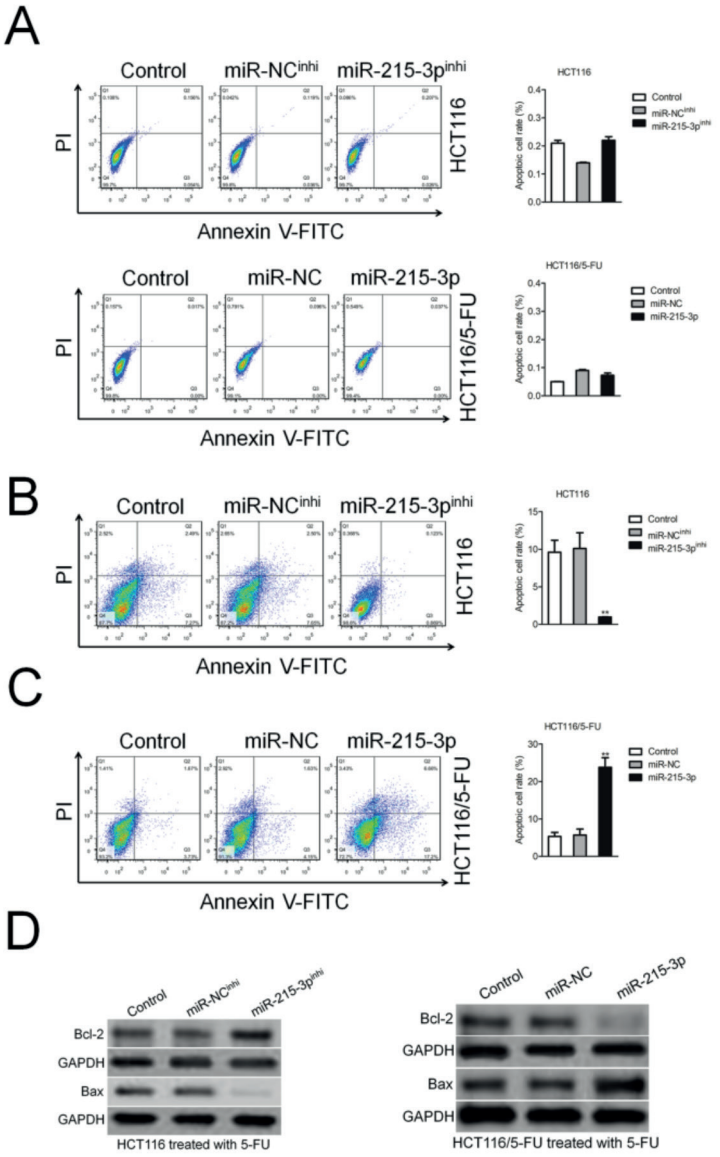

Figure 3. MiR-215-3p promotes apoptosis of colorectal cancer cells in the presence of 5-Fu. ***A,*** HCT116 cells transfected with miR-215-3p or miR-215-3p^{inhi}, and cell apoptosis was determined by Annexin V-FITC/PI apoptosis experiment; ***B-C,*** HCT116 or HCT116/5-Fu cells were treated with 0.5 μg/mL of 5-Fu, and cell apoptosis was examined; **D,** The expressions of Bcl-2 and Bax in cells were determined by Western blot. $^{**}p < 0.01$ as compared to control.

