MIR-873-5P REGULATES THE PROLIFERATION, MIGRATION AND INVASION OF COLORECTAL CANCER CELLS BY TARGETING ZEB1 TO REGULATE EMT

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ABSTRACT

Objective: *To explore the mechanism of mir-873-5p for regulating the proliferation, migration and invasion of colorectal cancer cells.*

Methods: *The colorectal cancer tissue and adjacent normal tissue of 36 patients with colorectal cancer were randomly selected. The expression levels of miR-873-5p and ZEB1 in colorectal cancer tissue and adjacent normal tissue were determined. Methods: human colorectal cancer cell line SW480 was cultured and transfected. The expression levels of mir-873-5p and ZEB1 in the mir-873-5p low-expression group and the control group were determined. The cells were divided into a control group, a mir-873-5p low-expression group and a mir-873-5p + ZEB1 low-expression group, with five wells in each group. Cell proliferation, invasion and migration, and the expression levels of vimentin, ZO-1, E-cadherin, β - Catenin, N-cadherin and ZEB1 were measured.*

Results: *Compared with adjacent normal tissue, the expression of mir-873-5p was significantly lower and ZEB1 was significantly higher in colorectal cancer tissues (P<0.01). Compared with the control group, the mir-873-5p low-expression group was significantly lower and the ZEB1 expression level was significantly higher (P<0.01). Compared with the control group, the expression level of ZEB1 in the ZEB1 low-expression group was significantly lower (P<0.05). Compared with the control group, the mir-873-5p low-expression group significantly enhanced cell proliferation, colony formation, invasion and migration, and significantly increased the expression levels of vimentin, N-cadherin and ZEB1, and significantly decreased the expression levels of ZO-1, E-cadherin and β - Catenin (P<0.05); compared with the mir-873-5p low-expression group, the mir-873-5p + ZEB1 low-expression group significantly increased cell proliferation and colony formation. The expression levels of vimentin, N-cadherin and ZEB1 were significantly decreased, and the expression levels of ZO-1, E-cadherin and β - Catenin were significantly increased (P<0.05).*

Conclusion: *Inhibiting the expression of mir-873-5p can significantly inhibit the proliferation, invasion and migration of colorectal cancer cells and regulate the progress of EMT, which may be achieved by targeting ZEB1.*

Keywords: *Mir-873-5p, ZEB1, EMT, colorectal cancer, cell biological behaviour, mechanism.*

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Introduction

Cancer is a biologically heterogeneous disease with obvious genetic abnormalities, and it seriously impacts people's quality of life and health. Colorectal cancer is one of the most common malignant tumours of the digestive tract in the world, and its morbidity and mortality are both high[(1)]. Presently, clinical treatment for colorectal cancer usually includes surgical resection and chemotherapy according to the patient's condition after the operation, which sometimes achieves beneficial results. However, because most patients in the intermediate and advanced stages of colorectal cancer have undergone distant metastasis, their treatment effect is poor, and the death rate is still high[(2)]. Therefore, early diagnosis and timely treatment play an important role in reducing the mortality of patients. The pathogenesis of colorectal cancer is currently unclear, and it is believed that it may be regulated by a variety of factors and genes. MicroRNAs (miRNAs) are a type of single-stranded small-molecule RNA that can induce the degradation of the target gene mRAN and inhibit the process of transcription of the target gene

into protein[3]. Studies have found that the expression of miRNA is an important regulatory factor for the occurrence and development of colorectal cancer; miR-200c, miR-193a-3p, miR-338-5p, etc. are all involved in the development of colorectal cancer[4].

However, the relevant role of miR-873-5p in colorectal cancer is still unclear. In this study, colorectal cancer cell lines were selected to explore the effect of miR-873-5p on the biological behaviour of colorectal cancer cells and its mechanism of action.

Methods

Experimental materials

The colorectal cancer tissue and adjacent normal tissue of 36 patients with colorectal cancer undergoing surgical resection in our hospital were randomly selected.

