Original Article
miR-143 regulates proliferation and apoptosis of colorectal cancer cells and exhibits altered expression in colorectal cancer tissue

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Abstract: Colorectal cancer is one of the most commonly diagnosed cancers and a leading cause of death. Studies have shown that abnormal expression of microRNAs, small non-coding RNA molecules that regulate gene expression, is linked to the occurrence of cancer. This study sought to determine the role of microRNA-143 (miR-143) in colorectal cancer. Reduced levels of miR-143 expression were detected in colorectal cancer tissues compared to normal adjacent tissue. Transfection of artificially synthesized miR-143 mimics into SW-480 cells, a colorectal cancer cell line, resulted in increased levels of cell proliferation and apoptosis. Further, cells transfected with miR-143 mimics showed a reduction in the proportion of cells in S phase and an increase in the proportion of cells in G1 phase. The altered expression levels of miR-143 in colorectal cancer and its ability to affect the behavior of colorectal cancer cells suggest miR-143 could be used as a new target for the diagnosis and treatment of colorectal cancer.

Keywords: miR-143, colorectal cancer, cell proliferation, apoptosis

Introduction

Colorectal cancer is one of most frequently diagnosed malignant tumor types in China, ranking 4th in urban China and 5th in rural China [1]. The overall incidence of colorectal cancer in China has risen markedly in recent years with the continuous improvement in the standard of living and corresponding changes in eating habits [1]. According to one survey, the number of cases of colorectal cancer in China increased by 120,000 between 2000 and 2005, with an overall increase of 19.1% in males and 17.7% in females [2]. In Shanghai, for example, the incidence of colorectal cancer rose by approximately 4.2%, from being the 7th most common cancer to the 3rd most common and approaching the rate seen in Western developed countries [2]. Therefore, it is of critical importance to discover new targets for the diagnosis and treatment of colorectal cancer.

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression by inhibiting the mRNA expression of a target gene or by blocking protein translation at the post-transcriptional level. By regulating gene expression, miRNAs help to control multiple physiological and pathological processes, including cell differentiation, proliferation, apoptosis, migration, and invasion [3]. Calin et al. first reported the close correlation between the abnormal expression of miRNAs and the occurrence and development of cancer [4]. Indeed, abnormal expression of miRNAs is found in many types of cancer, including pancreatic cancer [5], esophageal cancer [6], bladder cancer [7], cervical fibroids [8], and non-small cell lung cancer [9]. This underscores a role for abnormal miRNA expression in cancer etiology.

In this study, we examined the role of microRNA-143 (miR-143), a miRNA found on human chromosome 5 that is expressed at low levels in multiple cancer tissues and serves as a prognostic marker for cancer [10]. Target genes of miR-143, including COX-2 [11], MMP-13 [12],
and GLI3 [13], have also been identified in multiple cancers and act as tumor suppressor genes. We detected miR-143 at lower levels in colorectal cancer samples than in normal tissue. Additionally, when exogenous miR-143 mimics-synthetic double-stranded RNAs that mimic endogenous miRNAs-were transiently transfected to SW-480 colorectal cancer cells, cell proliferation, cell cycle, and apoptosis were all affected. This suggests a role for miR-143 in regulating the biological behavior of colorectal cancer cells.

Materials and methods

Synthesis of miR-143 mimics

The precursor sequence of miR-143 was obtained from miRBase (www.mirbase.org), and a negative control was made by scrambling this sequence. miR-143 mimic and the negative control were synthesized by GenePharma (Shanghai, China). Sequences are as follows:miR-143: 5′-GCCAGCGCCCTGTCTCCCAGCCT-3′; control: 5′-UUCUCCGA ACGUGUCACGUTT-3′.

Cell culture and transfection

SW-480 human colorectal cancer cells (Institute of Biochemistry and Cell Biology, Shanghai, China) were seeded in a 6-well culture plate (2 mL each well), grown in culture medium containing RPMI medium (Gibco, Grand Island, NY, USA) and 15% fetal bovine serum (Gibco), and placed in a 5% CO₂ incubator (311 Direct Heat model; Thermo Scientific, Waltham, MA, USA) at 37°C. Once the cells reached 50-70% confluence, they were transfected with miR-143 mimics (100 nmol/well) using Lipofectamine 2000 (Invitrogen, Grand Island, NY, USA) according to the manufacturer’s instructions. As a control, Lipofectamine 2000 was not added to one of the wells. The transfected cells were incubated at 37°C for 48 hours and collected for experimental analysis.

