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Expression of MiR-20a-5p and its target gene in colon cancer and its effect on the proliferation and apoptosis of colon cancer cells.

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Keywords: MicroRNA-20a-5p; similar gene of breast cancer metastasis suppressor gene 1; colon cancer; proliferation; apoptosis.

Abstract. We investigated the expression of micro ribonucleic acid (miR)-20a-5p and its target gene, breast cancer metastasis suppressor 1 like (BRMS1L), in colon cancer tissues and their effects on the proliferation and apoptosis of colon cancer cells. The dual luciferase assay was used to detect the targeted regulation of miR-20a-5p on BRMS1L. The expression levels of miR-20a-5p and BRMS1L in colon cancer tissues and cells were detected by quantitative real-time polymerase chain reaction (qRT-PCR). MiR-20a-5p mimic and mimic negative control (NC) were transfected into the colon cancer cell line SW480 by the liposome transient transfection method. The MTT assay, monoclonal formation of cancer cells, and flow cytometry were used to detect cell proliferation and apoptosis. The expression level of miR-20a-5p in colon cancer tissues was significantly higher than that in adjacent tissues, and the expression level of BRMS1L was significantly lower than that in adjacent tissues. The expression level of miR-20a-5p was significantly correlated with tumor-node-metastasis (TNM) stage, lymph node metastasis, invasion depth, and differentiation degree. The higher the expression level of miR-20a-5p, the more advanced the TNM stage and invasion depth, and the easier it is for lymph nodes to metastasize ($p<0.05$). Compared with the control and the miR-NC groups, the miR-20a-5p group's cell proliferation ability, expression of CyclinD1 and B-cell lymphoma-2 (Bcl-2) were significantly increased, while apoptosis ability and caspase-3 protein expression were significantly decreased ($p<0.05$). The expression of miR-20a-5p in colon cancer tissues and cells increased. Overexpression of miR-20a-5p could promote the proliferation of colon cancer cells and inhibit their apoptosis.

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Expresión del MiR-20a-5p y su gen objetivo en cáncer de colon y sus efectos sobre la proliferación y apoptosis de células cancerosas de colon

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Palabras clave: MicroARN-20a-5p; gen similar al gen supresor de metástasis del cáncer de mama 1; cáncer de colon; proliferación; apoptosis.

Resumen. Investigamos la expresión del ácido microribonucleico (miR)-20a-5p y su objetivo, el gen similar al supresor de metástasis del cáncer de mama 1 (BRMS1L), en tejidos de cáncer de colon y sus efectos sobre la proliferación y la apoptosis de las células de cáncer de colon. Se utilizó el ensayo de luciferasa dual para detectar la regulación específica del miR-20a-5p en BRMS1L. Los niveles de expresión de miR-20a-5p y BRMS1L en tejidos y células de cáncer de colon se detectaron mediante la reacción en cadena de la polimerasa cuantitativa en tiempo real (qRT-PCR). El MiR-20a-5p mímico y el control mímico negativo (NC) se transfectaron a la línea celular de cáncer de colon SW480 mediante el método de transfección transitoria de liposomas. Se utilizaron el ensayo MTT, la formación monoclonal de células cancerosas y la citometría de flujo para detectar la proliferación celular y la apoptosis. El nivel de expresión de miR-20a-5p en tejidos de cáncer de colon fue significativamente mayor que en los tejidos adyacentes, y el nivel de expresión de BRMS1L fue significativamente menor que en tejidos adyacentes. El nivel de expresión de miR-20a-5p se correlacionó significativamente con el estadio tumor-ganglio-metástasis (TNM), la metástasis al ganglio linfático, la profundidad de la invasión y el grado de diferenciación. Cuanto mayor era el nivel de expresión de miR-20a-5p, más avanzada eran la etapa TNM y la profundidad de la invasión, y más fácil era que los ganglios linfáticos hicieran metástasis ($p<0,05$). En comparación con los grupos control y miR-NC, la capacidad de proliferación celular del grupo miR-20a-5p, la expresión de CyclinD1 y el linfoma-2 de células B (Bcl-2) aumentaron significativamente; mientras que la capacidad de apoptosis y la expresión de la proteína caspasa-3 disminuyeron significativamente ($p<0,05$). La expresión del miR-20a-5p en células y tejidos de cáncer de colon aumentó. La sobreexpresión del miR-20a-5p podría promover la proliferación de células de cáncer de colon e inhibir su apoptosis.