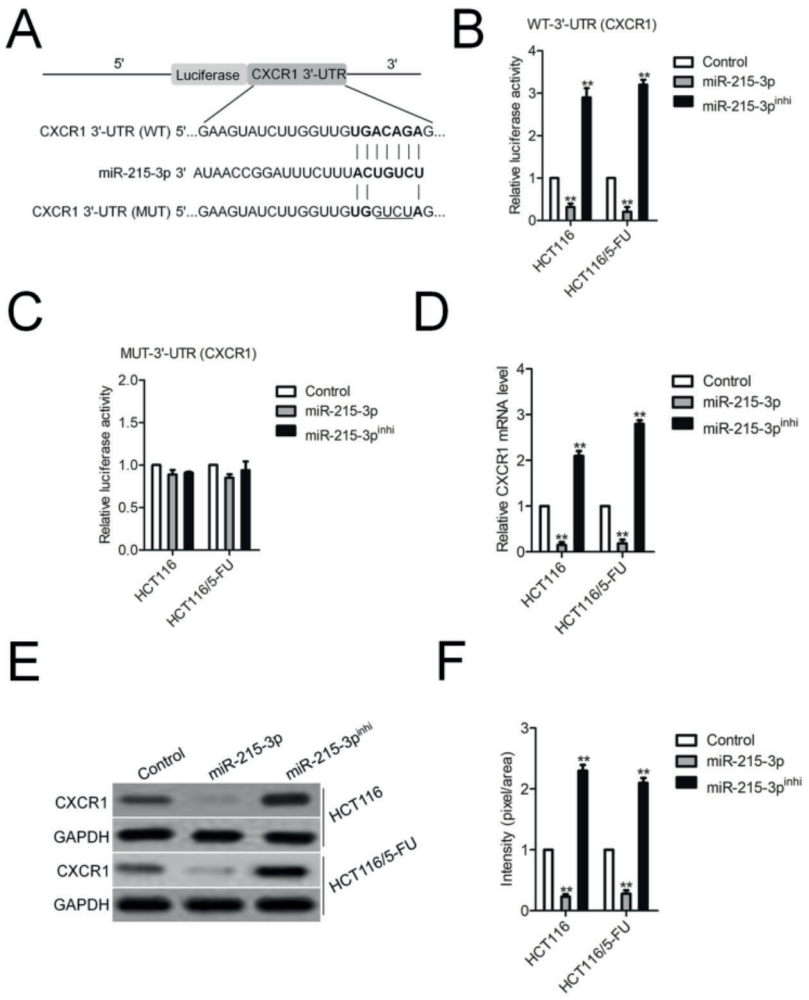

Figure 4. CXCR1 is a direct target of miR-215-3p. ***A,*** The 3'-UTR of wild-type (WT) CXCR1 mRNA contained the putative miR-3120-5p binding sites; ***B,*** Parental HCT116 cells or HCT116/5-FU cells were co-transfected with miR-215-3p or miR-215-3p^{inhi} and pmiRGLO-CXCR1-3'-UTR-WT luciferase vector. The luciferase reactivity was determined; ***C,*** Parental cells or HCT116/5-FU cells were co-transfected with miR-215-3p or miR-215-3p^{inhi} and pmiRGLO-CXCR1-3'-UTR-MUT luciferase vector. The luciferase reactivity was examined; ***D-F,*** CXCR1 was restrained in cells that transfected with miRNA-215-3p or was promoted in cells that transfected with miR-215-3p^{inhi}, as demonstrated by qRT-PCR and Western blot, respectively. $^{**}p <$ 0.01 as compared to control.

erase activities was abolished once the binding sites were altered (Figure 4C). Furthermore, the endogenous expression of CXCR1 in HCT116 or HCT116/5-Fu cells was controlled by miR-215-3p (Figure 4D-4F). Hence, CXCR1 was the target of miR-215-3p in HCT116 or HCT116/5-Fu cells.

MiR-215-3p-3p Affects Chemo-Resistance of HCT116 Cells Through Regulating CXCR1

Based on the above investigations, we presumed that the role of miR-215-3p in HCT116 cell chemo-resistance was possible via targeting CXCR1. To confirm the assumption, HCT116 cell was transfected with a CXCR1 ectopic expression vector to increase CXCR1 or shRNA targeting CXCR1 (shCXCR1) to inhibit CXCR1 (Figure 5A-5B). As shown in Figure 5C, inhibition of miR-215-3p strengthened 5-Fu resistance and the subsequent suppression of CXCR1 recovered the chemo-sensitivity. At the same time, the overexpression of CXCR1 neutralized the inhibited 5-Fu chemo-resistance which was mediated by miR-215-3p transfection in HCT116/5-Fu cells (Figure 5D). All the data indicated that the effect of 5-Fu chemo-resistance by miR-215-3p was via regulating CXCR1.