Real-time qRT-PCR

Cancerous colorectal tissue was collected from 16 patients and normal tissue was isolated from regions adjacent to cancerous tissue in 9 patients. Total RNA was extracted from the tissue or from transfected SW-480 cells using TRIzol (Invitrogen), according to the manufacturer’s protocol. The total RNA was reverse transcribed to cDNA using QIAGEN OneStep RT-PCR Kit (Code No. 210212) according to the manufacturer’s instructions (QIAGEN, Hilden, Germany). GAPDH was used as an internal control. Primers were purchased from Applied Biosciences (Foster City, CA, USA) and the forward (F) and reverse (R) primer sequences used were as follows: *miR-143*: F: 5′-ACACTCCAGCTGGGTGAGATGAAGCACTGTAG-3′; R: 5′-CTCAACTGGTGTCGATAGCATCTGC-3′; GAPDH: F: 5′-GGAACTTGGTAGTGCAGGC-3′; R: 5′-GAAGATGGGTGATGGGATTTC-3′.

The cDNA amplification conditions were as follows: pre-denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. Each sample was run in triplicate. Ct values were calculated for each well, and mean Ct values were calculated for each group. The relative expression levels of miR-431 in each sample were calculated by the 2^-ΔΔCT method after normalization to GAPDH.

MTT proliferation assay

An MTT proliferation assay was used to determine cell growth rates. SW-480 cells were diluted in culture medium to produce a single cell suspension and seeded into a 96-well plate (1000-10000 cells/well). When the cell density in the wells reached approximately 50%, the cells were transfected with either the scrambled negative control or miR-143 mimics. Each transfection was run in triplicate. After transfection, 20 µL (5 mg/mL) of MTT (Promega, Madison, WI, USA) were added to each well at 0, 24, 48, 72, and 96 h. After 4 h of continuous culture with MTT, 150 µL of dimethyl sulfoxide (DMSO) were added, and the plate was shaken for 10 min to fully dissolve the DMSO crystals. A microplate reader (Thermo Scientific) was used to measure the absorbance at 490 nm.

Flow cytometric analysis of cell cycle

After transfection of the SW-480 cells with miR-143 mimics or the negative control as described above, the cells were incubated for 48 h and then digested with 2.5 g/L pancreatin (Gibco) and made into a single cell suspension. The cells were washed 3 times with 1× PBS, the supernatant was discarded, and 1 mL of 1× PBS was added to resuspend the cells. The cells were then fixed by adding 2 mL of dehydrated alcohol, shaking the plate to mix thoroughly, sealing the plate with a sealing film, and
incubating overnight at 4°C. Before detection, the fixed cells were centrifuged at 800 r/min for 5 min, washed 3 times with 1× PBS, and 100 µL of 1× PBS were added to resuspend the cells. The cells were then treated with 0.1 g/L RNase and 5 g/L propidium iodide (BD Biosciences, San Jose, CA, USA), incubated at 37°C for 30 min, and filtered through a 300-mesh nylon net. The DNA content was measured at a wavelength of 488 nm and the experiment was repeated 3 times.

FACS analysis

SW-480 cells transfected with miR-143 mimics or the negative control were incubated for 48 h, digested with 2.5 g/L pancreatin, and made into a single cell suspension using culture medium containing 100 mL/L Fetal Bovine Serum (FBS). The cell suspension was centrifuged at 1000 r/min for 5 min, washed once with an incubation buffer (10 mmol/L HEPES/NaOH, pH 7.4, 140 mmol/L NaCl, 5 mmol/L CaCl₂), and centrifuged again at 1000 r/min for 5 min. The cells were resuspended with 100 µL of Annexin V and incubated in the dark at room temperature for 15 min. The cells were then centrifuged at 1000 r/min for 5 min, washed once with the incubation buffer, resuspended by adding 100 µL of propidium iodide, and incubated in the dark at 4°C for 20 min with intermittent shaking to mix the solution. A flow cytometer (BD FAC-S Calibur) was used an excitation wavelength set at 488 nm and emission detection wavelengths of 515 nm and over 560 nm. The experiment was repeated 3 times, and the final values were obtained with the averaged these repeated experiments.