The inclusive criteria were as follows:

• All patients met the relevant diagnostic criteria for colorectal cancer;

• This study met the approval of the hospital ethics committee, and conformed with ethical standards;

• The patients were recently diagnosed for the first time and had not undergone chemotherapy and other treatments. The human colorectal cancer cell line SW480 (Shanghai Beinuo Biotechnology Co., Ltd.) was selected.

Experimental reagents and instruments

Fetal bovine serum (Hangzhou Jiangbin Biotechnology Co., Ltd.); penicillin-streptomycin double-antibody solution (Wuhan Procell Life Science and Technology Co., Ltd.); DMEM medium (Thermo Fisher Scientific Biotechnology Co., Ltd.); real-time fluorescent quantitative PCR analyser (Shenzhen Sanli Technology Co., Ltd., model: TL988-IV); biological microscope (Suzhou Jingtong Instrument Co., Ltd., model: XSP-11CD); ultra–low temperature refrigerator (Zhongke Meiling Low Temperature Technology Co., Ltd., model: DW-HL398); cell incubator (Shanghai Bajiu Industrial Co., Ltd., model: BPN-240RWP); low-temperature high-speed centrifuge (Shanghai Luxiangyi Centrifuge Instrument Co., Ltd., model: TGL-17M).

Experimental methods

Real-time fluorescent quantitative PCR detection

The colorectal cancer tissue and paracancerous normal tissue in patients with colorectal cancer were fully ground, 150μL of chloroform was added, the solution was allowed to stand still and was then centrifuged; the supernatant was fetched, isopropanol was added and mixed well, the solution was incubated in a low-temperature environment overnight, centrifuged at 4°C, the supernatant was removed, 1.5 mL of 75% ethanol was added, the solution was centrifuged to dissolve the RNA precipitate, and the RNA was obtained. To detect the expression levels of miR-873-5p and zinc finger E-box binding protein 1 (ZEB1) in colorectal cancer tissues and paracancerous normal tissues, reverse transcription of RNA levels was performed and given the computer detection.

Cell culture

SW480 cells were into DMEM cell culture medium for culture, and sub-cultured when the cell culture reached about 75%. The cells in a good growth state and in the logarithmic growth phase were taken to prepare a cell suspension, centrifuged, the supernatant was removed, the cells were resuspended in a complete medium, and the culture was continued.

Cell transfection

Cells in a good growth state and in the logarithmic growth phase were inoculated into a 6-well plate at 10,000 cells/well and cultured in a cell incubator until the cell density reached about 70%. A miR-873-5p inhibitor, ZEB1 inhibitor, and blank plasmid were transfected into the cells for culture; four hours later, the complete medium-containing serum was changed and continued to culture for 48h. The miR-873-5p low-expression group, the ZEB1 low-expression group, and the control group were set with five replicate holes in each group.

CCK-8 method to determine cell capacity

The transfected cells were taken for subculture and inoculated into 96-well plates at 10,000 cells/well for culture. The 20μL/well of CCK-8 solution was added at 0h, 48h, and 96h, incubated for 2h in the dark, and the absorbance at 520nm was measured with a microplate reader.

Cell clone formation experiment

Cell suspension was taken with a cell density of 400 cells/mL and 1 mL/well was added into a 6-well plate and placed in a cell incubator for one week. When the cells grew visible to the naked eye, the culture was stooped, the supernatant was

removed and rinsed with phosphate buffer, fixed with formaldehyde solution for 10 minutes, stained with Giemsa stain, rinsed with tap water, naturally dried, and pictures were taken for observation.

Cell invasion test

The changes in cell invasion ability of each group were detected using the Transwell cell invasion test. The pre-cooled Matrigel glue with fetal bovine serum in an appropriate ratio was diluted and 200 μL of Matrigel was added to the upper chamber of the small chamber. Then 400μL of complete medium containing 10% fetal bovine serum was added to the lower chamber. The transfection group and the control group cells were inoculated in the upper chamber of the small chamber at 10,000 cells/well and placed in a cell culture incubator for 24 hours. It was stained with crystal violet dye and observed with a microscope.

Cell migration test

Changes in the cell migration ability of each group were measured using the Transwell cell migration test. The serum-free medium was taken to resuspend the cells and inoculated into the upper chamber of the small chamber at an appropriate density, then 400 μL of 10% complete medium containing 10% fetal bovine serum was added to the lower chamber and placed in an incubator for 24 hours. It was stained with crystal violet dye and observed with a microscope.

