miR-205-5p targeting SATB2 to promote survival and metastasis of colonic neoplasm cells

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Abstract: Colonic neoplasm is a common cancer type, and the relationship between the miR-205-5p/SATB2 axis and colonic neoplasms remains unclear. This study aims to discuss the molecular mechanism between miR-205-5p and SATB2 in colonic neoplasm. qPCR was applied to quantify miR-205-5p and SATB2 mRNA in colonic neoplasm tissues and cells. The vectors of miR-205-5p mimics, miR-205-5p inhibitor and SATB2 siRNA were constructed to observe the function of miR-205-5p and SATB2 on colonic neoplasm cells. Western blot was applied to determine marker proteins for apoptosis, invasion and migration. MTT assay was applied to detect cell activity, and dual luciferase reporter gene to prove the targeting relationship between miR-205-5p and SATB2. It was found that miR-205-5p decreased but SATB2 increased in colonic neoplasms. Upregulated miR-205-5p downregulated SATB2, while decreased miR-205-5p promoted SATB2. Increased miR-205-5p or decreased SATB2 promoted apoptosis increase but inhibited cell migration, invasion and proliferation. Downregulated miR-205-5p promoted the malignant expansion and growth of cells, and inhibited cell apoptosis. miR-205-5p could bind to SATB2 and negatively regulate SATB2. In summary, miR-205-5p promotes survival and metastasis of colonic neoplasm cells by inhibiting SATB2, hence, up-regulation of miR-205-5p or down-regulation of SATB2 may contribute to treatment for colonic neoplasms.

Keywords: Colonic neoplasms, miR-205-5p, SATB2, cell apoptosis

Introduction

Colonic neoplasm is one of the top three most common cancers in the world [1]. It can be caused by age, diabetes, abnormal expression or mutation of nucleic acid sequences, and gastrointestinal inflammatory bowel disease [2-6]. The pathogenesis of colonic neoplasm involves the activation of multiple pathways. In China, the incidence rate of colon cancer increases by 2.34% each year [7]. Therefore, the development of colonic neoplasm treatment strategies is extremely urgent. microRNA epigenetic regulation of cancer-related genes is an important link in tumorigenesis. Understanding the regulatory mechanism of microRNA can help improve the effectiveness of colonic neoplasm target therapy.

miR-205 is a segment of microRNA with the length of about 110 bp located on human chromosome. miR-205-5p, as a mature spliceo-
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5p may become a potential target for the treatment of colonic neoplasms. SATB2 (stabilin 2) is an adenosine phosphate serine receptor that promotes phagocytosis of apoptotic cells. A number of studies [17-19] have shown that the abnormal expression of SATB2 is involved in the cell life process and is highly likely to give rise to cancer. In colonic neoplasms, SATB2 is highly expressed and induces the occurrence of colonic neoplasms [20, 21].

Given the opposite expression trends of miR-205-5p and SATB2 in colon cancer and the involvement of colonic neoplasms, this paper speculates that miR-25-5p may have some regulatory relationship with SATB2. At present, the mechanism of miR-205-5p regulating SATB2 in colonic neoplasms is still unclear; therefore, this study aims to explore the correlation of miR-205-5p and SATB2 with colon cancer by regulating the differential expression of colonic neoplasm cells.

Materials and methods

Patients with colonic neoplasm

Cancer tissues were collected from 82 patients diagnosed with colon cancer in Xintai City People's Hospital. Inclusion criteria were as follows: Patients diagnosed with colon cancer according to clinical features or pathological sections and who cooperated with the treatment [5]. Exclusion criteria were as follows: Patients with mental illness. Patients with other tumors. Patients with previous history of surgery and chemoradiotherapy for colon cancer. Patients with colitis. The study was approved by the Xintai City People's Hospital ethics committee, and all patients were informed. The tissue samples were stored at -80°C before measurement.

Cell culture and transfection

Colonic neoplasm cell lines (SW620, SW480, HT29, HCT116, RKO) and human normal colon tissue cells (CCD-18Co) were purchased from the ATCC cell bank. Cell medium: DMEM basic medium (Hyclone) +10% fetal bovine serum (FBS) solution (Gibco) +1% penicillin/streptomycin solution (100 ×, Solarbio). Cells were inoculated in a T25 cell culture flask (Thermo Fisher). Five mL of medium was preheated to 37°C and was added to the flask. The cells were cultured at 37°C in 5% CO₂ in an animal cell incubator (Binder, Germany) until they grew well. The medium was replaced with medium without FBS before transfection. At transfection, cells were inoculated into 6-well plates with 1105 cells in each well. miR-205-5p mimic (miR mimic), miR-205-5p inhibitor (miR inhibitor) NC mimic, NC inhibitor, SATB2 siRNA and NC siRNA vectors were purchased from Shanghai Sangon Biotech Co., Ltd., (Shanghai, China). Lipofectamine 2000 transfection kit (Invitrogen, USA) was used to transfect the cell lines. Procedures were in accordance with the kit instructions. Fresh medium was replaced 8 h after transfection to avoid killing cells. The average transfection rate was 81.25 ± 2.67%.