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INTRODUCTION

Colon cancer (CC) is a common malignant tumor of the digestive tract, and its morbidity and mortality are increasing year by year. Every year, there are 1.2 million new colon cancer patients in the world, with 600,000 deaths [1]. The pathogenesis of colon cancer is complex, and the activation of carcinogenic pathways and the inhibition of defense mechanisms are important causes of the tumor, which not only affect the function of the digestive system but also involve the liver, lung, and other organs in

distant metastasis [2]. When colorectal cancer is diagnosed at an early stage, it can be cured by surgery, but when it is diagnosed at an advanced stage, the curative effect of chemotherapy drugs is very limited [3]. Therefore, finding new therapeutic methods and targets is very important. Micro ribonucleic acid (MiRNA) is an endogenous single-stranded non-coding RNA with a length of 20-25 nt, which combines with the messenger ribonucleic acid (mRNA) of the target gene through base complementary pairing, resulting in its degradation or translation stop, thus down-regulating the expression of the target gene [4]. MiRNA plays the role of oncogene or tumor suppressor gene in tumors and can regulate biological processes such as proliferation, differentiation, invasion, and apoptosis of tumor cells [5]. It has been reported that miR-20a-5p regulates tumor growth, cell differentiation, and apoptosis of many cancers. Luo Sheng *et al*. [6] pointed out that up-regulation of the expression of miR-20a-5p could promote the proliferation of pancreatic cancer cells and inhibit their apoptosis. Liu Xiao dong *et al*. [7] found that HAGLROS inhibited the expression of miR-20a-5p, thus inhibiting the proliferation of cardiomyocytes induced by high glucose and promoting their apoptosis. MiR-20a-5p was sheared from miR-20a. At present, miR-20a is widely studied in the serum of colon cancer patients, but the expression of miR-20a-5p in colon cancer tissues and its effect on colon cancer cells are rarely reported. Therefore, this study intended to explore the expression level of miR-20a-5p and its target gene in colon cancer tissues and cells, as well as its effects on proliferation and apoptosis of colon cancer cells, so as to provide theoretical basis for early diagnosis and treatment of colon cancer.

MATERIALS AND METHODS

Objectives

This study belongs to a single-center study, and the research objects are collected in a continuous way. Inclusive criteria: All patients were diagnosed as colon cancer by histopathology, and had not received radiotherapy, chemotherapy or other anti-tumor adjuvant therapy before operation. Exclusion criteria: Other digestive diseases (colon polyps, chronic enteritis, etc.) and other systemic malignant tumors (lung cancer, breast cancer, etc.). From January 2021 to October 2022, 87 cases of colon cancer patients in our hospital were collected. The specimens of colon cancer tissues and adjacent tissues >5cm from the tumor edge were collected. The collected specimens were kept in liquid nitrogen. General clinical data include age, sex, smoking history, drinking history, tumor diameter, tumor location, tumor-node-metastasis (TNM) stage, lymph node metastasis, infiltration depth, differentiation degree and other information were collected.

Cells

Human normal colon epithelial cell lines FHC, colon cancer cell lines SW480, HT-29 and HCT-116 (Beina Biotechnology Co., Ltd.) (Wuhan, China).

Reagents and Equipment

MiR-20a-5p mimic (MiR-20a-5p mimic) and mimic negative control (NC) (Shanghai Jima Pharmaceutical Technology Co., Ltd.); MiR-20a-5p and BRMS1L primers (Shanghai Shenggong Bioengineering Co., Ltd.); Monoclonal antibodies against CyclinD1, B-cell lymphoma-2(Bcl-2), caspase-3 and glyceraldehyde-3-phosphate dehydrogenase(GAPDH) (Cell Signal Technology Company, USA); Trizol ribonucleic acid(RNA) extraction kit, Lipofectamine TM3000 liposome and reverse transcription kit (Dalian Bao Biotechnology Co., Ltd.); Bicinchoninic acid(BCA) protein assay kit (Shanghai Biyuntian Biotechnology Co., Ltd.); Fetal serum, Dulbecco's modification of Eagle's medium (DMEM) medium and trypsin (GIBCO Company, USA); Net Airtech clean bench (Thermo Fisher Company, USA); DYCZ 425D double vertical elec-

trophoresis apparatus (Beijing Liuyi Instrument Factory); Carbon dioxide incubator (Thermo Company, USA); SpectraMax i D5 microplate reader (Thermo, USA); IXplore Standard inverted microscope (Olympus, Japan).