Western blotting method

SW480 cells were taken to be fully lysed and centrifuged. The supernatant was taken, the BCA working solution was prepared, added into each well and mixed thoroughly. The total protein level was measured. After SDS-PAGE electrophoresis, the membrane was transferred and blocked, then the primary antibody was added and washed with TBST. In a shaker at 4°C, secondary antibody was added and PVDF membrane rinsed for antibody incubation, ECL developer solution was dropped, the optical density value of the band was analysed by the gel image processing system.

Observation indicators

The expression levels of miR-873-5p and ZEB1 in colorectal cancer tissue and paracancerous normal tissue were measured using real-time fluorescence quantitative PCR. The expression levels of miR-873-5p and ZEB1 in the low-expression group of miR-873-5p and the control group were determined by real-time fluorescence quantitative PCR. The ZEB1 expression levels in the ZEB1 low-expression group and the control group were determined using Western blotting. The cells were divided into a control group, a miR-873-5p low-expression group and a miR-873-5p+ZEB1 low-expression group, with five replicate wells in each group. The changes in cell proliferation in each group were determined using the CCK-8 method and cell clone formation experiment. The changes in the cell invasion and migration abilities of each group were determined using the Transwell cell invasion and migration experiment.

The epithelial-mesenchymal transition (EMT) -related proteins vimentin (Vimentin), tight junction protein (zonula occludes protein, ZO-1), E-cadherin (E-cadherin), β-catenin (β-catenin), nerve cadherin (N-cadherin), and ZEB1 expression levels were determined using Western blotting.

Statistical methods

In this study, the expression levels of miR-873-5p and ZEB1 in colorectal cancer tissue and paracancerous normal tissue, as well as the cell proliferation, invasion, and migration abilities, were in line with normal distribution. The comparison between multiple groups was performed by single-factor multiple-sample mean comparison, and the comparison between two groups was performed by an independent sample t test, with results expressed as ($\bar{x}\pm s$). In this study, the SPSS22.0 software package was used for statistical data analysis, and the results $P<0.05$ were regarded as statistically significant.

Results

The expression levels of miR-873-5p and ZEB1 in colorectal cancer tissue and paracancerous normal tissue

Compared with paracancerous normal tissue, the expression level of miR-873-5p in colorectal cancer tissue was significantly reduced, and the expression level of ZEB1 was significantly increased ($P<0.01$). See Table 1.

The expression levels of miR-873-5p and ZEB1 in the miR-873-5p low-expression group and the control group

Compared with the control group, the miR-873-5p low-expression group was significantly reduced and the ZEB1 expression level was significantly increased ($P<0.01$). See Table 2.

Groups	Cases	miR-873-5p	ZEB1
Colorectal cancer tissue	36	0.34±0.10	7.23±0.52
Paracancerous normal tissue	36	0.86±0.13	1.00±0.07
t		19.023	71.242
P		<0.001	<0.001

Table 1: The expression levels of miR-873-5p and ZEB1 in colorectal cancer tissue and paracancerous normal tissue ($\bar{x}\pm s$).

Groups	Cases	miR-873-5p	ZEB1
miR-873-5p low-expression group	5	0.31±0.04	2.96±0.27
Control group	5	1.01±0.01	0.97±0.02
t		37.963	16.436
P		<0.001	<0.001

Table 2: The expression levels of miR-873-5p and ZEB1 in the low-expression miR-873-5p group and the control group ($\bar{x}\pm s$).

ZEB1 expression level in the ZEB1 low-expression group and the control group

Compared with the control group, the ZEB1 expression level in the ZEB1 low-expression group was significantly reduced (P<0.05). See Table 3.

Group	n	ZEB1expression
Control	36	0.97±0.31
ZEB1 low expression	36	0.57±0.30
t		5.563
P		<0.001

Table 3: ZEB1 expression level in ZEB1 low-expression group and control group.

