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SPTBN2 regulated by miR-214-3p inhibits the proliferation and migration of colorectal cancer cells

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ABSTRACT

Colorectal cancer (CRC) is one of the most common and lethal malignancies. According to our analysis in the GEPIA database, SPTBN2 was found to be significantly elevated in COAD patients.Western blot also verified this result, and SPTBN2 was highly expressed in two types of colorectal cancer cells, Caco2 and HCT-8. Therefore, we knocked down SPTBN2 to investigate its function in colorectal cancer, and the results of CCK-8 and Transwell assays showed that SPTBN2 deletion inhibited the proliferation, migration and invasion of CRC cells. In addition, we found that SPTBN2 may be a target of miR-214-3p through the staebase database. miR-214-3p inhibitors promote CRC cell proliferation, migration and invasion. And inhibition of SPTBN2 partially reversed the effect of miR-214-3p in CRC. Taken together, we demonstrated that SPTBN2 acts as an important target of miR-214-3p in CRC. Our study lays the foundation for the mechanism of CRC.

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Introduction

In terms of incidence, colorectal cancer (CRC) ranks third worldwide, but it is the second leading cause of cancer-related death. According to multiple health institutes on the incidence of cancer in various countries, there would be around 1.9 million new cases of colorectal cancer in 2020, with an estimated 940,000 related deaths (1). The high incidence of colorectal cancer is associated with age, obesity, diet and lifestyle (2-7). The current treatment options for colorectal cancer include radical surgical resection, local radiotherapy and systemic or targeted chemotherapy. Despite the numerous current treatment options, most clinical patients fail to achieve good therapeutic benefits. Therefore, there is an urgent need for an in-depth study of the pathogenesis of colorectal cancer.

SPTBN2, which is made up of two and two spectrin subunits, is an important part of the cell membrane cytoskeleton. A protein of the spectrin family is encoded by the SPTBN2 gene. SPTBN2 is implicated in a number of biological processes related to carcinogenesis, and studies have revealed that it is increased in MPNST, colorectal cancer, and ovarian cancer(8-10). SPTBN2 is essential to cancer pathogenesis and has been discovered as a marker gene that can aid in the study and early diagnosis of the disease (11). By interacting with CLDN4, SPTBN2 activates the PI3K/AKT pathway, which in turn stimulates the growth of endometrial cancer tumors, according to Wang et alresearch .'s (12). Zhou et al. found that inhibiting SPTBN2 expression led to a decrease in thyroid cancer cell proliferation, migration, and invasion, as well as apoptosis (13). Notably, Zhao et al. discovered that colorectal cancer cells had considerably higher levels of SPTBN2 expression and that overexpressing SPTBN2 reversed the malignant behavior that was brought on by CERS6-AS1 deletion in these cells (14). SPTBN2's exact molecular mechanism in colorectal cancer is unclear, nevertheless.

MicroRNAs (miRNAs) usually consist of 20-30 nucleotides, which are not involved in encoding protein molecules and typically act on mRNAs to influence cellular functions, mainly regulating mRNA stability and translation processes. Mammalian miRNAs generally act in vivo to buffer cells and tissues against stress, thus maintaining dynamic homeostasis in the body. miRNAs are important diagnostic indicators and therapeutic targets in human illness states to maintain their function in stress-activated pathways (15). MiRNAs have been linked to tumor development and metastasis in a number of human malignancies, according to several studies (16, 17). As regulators of vital biological processes like tumor cell proliferation, migration, invasion, and apoptosis, miRNAs play a pivotal role in the etiology of colorectal cancer (18-22). For example, in colorectal cancer, miR-15a and miR-16-1 inhibit B-cell aggregation and promote CDB+ T-cell proliferation and activation (23). miR-145 overexpression reduced the migratory ability and invasion of human CRC cells (24). miR-21 antagonizes the PI3K/Akt pathway through the downregulation of PTEN and RECK, thereby affecting colorectal cancer cell proliferation, adhesion, migratory invasion, metabolism and anti-apoptosis (25). miR-29b inhibits CRC cell proliferation, induces apoptosis, and mediates epithelial-mesenchymal transformation (EMT) inhibition (18, 19). miR-29a inhibits colorectal cancer cell metastasis by targeting KLF4 to inhibit MMP2 and

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upregulating E-calmodulin (20). The suppression of CRC cell migration and invasion can be prevented by lowering AMFR and NOTCH1 expression (21). There are only a few findings on crucial miRNAs in CRC, though.

According to the current study, SPTBN2 was increased in CRC and its knockdown greatly reduced colon cancer cells' ability to proliferate, migrate, and invade. Inhibition of miR-214-3p reversed the suppression of colon cancer caused by low levels of SPTBN2, as suggested by the underlying mechanism. SPTBN2 may therefore cause cancer through miR-214-3p. Therefore, we provide the groundwork for CRC carcinogenesis by illuminating the function and probable mechanism of SPTBN2 in CRC.

Materials and Methods

Cultured cells

The American Type Culture Collection (ATCC) provided the human normal colorectal epithelial cell line FHC, as well as the colorectal cancer cell lines Caco-2, T84, and HCT-8. Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin at 37 °C in a 5% CO2 incubator.