Cell culture

Colon cancer cell lines (SW480, HT-29, HCT-116) and human normal colon epithelial cell line FHC were resuscitated, then added into DMEM medium containing 10% fetal bovine serum, and cultured in an incubator at 37℃ and 5% CO2. When the cell fusion rate reached 80%, the cells were digested and passaged with trypsin.

Grouping processing

SW480 cells were divided into three groups: control group: untransfected SW480 cells; MiR-NC group: SW480 cells transfected with mimic NC; miR-20a-5p group: SW480 cells transfected with miR-20a-5p mimic.

10 mL SW480 cell fluid were inoculated into 6-well plate, 100 pmol of miR-20a-5p mimic and mimic NC were added into DMEM medium, and 4μL Lipofectamine 3000 were added to incubate for 30 min. The cells were washed with the mixture, cultured in DMEM medium for 24 h, and the transfection efficiency was detected by flow cytometry.

Bioinformatics prediction and double luciferase experiment

Using bioinformatics database TargetScanHuman (http://www.targetscan.org/vert_72/) to predict the related target genes of miR-20a-5p, it was found that BRMS1L might be the target gene of miR-20a-5p. The wild-type 3'UTR vector (pgl3-BRMS1L-3'-UTR-wt) and the mutant 3'UTR luciferase reporter vector (pGL3-BRMS1L-3'UTR-MUT) were constructed in turn. SW480 cells in logarithmic growth phase were inoculated into 6-well plates, and the two plasmids were mixed with mimic NC and miR-20a-5p mimic respectively, and then co-transfected into the cells by Lipofectamine TM3000 for 48 hours. Collect cells from each group to prepare cell lysate, and detect luciferase activity according to the requirements of the kit, to determine whether miR-20a-5p binds to 3'UTR of BRMS1L.Each group had three parallel settings.

Detection of miR-20a-5p and BRMS1L in colon cancer tissues and cells by qRT-PCR

Total RNA was extracted from tissues and cells by Trizol reagent. RNA was reverse transcribed into cDNA by reverse transcription kit, and amplified by SYBR Green method. The U6 was used as internal reference. The reaction conditions: pre-denaturation at 95℃ for 5min, pre-denaturation at 95℃ for 30s and pre-denaturation at 60℃ for 30 s, a total of 40 cycles. The relative expression levels of miR-20a-5p and BRMS1L were calculated by $2^{-\Delta\Delta CT}$ formula. PCR primers: miR-20a-5p forward primer, 5'-AGTCTATACAAGGGCAAGCTCTC-3', reverse primer, 5'-CCCAATACGACCAAATCCGTT-3'. U6 forward primer 5'-CTGCTTCGGCAGCACA-3', reverse primer 5'-AACGCTTCACGAATTTGCGT-3'. BRMS1L forward primer, 5'-GGCACAGCATTGATATTACCTCA-3', reverse primer, 5'-TATGGACCTGAAACAACAACTGG-3'. Each group had three parallel settings.

Detection of cell proliferation by MTT assay and cell cloning

After 24 hours of transfection, the cells were digested and inoculated in 96-well plates at the density of 1×10^3/ well. Each well was equipped with three duplicate wells, which were cultured for 24, 48, 72 and 96 hours, respectively. MTT solution was added, incubated at 37℃ for 4 hours, centrifuged to remove supernatant, DMSO was added, and the absorbance value was detected at 490 nm with enzyme-labeled instrument. Each group had three parallel settings.

The cell density was adjusted to 1×10^3/ well, and the cells were inoculated in 6-well culture plates, and each well was provided with three duplicate wells. After culturing for ten days, it was fixed with 4% paraformal-

dehyde solution, stained with crystal violet, and the colony formation was observed under microscope and counted. Each group had three parallel settings.

Detection of the apoptosis of cell lines by flow cytometry

The adjusted cell density was 1×10^5/well, which was inoculated into 6-well plates, cultured for 48 h, washed with PBS, and suspended with binding buffer. AnnexinV FITC and PI were added, incubated in the dark for 10 min, and cell apoptosis was detected by flow cytometry. Each group had three parallel settings.