Changes in cell proliferation ability in each group

There was no significant difference in the cell proliferation ability of cells in the three groups at 0h (P>0.05). From 48h to 96h, compared with the control group, the cell proliferation ability in the miR-873-5p low-expression group was significantly increased (P<0.05). Compared with the miR-873-5p low-expression group, the cell proliferation ability of the miR-873-5p+ZEB1 low-expression group was significantly decreased (P<0.05). See Table 4.

Cell clone formation experiment

Compared with the control group, the cell clone formation ability of the miR-873-5p low-expression group was significantly enhanced (P<0.05); compared with the low-expression group of miR-873-5p, the ability of cell clone formation in the low-expression group of miR-873-5p+ZEB1 was significantly decreased (P<0.05). See Figure 1.

Groups	Case	Cell proliferation ability		
		0h	48h	96h
Control group	5	0.40±0.02	0.73±0.03	1.16±0.08
miR-873-5p low-expression group	5	0.41±0.03	1.04±0.05[a]	2.53±0.26[a]
miR-873-5p+ZEB1 low-expression group	5	0.40±0.03	0.87±0.05[ab]	1.76±0.11[ab]
F		0.23	61.27	82.17
P		0.800	<0.001	<0.001

Table 4: Changes of cell proliferation ability in each group ($\bar{x}\pm s$).
Note: Compared with the control group, [a]P<0.05; compared with the miR-873-5p low-expression group, [b]P<0.05.

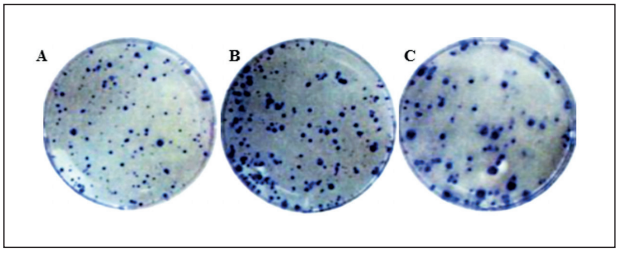

Figure 1: Cell clone formation experiment.
Figure A: The control group; Figure B: miR-873-5p low-expression group; Figure C: miR-873-5p+ZEB1 low-expression group.

Changes in cell invasion and migration ability in each group

Compared with the control group, the cell invasion and migration ability of the miR-873-5p low-expression group was significantly enhanced (P<0.05); compared with the miR-873-5p low-expression group, the cell invasion and migration ability of the miR-873-5p+ZEB1 low-expression group was significantly reduced (P<0.05). See Table 5.

Groups	Cases	Cell invasion numbers	Cell migration numbers
The control group	5	86.14±7.36	77.58±12.37
miR-873-5p low-expression group	5	173.85±44.74[a]	137.47±25.51[a]
miR-873-5p+ZEB1 low-expression group	5	128.65±13.49[ab]	106.69±13.58[ab]
F		12.90	13.61
P		0.001	0.001

Table 5: Changes to cell invasion and migration ability in each group ($\bar{x}\pm s$).
Note: Compared with the control group, [a]P<0.05; compared with the miR-873-5p low-expression group, [b]P<0.05.

Expression levels of EMT-related proteins Vimentin, ZO-1, E-cadherin, β-catenin, N-cadherin, and ZEB1 of cells in each group

Compared with the control group, the expression levels of Vimentin, N-cadherin, and ZEB1 in the miR-873-5p low-expression group were significantly increased, while the expression levels of ZO-1, E-cadherin, and β-catenin were significantly decreased (P<0.05); compared with the miR-873-5p low-expression group, the expression levels of Vimentin, N-cadherin, and ZEB1 in the miR-873-5p+ZEB1 low-expression group were significantly lower, and the expression levels of ZO-1, E-cadherin, and β-catenin were significantly increased (P<0.05). See Table 6.