qPCR

Trizol was used to extract total RNA from tissues and cells. The OD value of total RNA was detected at 260-280 nm by UV spectrophotometer, and OD260/OD280 > 1.8 was taken for subsequent qPCR quantification. FastKing one-step reverse transcription-fluorescence quantitative kit (Beijing Tiangen Biotech Co., Ltd., FP314) and ABI PRISM 7000 (Applied Biosystems, USA) were used for quantitative analysis of RNA. miR-205-5p and SATB2 mRNA primers were designed and synthesized by Shanghai Sangon Biotech Co., Ltd. miR-205-5p, F: 5'-GCGCCGGGTAGTGTTTCCTA-3', R: 5'-GTGCAGGGTGTAAGTTTTTCCA-3'. SATB2 mRNA, F: 5'-GGTCCAGGGTGTTTTCGTT-3', R: 5'-GCTGTCGGTGTCGAGGTTT-3'. The qPCR reaction system (50 µL) was as follows: upstream primer 1.25 µL, downstream primer 1.25 µL, probe 1.0 µL, RNA template 10 pg/µg, 50 × ROX Reference Dye ROX 5 µL, and RNase-Free ddH₂O was added to the total reaction system to supplement to 50 µL. Reaction process was as follows: reverse transcription at 50°C for 30 minutes, with one cycle, pre-denaturation at 95°C for 3 minutes, with one cycle, denaturation at 95°C for 15 s, annealing at 60°C for 30 s, with 46 cycles. The results were analyzed by ABI PRISM 7000 instrument. The internal reference genes were U6 and GAPDH, which were standardized using 2⁻ΔΔCt.

Western blot

Preparation of cell protein extract: 20 mM Tris-HCl solution (pH7.5, Solarbio), protein inhibitors
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Cells were digested with trypsin and prepared in a cell suspension. One mL cell protein extract was added to the suspension of lysed cells, and blowing upon the solution repeatedly until the cells were completely lysed. The centrifuge was pre-cooled at 4°C and the extract was centrifuged for 20 min, 1.6 × 10^4 × g. The supernatant was taken to determine the protein concentration by BCA. The proteins were separated by SDS-PAGE electrophoresis and transferred to NC membrane, standing at room temperature for 1 h (sealing with 5% skim milk-PBS solution). Then the protein to be measured and β-actin primary antibodies were added and left overnight at 4°C. NC membrane was washed three times with PBS solution, and then goat anti-rabbit secondary antibody (HRP cross-linking) was added, kept for 1 h at room temperature. At last, the NC membrane was washed with PBS solution and visualized with ECL luminescent solution. The internal reference protein was β-actin, and the relative expression level of the protein to be measured = the gray value of the band to be measured/ the gray value of β-actin band. SATB2, Caspase 3, Caspase 9, E-cadherin, Bax, Bcl-2, N-cadherin, β-catenin, β-actin primary antibody and secondary antibody goat anti-rabbit (HRP cross-linking) were purchased from Abcam, Shanghai.

Transwell assay

Cells were inoculated with 2 × 10^4 cells per well in the upper migration chamber (200 µL 10% FBS +1% DMEM was added in advance), and the lower chamber had DMEM (containing 10% FBS, with a total volume of 500 µL). After 24 h of cell culture, the upper chamber fluid was removed, and the cells on the wall were wiped off. Cells on the opposite side of the Transwell chamber were fixed with 4% paraformaldehyde for 20 min. The crystal violet stain was used for 15 min, and the Transwell chamber was washed with PBS buffer solution. Cell migration photos were collected under a 200-fold microscope, and 3 fields were randomly selected to calculate the number of cells. The average value was taken as the number of transmembrane cells. The experiment was repeated three times. The invasion was performed with 8% matrigel on the basis of the steps above and the number of cells per well was increased to 5 × 10^4.

MTT assay

Four 96-well plates were taken and transfected cells were inoculated into the plates according to the specification of 5 × 10^3/100 µL per well, with 3 wells in each group. One plate was removed every 24 h, 5 mg/ml MTT solution (MTT dissolved in DMSO, Solarbio) was added at 10 L/well, and cultured for 1 h. Then the culture medium was removed, and OD value at 570 nm was measured by microplate. OD-time curve was drawn.

Dual luciferase reporter gene

pmirGLO-SATB2-wt and pmirGLO-SATB2-mut vectors were constructed and co-transfected with miRR-205-5p mimics and NC mimics, respectively. The cells were cultured on a 96-well plate for 48 h. The intensity was measured using the dual luciferase reporter gene system (Promega).