Analysis with bioinformatics

SPTBN2 expression levels in COAD and READ tissues were predicted using the GEPIA website (http://gepia.cancer-pku.cn/). The expression levels of SPTBN3 and miR370-3p in COAD tissues were analyzed using Starbase (http://starbase.sysu.edu.cn/).

Transfection of cells

We purchased short hairpin RNAs targeting miR-214-3pb-3p and SPTBN2 from Tsingke Biotechnology in Beijing, China. Colorectal cancer cells were transfected with the help of Lipofectamine 2000 (Invitrogen, Carlsbad, CA).

qRT-PCR

Trizol was used to extract RNA from colon cancer cells for 2.4 qRT-PCR. Total RNA was used to generate cDNA, which was subsequently used as a template for quantitative polymerase chain reaction. The ABI one-step plus real-time PCR system employed the SYBR® Premix Ex TaqTM kit for detection. The impact of miR-214-3p on SPTBN2 expression was analyzed. Here, the 2-t method is used to examine the amount of target mRNA expression. The order of these primers is shown in Table 1.

CCK-8

Cells were transfected and inoculated in 96-well plates for cell culture, and approximately 5000 cells were implanted in each well. After transfection and different treatment of each group, the cells were incubated for 48 h at room temperature. 1:9 (CCK-8 solution:medium) pre-warmed mixed medium was added to each well. 1-4 h later, the proliferation level of each group of colon cancer cells was detected by an enzyme plate analyzer.

Transwell assays

Transwell tests were carried out in Corning, USA, Transwell chambers using Matrigel for migration and without Matrigel for invasion. The lower chamber was filled with 10% fetal bovine serum-containing DMEM. In the upper wells, cells were grown for 12 hours in serum-free media. In wells covered with matrix gel and left in place for 24 hours, cell invasion was evaluated. Cells on the lower side of the membrane were stained for 30 minutes with 0.1% crystal violet after being fixed in 4% paraformaldehyde. Cell quantification was then estimated using images taken under a microscope.

Statistical analysis

The data was processed using Graphpad 8.0 and SPSS (version: 26.0). One-way ANOVA and a t-test were used to compare the data from various groups to one another. $*p < 0.05$, $**p < 0.01$,$***p < 0.001$.

Results

SPTBN2 is highly expressed in CRC cells

SPTBN2 was considerably elevated in COAD and READ tissue samples, according to GEPIA and Starbase database studies, which were utilized to determine whether SPTBN2 is involved in CRC (Figure 1A, B). Western blot results analysis proved that compared to FHC cells, colorectal cancer cell lines in which SPTBN2 was upregulated (Figure 1C-D).

Knockdown of SPTBN2 inhibits proliferation, migration, and invasion of colorectal cancer cells

To investigate the role of SPTBN2 in colon cancer, we used knockdown of SPTBN to explore its effect on the phenotype of colon cancer cells. First, short hairpin RNA was transfected to silence SPTBN2 expression, and the knockdown efficiency of sh-SPTBN2-1/-2 in colorectal cancer cells was verified by western blot (Figure 2a-b). The sh-SPTBN2-1 with higher knockdown efficiency was selected to investigate its function. CCK-8 showed that the knockdown of SPTBN2 inhibited the proliferation of colon cancer cells (Figure 2C). Using transwell assays, the knockdown of SPTBN21 was found to reduce the number of CRC cells that had migrated or invaded (Figure 2D-G). These results demonstrate SPTBN2's oncogenic function in CRC.

Table 1. Primer sequence for RT-PCR.

Gene	Primer sequence
SPTBN2	Forward: 5′- AGAGATACTGCCAAAGCCT-3′
	Reverse: 5′-TCTGCTCCTTGAGGAACTG-3′
miR-214-3p	Forward: 5′-ACAGCAGGCACAGACAGG-3′
	Reverse: 5′-GTGCAGGGTCCGAGGT- 3′
GAPDH	Forward: 5′–CTTCTTTTGCGTCGCCAGCC–3′
	Reverse: 5′-TTCTCAGCCTTGACGGTGCC- 3′

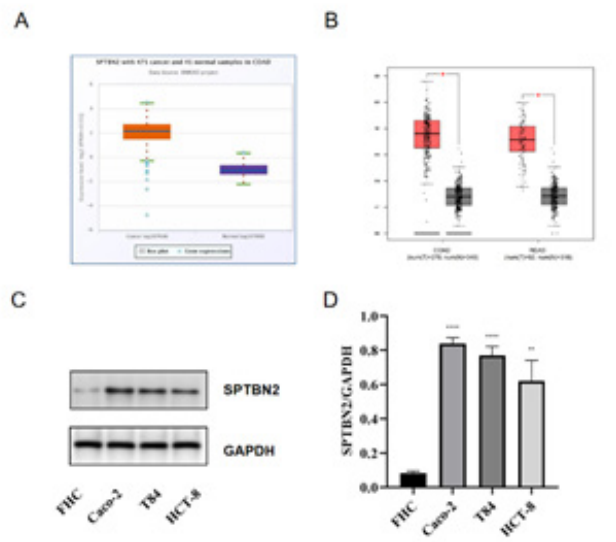

Figure 1. SPTBN is upregulated in CRC. A. GEPIA predicts the expression profile of SPTBN2 in COAD and READ. B. Starbase shows SPTBN2 expression in COAD. C-D. Western blot detects SPTBN2 expression in CRC cells and FHC. ImageJ Quantitative analysis.