Group	Vimentin	ZO-1	E-cadherin	β-catenin	N-cadherin	ZEB1
Group	0.99±0.06	1.01±0.04	1.09±0.11	1.06±0.05	1.20±0.11	0.89±0.07
miR-873-5p low expression	2.54±0.08[a]	0.59±0.08[a]	0.31±0.10[a]	0.43±0.01[a]	0.61±0.05[a]	1.78±0.06[a]
miR-873-5p+ZEB1 low expression	1.46±0.06[ab]	0.72±0.09[ab]	0.82±0.03[ab]	0.66±0.03[ab]	0.75±0.04[ab]	1.42±0.01[ab]

Table 6: Expression levels of EMT-related proteins Vimentin, ZO-1, E-cadherin, β-catenin, N-cadherin and ZEB1 of cells in each group.
Note: Compared with the control group, [a]P<0.05; compared with the miR-873-5p low-expression group, [b]P<0.05.

Discussion

Colorectal cancer is one of the most common gastrointestinal malignancies in clinical practice. With improvements to people's living standards and changes in common lifestyle habits, the incidence of colorectal cancer has increased yearly and now ranks fourth in the world among common cancers, following lung cancer, liver cancer, and stomach cancer[5]. Currently, in the clinical treatment of colorectal cancer, surgical resection combined with radiotherapy and chemotherapy is the typical treatment approach, but the clinical effect is poor for patients in advanced stages of the disease. With the rapid development of technology related to molecular biology, the diagnosis and treatment of colorectal cancer have been significantly improved, but the pathogenesis has still not been fully elucidated. Therefore, in-depth exploration of its pathogenesis and searching for effective treatments play an important role in increasing the survival rate of patients and improving the prognosis of patients.

miRNAs are a class of non-coding small molecule RNAs, which, as a new biomarker or therapeutic target, are increasingly used in the diagnosis of lung cancer, ovarian cancer, breast cancer and other cancers[6]. Rahmani et al.[7], in a study of lung cancer, found that miRNAs can participate in the occurrence and development of colorectal cancer by affecting the biological behaviour of cancer stem cells, angiogenesis, epithelial-mesenchymal, and mesenchymal-epithelial transformation or drug resistance. miR-873-5p is a member of the miRNAs family and was first discovered in the hippocampus of rats with temporal lobe epilepsy and memory impairment. It can inhibit neuronal apoptosis, thus playing a certain protective role[8].

Guo et al.[9], in a study of gastric cancer, found that overexpression of miR-873-5p can significantly block the development of the cell cycle, promote cell apoptosis, and inhibit cell proliferation. However, there are few reports about its role in colorectal cancer. In this study, its expression in colorectal cancer and related mechanisms was analysed and discussed by observing the expression level of miR-873-5p in colorectal cancer tissue and cells.

EMT is an important biological process in which epithelial-derived tumour cells acquire invasion and migration abilities[10]. Studies have found that EMT plays an important role in the invasion and migration of colorectal cancer[11]. ZEB1 is a nuclear transcription factor and one of the important regulatory genes for EMT. It can promote the epithelial-mesenchymal transition of tumour cells and is expressed in lung cancer, liver cancer, breast cancer, pancreatic cancer, and other tumours[12]. By studying glioma cells, some researchers have found that inhibiting the expression of ZBE1 can regulate the EMT process of cancer cells[13]. Vimentin, ZO-1, E-cadherin, β-catenin, N-cadherin, etc. are all related proteins of EMT. According to related reports, when a tumour develops EMT, the expression levels of Vimentin and N-cadherin are significantly increased, and the expression levels of ZO-1, E-cadherin, and β-catenin are significantly reduced.

Cells are gradually epithelialised, losing cell polarity and cell-to-cell connection, thus promoting cell escape and apoptosis, which in turn leads to cell migration and invasion[14-15].

This study found that the expression level of miR-873-5p in colorectal cancer tissue and cells was significantly reduced, and the expression level of ZEB1 was significantly increased; after inhibiting the expression of miR-873-5p in the colorectal cancer cell line SW480, the expression level of ZEB1 was significantly increased, the cell proliferation, invasion, and migration ability were significantly enhanced, and the EMT process was accelerated; after inhibiting the expression of ZEB1, the above trends were reversed. miR-873-5p can negatively regulate the expression of ZEB1, and ZEB1 may be the downstream target of miR-873-5p.

In conclusion, inhibiting the expression of miR-873-5p can significantly inhibit the proliferation, invasion and migration of colorectal cancer cells, and regulate the progress of EMT, which may be achieved by targeting ZEB1 regulation.

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