Statistics and analysis

SPSS 20.0 software (Asia Analytics Formerly SPSS China) was applied to analyze and compare data difference. GraphPad Prism 6.0 software was used to draw the illustrations. The experiment was repeated three times. The measurement data were expressed as Mean ± SD. The independent sample t-test was used to compare the statistical differences between colon cancer tissues and normal para-carcinoma tissues, and the statistical differences between the NC siRNA group and the SATB2 siRNA group. One-way ANOVA was used to compare the differences between multiple groups. Pairwise comparison was qualified by LSD-t, and all data were qualified by two-tailed test. Set at 95% was the confidence interval, and statistical significance was at P<0.05.

Results

Downregulated miR-205-5p and upregulated SATB2 may be involved in the occurrence of colonic neoplasms

First, a total of 82 colonic neoplasms tissues and 66 normal para-carcinoma tissues were collected, and miR-205-5p and SATB2 mRNA were quantified by qPCR. According to Figure 1A, colonic neoplasm tissues showed a decrease of miR-205-5p and an increase of SATB2 mRNA compared to normal para-carcinoma tissues. Subsequently, the level of miR-
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miR-205-5p and SATB2 mRNA in normal human colon tissue cells and colonic neoplasms were detected. Figure 1B showed that miR-205-5p decreased and SATB2 increased. Since miR-205-5p was the least and the second least expressed in SW480 and HCT116 cells, respectively, SW480 and HCT116 cells were selected as the study tools. In this study, miR-205-5p mimic and miR-205-59 inhibitor vectors were constructed to transfect colon cancer cells. These results suggested that downregulated miR-205-5p and upregulated SATB2 may be involved in the occurrence of colonic neoplasms.

In order to understand the function of miR-205-5p in colonic neoplasm cells, miR-205-5p mimic and miR-205-5p inhibitor vectors were constructed to regulate miR-205-5p levels in SW480 and HCT116 cell lines. In this study, MTT assay was applied to determine the function of miR-205-5p on cell activity, and Western blot was applied to detect the function of miR-205-5p on Caspase 3, Caspase 9, Bax, E-cadherin, Bcl2, N-cadherin and β-catenin. Figure 2A showed that upregulated miR-205-5p promoted cell apoptosis but inhibited cell malignant expansion and growth.
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Figure 2. miR-205-5p promoted cell apoptosis but inhibited cell malignant expansion and growth. A. Increased miR-205-5p resulted in decreased cell activity, while decreased miR-205-5p resulted in increased cell activity, compared with the NC group *P<0.05. B. Increased miR-205-5p promoted Caspase 3, Caspase 9, Bax, E-cadherin and inhibited Bcl2, N-cadherin and β-catenin; while decreased miR-205-5p inhibited Caspase 3, Caspase 9, Bax, E-cadherin and promoted Bcl2, N-cadherin and β-catenin, compared with NC group *P<0.05. C. Increased miR-205-5p inhibited cell migration and invasion, but decreased miR-205-5p promoted cell invasion and migration, *P<0.05. D. Relative expression of miR-205-5p in cells, *P<0.05, **P<0.01.
miR-205-5p led to decreased cell activity, while downregulated miR-205-5p led to increased cell activity. Figure 2B showed that up-regulated miR-205-5p promoted Caspase 3, Caspase 9, Bax, E-cadherin, and inhibited Bcl2, N-cadherin, and β-catenin; while downregulated miR-205-5p inhibited Caspase 3, Caspase 9, Bax, E-cadherin, and promoted Bcl2, N-cadherin, and β-catenin. E-cadherin, N-cadherin, and β-catenin are proteins involved in cell migration and invasion. Because of the influences of miR-205-5p on the above three, the Transwell assay was used to determine the function of miR-250-5p on cell migration and invasion. Figure 2C showed that up-regulated miR-205-5p inhibited cell migration and invasion, but down-regulated miR-205-5p promoted it. These results indicated that miR-205-5p promoted cell apoptosis but inhibited cell malignant expansion and growth.

SATB2 inhibits cell apoptosis and promotes cell malignant expansion and growth

Since SATB2 showed a down-regulated trend in colonic neoplasms and was associated with the occurrence of colon cancer, this paper constructed SATB2 siRNA vectors to study the role of SATB2 in colonic neoplasms cells, as shown in Figure 3. The results showed that down-regulation of SATB2 resulted in up-regulation of Caspase 3, Caspase 9, Bax, E-cadherin, as well as down-regulation of Bcl2, N-cadherin, and β-catenin, and decreased cell activity, migration, and invasion. Results above indicated that SATB2 prevented cell apoptosis and promoted cell proliferation, migration and invasion.

miR-205-5p targeted binding to SATB2

The Targetscan database predicted the presence of a miR-205-5p pairing site in SATB2 sequence (Figure 4A). At the same time, increased miR-205-5p led to SATB2 down-regulation, and decreased miR-205-5p led to SATB2 upregulation (Figure 4B). Therefore, we speculated that miR-205-5p targeted binding and negatively regulated SATB2. The dual luciferase reporter gene was applied to prove the authenticity of the above site. Figure 4C showed that luciferase activity decreased only when SATB2 wt was co-transfected with miR-205-5p mimics in colonic neoplasm cells, while the fluorescence activity of other co-transfected combinations was not significantly changed. Results above suggested that miR-205-5p negatively regulated SATB2 in colonic neoplasms by binding to SATB2.