Detection of CyclinD1, Bcl-2 and caspase-3 by Western blotting

Cell precipitation was harvested and added with precooled protein lysate, followed by incubation on ice for 30 min and centrifugation at 10,000 rpm at 4°C. Subsequently, the supernatant was taken for protein quantification using the BCA Protein Assay Kit (Thermo Fisher), and the total protein content was adjusted for sample preparation. After electrophoresis at 90 V, the proteins were transferred onto a PVDF membrane. The target band was cut, blocked with 5% skim milk, and incubated on a shaker at room temperature for 1 h. After antibody corresponding incubation solution (CyclinD1, Bcl-2, caspase-3, GAPDH, 1: 2000) was added, the membrane was incubated on a shaker at room temperature for 30 min, and then 4°C overnight. On the second day, after returning to room temperature, the membrane was washed. Next, the membrane was added with secondary antibody corresponding incubation solution (1:5,000), and incubated on a shaker at room temperature for 2 h. After washing, the membrane added with an appropriate amount of ECL solution, followed by reaction for 5 min avoiding light. Then, fluorescence results were gathered using a quantitative imager. Each group had three parallel settings.

Statistical analysis

The SPSS 26.0 software (International Business Machines Corporation, New York, USA) was utilized for statistical analysis, and GraphPad Prism 5.0 software was employed for plotting. The *t*-test was adopted for comparison between groups, and one-way ANOVA was used for comparison among groups. A difference of $p<0.05$ was considered as statistically significant.

RESULTS

Bioinformatics prediction and double luciferase experiment

TargetScanHuman prediction showed that miR-20a-5p and BRMS1L had targeted binding sites. The double luciferase experiment showed that the luciferase activity of wild-type BRMS1L was significantly decreased ($p<0.05$) after transfection of miR-20a-5p, while that of mutant BRMS1L was no significantly different ($p>0.05$) (Fig. 1), indicating that there was a targeted regulation relationship between miR-20a-5p and BRMS1L.

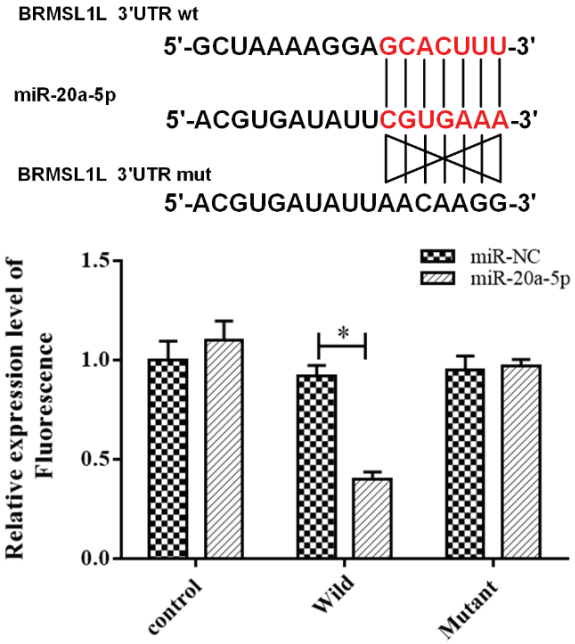

Fig. 1. Double luciferase verification experiment (compared with miR-NC group, *: $p<0.05$).

Expression of miR-20a-5p and BRMS1L mRNA in colon cancer tissues and colon cancer cell lines

The results of qRT-PCR (Fig. 2) showed that the expression of miR-20a-5p in colon cancer tissue was significantly higher than that in para-cancerous tissues ($p<0.05$), and the expression of BRMS1L was significantly lower ($p<0.05$). Compared with human normal colon epithelial cell line FHC, the expression of miR-20a-5p in colon cancer cell lines SW480, HT-29 and HCT-116 increased significantly, and the expression of BRMS1L decreased significantly ($p<0.05$).

Relationship between the expression of miR-20a-5p and clinicopathological features of patients

To verify the role of miR-20a-5p in the occurrence and development of colon cancer, 87 patients were divided into a high-expression group (n=43) and a low-expression group (n=44) according to the p median expression of miR-20a-5p. Results (Table 1) showed that age, sex, smoking history, drinking history, tumor size, and tumor location had no significant differences between the high-expression group and the low-expression group of miR-20a-5p ($p>0.05$), but had