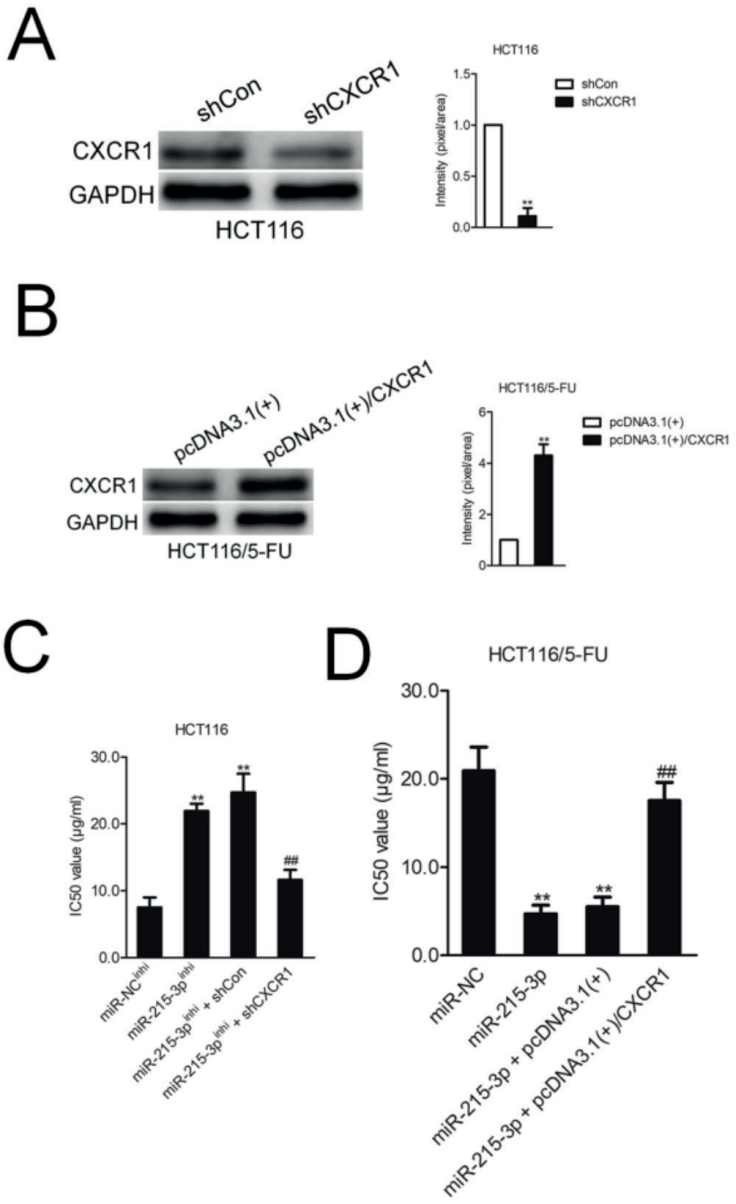

Figure 5. MiR-215-3p influences 5-Fu chemo-sensitivity of colorectal cancer cells through targeting CXCR1. ***A,*** pcDNA3.1(+)/CXCR1 was transfected into HCT116 cells and the expression was determined by Western blotting analysis; ***B,*** Small hairpin RNA for specific knockdown of CXCR1 (shCXCR1) was transfected into HCT116/5-Fu cells. CXCR1 was determined by Western blot; ***C,*** HCT116 cells were transfected with miR-215-3p and shCXCR4. After this, the cytotoxicity experiment was conducted and IC50 were shown; ***D,*** HCT116/5-Fu cells were transfected with miR-215-3p mimics and CXCR1 overexpressing vector. The cytotoxicity examination was conducted and IC50 were shown. $^{**}p < 0.01$ as compared to control. $^{\#\#}p < 0.01$ as compared to R-215-3p.