Discussion

MiR-205-5p was down-regulated while SATB2 was up-regulated in the investigation of colonic neoplasm tissue samples in this study. Combined with previous studies [15, 16, 20, 21] and the differential expression observed in this study, we assumed that miR-205-5p and SATB2 were related to the occurrence of colon cancer. At the same time, upregulated miR-205-5p decreased SATB2, but downregulated mir-205-5p increased SATB2. The Targetscan database predicted the presence of miR-205-5p pairing sites at the 3’UTR of SATB2. To verify the authenticity of this site, we constructed SATB2-wt and SATB2-mut vectors and co-transfected colonic neoplasm cells with miR-205-5p mimics, respectively. When miR-205-5p mimics were co-transfected with SATB2-wt, luciferase activity decreased statistically, suggesting that miR-205-5p could bind to SATB2. Results above showed that miR-205-5p might be involved in the progression of colonic neoplasms by inhibiting SATB2.

In order to confirm the above conjectures, we constructed miR-205-5p mimics, miR-205-5p inhibitor and SATB2 siRNA to change the level of miR-205-5p and SATB2 in colonic neoplasm cells. When miR-205-5p was increased or SATB2 was decreased, cell malignant expansion and growth were decreased, and apoptosis was increased. However, when miR-205-5p was down-regulated, cell malignant expansion and growth were increased, and apoptosis was decreased. The results above proved our hypothesis that miR-205-5p inhibits the cancerization of colonic neoplasm cells by down-regulating SATB2.

SATB2 is an important regulator of cellular programs and involves many downstream functional proteins. During cell growth, N-cadherin and β-catenin are essential proteins for cell migration and invasion, while Bcl2 prevents cells from entering the apoptotic process and promotes cell survival. SATB2 promotes cell survival, migration, and invasion by inducing N-cadherin, β-catenin, and Bcl2 [22-24]. The excessive activation of the above-mentioned
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Figure 3. SATB2 inhibits cell apoptosis and promotes cell malignant expansion and growth. A. Decreased SATB2 resulted in decreased cell activity, compared with the NC siRNA group *P<0.05. B. Decreased SATB2 resulted in up-regulation of Caspase 3, Caspase 9, Bax and N-cadherin, as well as down-regulation of Bcl2, N-cadherin and β-catenin, compared with the NC siRNA group, *P<0.05. C. Down-regulated SATB2 resulted in decreased cell migration and invasion, compared with the NC siRNA group, *P<0.05. D. Relative expression of STAB2 mRNA in cells, *P<0.05 and **P<0.01.
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cell behavior directly leads to cancerization of normal cells and gives rise to tumor formation. For the results of this paper, miR-205-5p may regulate colon cancer cell behavior through SATB2-mediated cellular programs. In this regulation, miR-205-5p down-regulates the activity of downstream functional proteins such as N-cadherin, β-catenin, and Bcl2 by inhibiting SATB2, and the inactivation of these functional proteins leads to programmed changes in cell behavior and ultimately to the reduction of malignant cell activity. Therefore, the miR-205-5p/SATB2 axis is an effective factor in regulating the behavior of colon cancer cells.

This study showed the correlation between miR-205-5p and SATB2 in colonic neoplasms,
and suggested that miR-205-5p regulated the life course of colonic neoplasm cells through SATB2. Although the function of miR-205-5p/SATB2 axis in colonic neoplasms has been fully described in this article, the upstream regulatory factors of this axis have not been fully studied. Therefore, the upstream regulation on miR-205-5p will be investigated in future research. In addition, SATB2 is closely related to the pathways such as Wnt/β-catenin, TGF-β, and NF-κB in many cancers [25, 26]. This paper speculates that miR-205-5p may regulate these pathways through SATB2 in colon cancer, and this conjecture will also be systematically studied in the future.

In summary, it is found that miR-205-5p induced colonic neoplasm cell apoptosis, and suppressed cell proliferation, migration and invasion through inhibiting SATB2. This mechanism will provide possible therapeutic ideas for the treatment of colonic neoplasms, and miR-205-5p upregulation or SATB2 downregulation may help improve and inhibit disease in patients with colonic neoplasms.

Disclosure of conflict of interest

None.

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