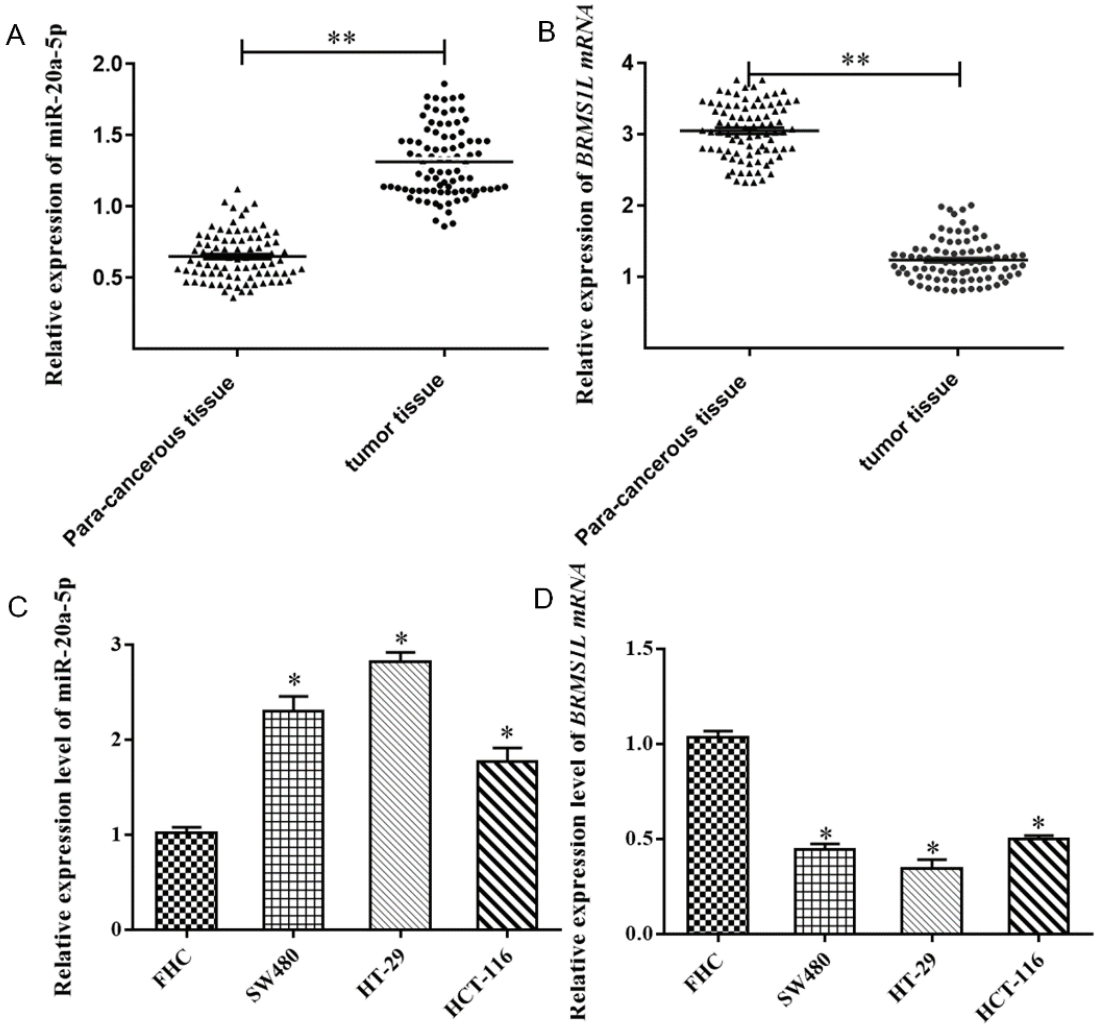

Fig. 2. The expression of miR-20a-5p and BRMS1L in colon cancer tissues (A, B, Compared with normal tissues, *:$p<0.05$, **: $p<0.001$) and colon cancer cells (C, D, Compared with FHC, *: $p<0.05$, **: $p<0.001$).

Table 1
Relationship between expression level of miR-20a-5p in colon cancer and clinicopathological parameters of patients.

	n	miR-20a-5p		χ^2	*p*
		Low expression n (%)	High Expression n (%)		
Age				1.299	0.254
<65	22	13 (52.00)	9 (36.00)		
≥65	28	12 (48.00)	16 (64.00)		
Gender				0.725	0.395
Male	27	15 (60.00)	12 (48.00)		
Female	23	10 (40.00)	13 (52.00)		
History of smoking				0.368	0.544
Yes	16	7 (28.00)	9 (36.00)		
No	34	18 (72.00)	16 (64.00)		
History of drinking				0.439	0.508
Yes	12	5 (20.00)	7 (28.00)		
No	38	20 (80.00)	18 (72.00)		
Tumor diameter (cm)				1.389	0.239
≤5	18	11 (44.00)	7 (28.00)		
>5	32	14 (56.00)	18 (72.00)		
Tumor location				3.949	0.139
Gastric antrum	23	15 (60.00)	8 (32.00)		
Gastric body	11	4 (16.00)	7 (28.00)		
Gastric fundus and cardia	16	6 (24.00)	10 (40.00)		
TNM				6.65	0.01
I～II	29	19 (76.00)	10 (40.00)		
III～IV	21	6 (24.00)	15 (60.00)		
Lymph node metastasis				5.195	0.023
Yes	28	10 (40.00)	18 (72.00)		
NO	22	15 (60.00)	7 (28.00)		
Infiltration depth				6.876	0.009
Mucosa layer	31	11 (44.00)	20 (80.00)		
Submucosal layer	19	14 (56.00)	5 (20.00)		
Degree of differentiation				5.882	0.015
poorly	34	13 (52.00)	21 (84.00)		
Medium/high	16	12 (48.00)	4 (16.00)		