MiR-215-3p-3p Inhibits HCT116 Cells Derived Xenograft Tumor Growth in the Presence of 5-Fu

The *in vitro* examinations showed the overexpression of miR-215-3p alleviated the 5-Fu resistance in colorectal cancer cells. Subsequently, we investigated the effect of miR-215-3p on colon cancer cells growth in the xenograft model. HCT116/5-Fu cells that transfected with miR-215-3p or miR-NC were injected subcutaneously into the nude mice. As shown in Figure 6A, miR-215-3p restrained the xenograft tumor growth after the mice were treated with 5-Fu. However, in the mice that treated with saline, there was no

discrepancy of tumor volume between miR-215-3p transfected HCT116/5-Fu cells and miR-NC transfected HCT116/5-Fu cells (Figure 6B). Furthermore, the expression of Bcl-2 was down-expressed in miR-215-3p transfected HCT116/5-Fu cells whereas the level of Bax was up-regulated in the presence of 5-Fu (Figure 6C-6D). In brief, these findings revealed that miR-215-3p inhibited the growth of colorectal cancer cells *in vivo* and strengthened the chemo-sensitivity of colorectal cancer cells to 5-Fu.

Discussion

As common cancer, colorectal cancer results in big numbers of cancer-related deaths annually. Chemo-resistance even now remains as one of the major limitations in the treatment of colorectal cancer. Increasing studies reveal that miRNAs participate into the regulation of chemo-sensitivity in cancer cells[15]; however, the underlying molecular mechanism of specific miRNA in colon cancer chemo-resistance remains largely unknown. In this study, we demonstrated that miR-215-3p was down-expressed in colon cancer. Overexpression of miR-215-3p in colorectal cancer cell inhibited the growth and induced cellular apoptosis *in vitro* and *in vivo*. Mechanistically, it was proved that CXCR1 acted as the target of miR-215-3p and miR-215-3p negatively regulated the expression of CXCR1 in the colorectal cancer cell. Overexpression or knock-down of CXCR1 altered the effects of miR-215-3p in the sensitivity of 5-Fu. Previous studies demonstrate that miRNAs behave as either oncogenic miRNAs or cancer suppressor miRNAs relaying on their targets in the malignant cancer[16]. Recently, miRNAs have been investigated for associations with chemo-sensitivity in cancer[17]. For example, up-regulation of miR-21 inhibits cell growth and invasion via the JNK-1/c-Jun signaling pathway. Furthermore, miR-136 induces chemo-resistance in epithelial ovarian cancer (EOC) partly by down-regulating apoptosis and enhancing the repair of cisplatin-induced DNA damage. MiR-215, which is a transcript of chromosome 11q13.1, has been demonstrated to be down-regulated in pancreatic cancer, esophageal adenocarcinoma and renal cell carcinoma[18]. Moreover, the previous study[19] proves that miR-