Statistical analysis

SPSS 17.0 statistical software (IBM, Armonk, NY, USA) was used for statistical analysis, and the data were expressed as mean ± standard deviation ($\overline{x} \pm s$). A two-tailed, independent-samples t test was used to compare the differ-
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Figure 5. Apoptosis levels are higher in SW-480 cells transfected with miR-143 mimics. Note: Graph of the percentage of cells ($X \pm s$) undergoing apoptosis in SW-480 cells transfected with miR-143 mimics or the scrambled negative control. $^p < 0.05$, vs the numbers of apoptotic cells of the scrambled negative control-transfected cells by using t test.

ences among various groups, where $\alpha = 0.05$ denoted a significance level and $P < 0.05$ was considered statistically significant.

Results

Expression of miR-143 is reduced in colorectal cancer tissues

qRT-PCR was used to detect the expression of miR-143 in colorectal cancer tissues and normal tissues from regions adjacent (control) to cancerous colorectal tissue. The expression level of miR-143 in colorectal cancer tissue was significantly lower than the levels found in normal tissue ($P < 0.05$; Figure 1).

Expression of miR-143 in SW-480 cells transfected with a miR-143 mimic

To detect the expression level of miR-143 in SW-480 transfected cells, qRT-PCR was performed 48 h after transfection with miR-143 mimics or a negative control. The expression level in the miR-143 mimic-transfected group was significantly lower than in the group transfected with a scrambled negative control ($P < 0.05$; Figure 2).

Exogenous miR-143 increases proliferation of SW-480 cells

The amount of cell proliferation was measured at 24-h intervals in miR-143 mimic-transfected and negative control-transfected groups. At 0 and 24 h after transfection, there were no significant differences in proliferation between the two groups. However, 48 h after transfection, the proliferative capacity of miR-143 mimic-transfected group was significantly stronger than the scrambled negative control-transfected group ($P < 0.05$; Figure 3). The increase in proliferation in miR-143-transfected cells compared to the negative control was also observed at the 72-h and 96-h time points (Figure 3).

Cell cycle of SW-480 cells is altered by miR-143 mimic transfection

To examine the distribution of cells through the cell cycle, flow cytometry was performed on SW-480 cells transfected with either miR-143 mimics or a negative control. Compared with cells transfected with the negative control, the miR-143 mimic-transfected group underwent a statistically significant decrease in the proportion of cells in S phase and an increase in the proportion in G1 phase ($P < 0.05$; Figure 4).

Apoptosis is increased in miR-143 mimic-transfected SW-480 cells

FACS analysis of transfected SW-480 cells showed that the number of apoptotic cells was markedly higher in the miR-143 mimic-transfected group compared to the sequence-transfected group ($P < 0.05$; Figure 5).

Discussion

Malignant tumors are one of main causes of human mortality. With the development of molecular biology and human genomics, awareness of tumors and their health risk has reached a heightened level. Studies have shown that the occurrence and development of malignant tumors is accompanied by abnormal miRNA expression [14, 15]. Similarly, we have found that the expression of miR-143 in colorectal cancer tissues was reduced compared to expression levels in normal tissues. In SW-480 cells transfected with miR-143 mimics, cell proliferation was enhanced and the rate of apoptosis was increased compared to controls. Further, flow cytometry revealed a marked decrease in S phase and increase in G1 phase in miR-143 mimic-transfected cells compared to controls. This study has provided an experimental and theoretical basis for miR-143 as a new target for diagnosis and treatment of colorectal cancer. However, further studies are still required to investigate why miR-143 expression is reduced in colorectal cancer and wheth-
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It regulates the biological behavior of colorectal cancer by inhibiting downstream target genes.

Disclosure of conflict of interest

None.

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References