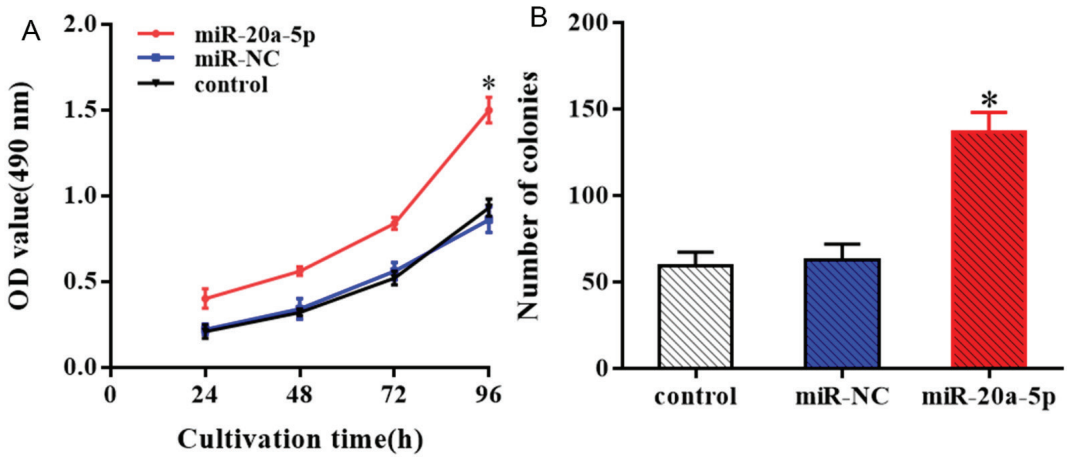

Fig. 3. MTT assay (A) and cell cloning (B) were used to detect the cell proliferation ability (compared with miR-NC group, *: $p<0.05$).

statistical differences with the TNM stage, lymph node metastasis, infiltration depth, and differentiation degree between the high-expression group and the low-expression group of miR-20a-5p.

Results of cell proliferation detected by MTT assay and cell cloning

The results of the MTT assay (Fig. 3A) showed that at 24h, 48h, 72h and 96h, the OD value of the miR-20a-5p group was significantly higher than that of the control and miR-NC groups ($p<0.05$). However, there was no significant difference in OD between the control group and the miR-NC group ($p>0.05$). The monoclonal formation experiment (Fig. 3B) showed that at 48h, compared with the control and miR-NC groups, the number of cell monoclonal formation in the miR-20a-5p group was significantly higher than that in the control and miR-NC groups, and the difference was statistically significant ($p<0.05$). However, there was no significant difference in the number of cell clones between control group and miR-NC group ($p>0.05$).

Results of cell apoptosis detected by flow cytometry

The results of flow cytometry (Fig. 4) showed that compared with control group and miR-NC group, the apoptosis rate of miR-20a-5p group decreased significantly ($p<0.05$). However, there was no significant difference in apoptosis between control group and miR-NC group ($p>0.05$).

Results of CyclinD1, Bcl-2 and caspase-3 expressions detected by Western blotting.

The Western blot results (Fig. 5) showed that compared with the control and miR-NC groups, the expression levels of CyclinD1 and Bcl-2 in the miR-20a-5p group were significantly increased, and the expression levels of caspase-3 were significantly decreased ($p<0.05$).