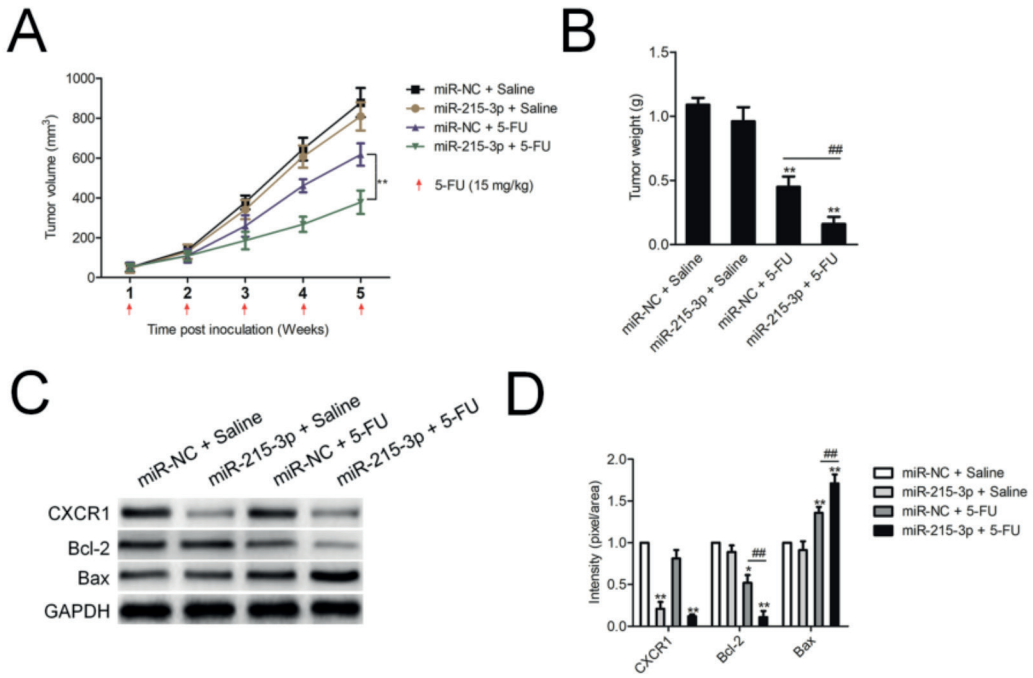

Figure 6. miR-215-3p suppresses colorectal cancer cells growth *in vivo*. ***A***, miR-215-3p transfected HCT116/5-Fu cells were injected into nude mice. After the tumor volume reached 100 mm^3, two group mice were treated with 15 mg/kg 5-Fu by the intragastric administration. The tumor mass was checked every week. $^{**}p < 0.01$ as compared to miR-NC + 5-FU; ***B***, On the 35 days, tumor mass was weighted; ***C-D***, The protein of the tumor mass was collected and the levels of CXCR1, Bcl-2 and Bax were determined by Western blot assay. $^{**}p < 0.01$ as compared to miR-NC + Saline. $^{\#\#}p < 0.01$ as compared to miR-NC + 5-FU.

215 is remarkably decreased in stage II colon cancer compared to the corresponding normal tissues and miR-215 inhibits colon cancer cell growth and aggressiveness. However, the precise roles of miR-125 in the chemo-resistance of colon cancer and the underlying molecular mechanisms still remain to be investigated.

In the current research, we demonstrated that miR-215-3p could sensitize the colorectal cancer cells to 5-Fu via controlling CXCR1. Firstly, the level of miR-215-3p was related to 5-Fu chemo-sensitivity of colorectal cancer cells. Increased or reduced the level of miR-215-3p in colorectal cancer cells promoted or attenuated cell sensitivity to 5- Fu, respectively. Secondly, miR-215-3p bound to 3'-UTR of CXCR1 and negatively regulated the expression of CXCR1 in both HCT116 and HCT116/5-Fu cells. Thirdly, miR-215-3p mediated chemo-sensitivity regulation relayed on its regulation on CXCR1 expression since overexpression or knock-down of CXCR1 reversed 5-Fu sensitivity mediated by miR-215-3p alteration. The mechanism of miR-215-3p induced 5-Fu chemo-sensitivity was, in part, owing to its apoptosis promotion reactivity. Altogether, these data demonstrated the role of miR-215-3p/CXCR1 in the chemo-sensitivity of colon cancer cells. CXCR1 as a cell surface receptor of interleukin-8 is shown to be up-regulated and associated with the progression of several cancers[14]. In gastric cancer, CXCR1 enhances the malignant activity of gastric tumor cells via regulating Protein kinase B (AKT) and extracellular regulated protein kinase 1/2 (ERK1/2) phosphorylation *in vitro* and *in vivo*[20]. Additionally, CXCR1 knock-down in osteosarcoma cells improves the sensitivity to chemotherapy partly via interleukin 8 (IL-8)/CXCR1/AKT signaling pathway[21]. In pancreatic cancer, the IL-8/CXCR1 axis is associated with the cancer stem cell (CSC)-like properties of pancreatic cancer cells and the prognosis in patients with pancreatic cancer[22]. These investigatons indicate that CXCR1 might play important roles during cancer procession. Our results indicated that miR-215-3p was significantly related to 5-FU response in colon cancer and also exerted its important functions dependent on CXCR1.

Conclusions

We demonstrated that the tumor suppressor role of miR-215-3p in colorectal cancer. MiR-215-3p sensitized colorectal cancer cells to 5-Fu, and this reactivity partly through suppression of its target, CXCR1. Nevertheless, because of the intricacy of miRNA regulation network in cancer, the single regulation pathway controlled by miR-215-3p is not adequate to influence the chemo-resistance independently. It is expected that more pathways associated with miR-215-3p could be explored in the chemo-resistance of colorectal cancer.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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