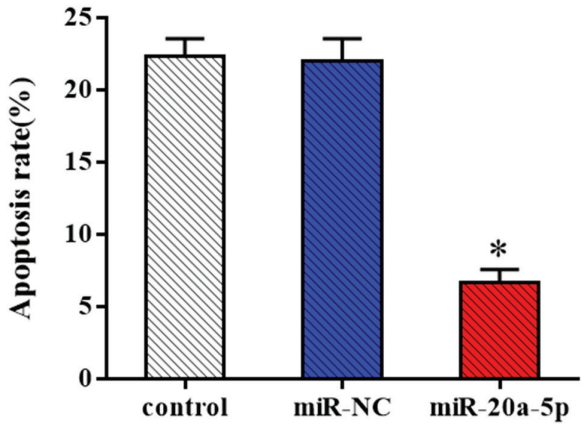

Fig. 4. Fow cytometry was used to detect apoptosis (compared with miR-NC group, *: $p<0.05$).

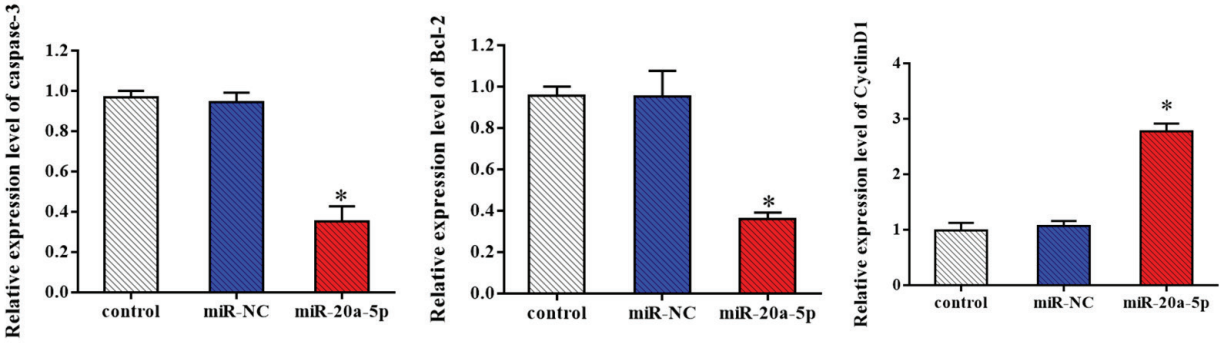

Fig. 5. The expression levels of CyclinD1, Bcl-2 and caspase-3 were detected by protein blotting (compared with miR-NC group, *: $p<0.05$).

Mechanism of miR-20a-5p affecting malignant phenotype of colon cancer cells

By detecting the levels of miR-20a-5p and BRMS1L in colon cancer tissues and cells, we found that the expression of miR-20a-5p in colon cancer tissues and cells was up-regulated, while that of BRMS1L was down-regulated, and the level of miR-20a-5p was significantly correlated with the TNM stage, lymph node metastasis, invasion depth and differentiation degree of patients. miR-20a-5 played the role of an oncogene, and BRMS1L played the role of tumor suppressor. It indicated that miR-20a-5 and BRMS1L were related to the progression of colon cancer. To further observe the effect of miR-20a-5p on the malignant behavior of colon cancer cells, we tested the proliferation and apoptosis of cancer cells, and found that overexpression of miR-20a-5p promoted cell proliferation and inhibited cell apoptosis. At the same time, miR-20a-5p could increase the protein levels of CyclinD1 and Bcl-2 and decrease the expression of caspase-3. Therefore, we concluded that miR-20a-5p could promote the proliferation of colon cancer cells and inhibit the apoptosis of colon cancer cells by targeted regulation of BRMS1L (Fig. 6).

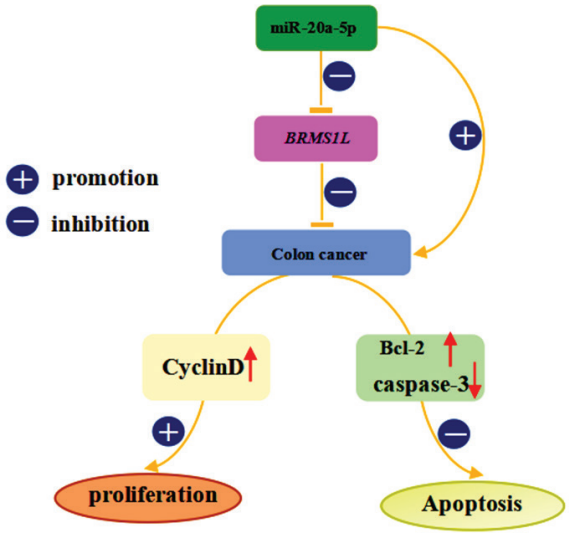

Fig. 6. Mechanism of action of miR-20a-5p on malignant phenotype of colon cancer cells.

DISCUSSION

MiRNA can regulate the expression of target genes, and participate in the proliferation, migration, invasion and apoptosis of tumor cells as oncogenes or tumor suppressor genes [8,9].

Many studies have shown that miR-20a-5p has the function of oncogene. The research of Wang Xiaojing *et al.* [10] showed that miR-20a-5p could alleviate the endothelial cell injury induced by oxidized low density lipoprotein (ox-LDL) by targeting and regulating cardiac myosin-related transcription factor A(MRTFA). Zheng Hui e *et al.* [11] found that acupuncture can down-regulate the expression of miR-20a-5p, thus promoting

cell proliferation and inhibiting cell apoptosis in rats with cerebral ischemia-reperfusion injury.

Bai *et al*. [12] found that miR-20a-5p was highly expressed in triple-negative breast cancer, and promoted the growth of tumor cells by targeting human-related transcription factor 3 (RUNX3).

However, Yu *et al.* [13] found that miR-20a-5p showed low expression in neuroblastoma, which inhibited the proliferation of tumor cells through targeted regulation of autophagy related gene 7(ATG7). It was suggested that miR-20a-5p had different regulatory effects on the occurrence and development of tumors. The results showed that the expression of miR-20a-5p in colon cancer was significantly higher than that in adjacent tissues. MiR-20a-5p was significantly correlated with the TNM stage, lymph node metastasis, invasion depth and differentiation degree, and the higher the expression of miR-20a-5p, the more serious the TNM stage and invasion depth, and the easier it was for lymph nodes to metastasize ($p<0.05$). The cell experiment showed that after miR-20a-5p mimic was transfected, the proliferation ability of SW480 cells was obviously enhanced, but the apoptosis ability was obviously weakened, which indicated that miR-20a-5p played a cancer-promoting role in colon cancer.

BRMS1L is the homologous gene of BRMS1, which can promote the deacetylation of histone deacetylase and combine with specific transcription factors, so as to regulate the expression of related genes and inhibit the proliferation and metastasis of various tumor cells. Cao *et al*. [14] found that BRMS1L inhibited the invasion and metastasis of ovarian cancer cells by inhibiting β-catenin-wnt signaling pathway. Lv *et al*. [15] found that BRMS1L inhibited the invasion of tumors of the nervous system and played an anti-cancer gene role in these tumors. Wang Jihong *et al*. [16] found that miR-17-5p could promote the proliferation, invasion, migration and apoptosis of CNE2 cells of nasopharyngeal carcinoma by down-regulating the expression of BRMS1L. Chen Jie [17] found that miR-20a-5p targeted and regulated the expression of BRMS1L, inhibited the proliferation of the human colorectal cancer cell line SW480 and promoted its apoptosis. In this study, the online database of TargetScanHuman was used to predict that miR-20a-5p has a binding site with BRMS1L, and the double luciferase gene reporting experiment verified that miR-20a-5p can effectively target the negative regulation of BRMS1L.

The growth of a tumor is closely related to cell proliferation and apoptosis. When the expression of proliferation and apoptosis genes that affect tumor growth changes, the progress of cancer will also change accordingly [18]. CyclinD1 is a proliferation-promoting gene, which is involved in the regulation of cell cycle. Its coded products can bind to the corresponding kinases, thus promoting the development of cell cycle and enhancing cell vitality [19]. Bcl-2 is an apoptosis-inhibiting gene, which plays a role by antagonizing the apoptosis-promoting gene Bax [20]. Caspase-3 is a pro-apoptosis gene, which is activated by the mitochondrial and death receptor pathways, and then causes cell apoptosis [21]. In this study, the expression of CyclinD1, Bcl-2 and caspase-3 in miR-20a-5p group were significantly increased, which indicated that miR-20a-5p induced the proliferation of cancer cells and inhibited their apoptosis by regulating the expression of CyclinD1, Bcl-2 and caspase-3.

To sum up, the expression of miR-20a-5p in colon cancer tissues and cells increased, and the expression of its target gene BRMS1L in colon cancer tissues and cells increased. Over-expression of miR-20a-5p could enhance the proliferation of colon cancer cells and inhibit their apoptosis, which provided a theoretical and experimental basis for the treatment of colon cancer by inhibiting the expression of miR-20a-5p.

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Conflicts of interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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Author contributions

XT and YL performed the experiments and wrote the article. YW, JH, XL, JL and XZ performed the experiments.

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