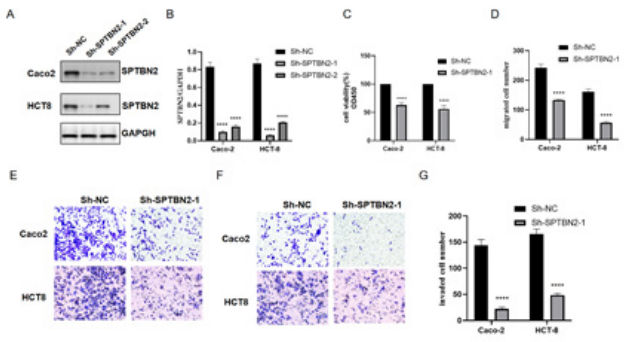

Figure 2. Lack of SPTEN2 inhibits cell proliferation, migration and invasion in Caco2 and HCT-8. A-B. Western blot assay to detect the transfection efficiency of SPTBN2. imagaJ quantitative analysis. C. CCK-8 assessment of the proliferative capacity of sh-SPTBN2-1 transfected colorectal cancer cells. D-G, transwell assay to assess the migration and invasion of CRC cells.

SPTBN2 is associated with miR-214-3p in colorectal cancer cells

The rationale for aberrant SPTBN2 expression in colorectal cancer was investigated by examining miRNA dysregulation. SPTBN2 was predicted to bind to miR-214-3p using starbase, and expression was reduced in COAD (Figure 3A-B). qRT-PCR analysis, miR-214-3p expression was also reduced in colorectal cancer cells (Figure 3C). To further investigate whether SPTBN2 is regulated by miR-214-3p. We used miR-214-3p inhibitor (figure 3D) and detected the expression of SPTBN2 after downregulating miR-214-3p. The results showed that miR-214-3p inhibitor increased the expression of SPTBN2 (Figure 3E).

The role of SPTBN2 in colorectal cancer is regulated by miR-214-3p

Using rescue studies, we tested the hypothesis that SPTBN2 is a miR-214-3p target with functional significance. Experiments using the cell proliferation assay CCK-8 demonstrated that miR-214-3p inhibitor enhanced CRC cell growth, but the knockdown of SPTBN2 reversed the impact of miR-214-3p on CRC cell proliferation (Figure 4A). Meanwhile, cutting down SPTBN2 reversed miR-214-3p's effects on colorectal cancer cells' migration and invasiveness. These results imply that miR-214-3p's influence on colorectal cancer cell proliferation and migration can be abolished by silencing SPTBN2(Figure 4B-E).

Discussion

CRC is a common gastrointestinal tumor worldwide and a serious health problem that threatens the health of different populations in recent years. Because of its high morbidity and mortality, CRC has always been a topic of concern and research for biologists and medical scientists. The incidence of CRC is reported to be low in people under 50 years of age, but it increases significantly with age. In industrialized nations, a CRC diagnosis often occurs at the age of 70. Some nations in South Asia, Central Asia, and Africa have the lowest incidence of CRC, whereas other countries in Europe, North America, and Oceania have the highest (1). There is no single risk factor that can be used

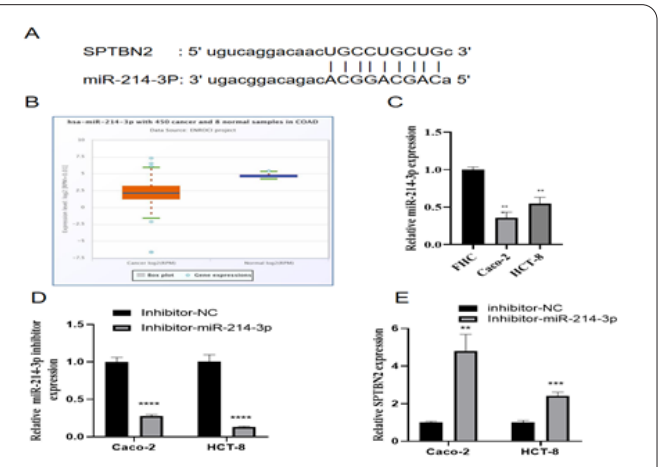

Figure 3. miR-214-3p targets SPTBN2 in colorectal cancer. The binding location of SPTBN2 to miR-214-3p has been predicted using Starbase. MiR-214-3p is expressed in COAD, as shown by B. Starbase. The expression of miR-214-3p may be detected in CRC and FHC cells using quantitative real-time polymerase chain reaction (qRT-PCR). D, quantitative RT-PCR to evaluate miR-214-3p inhibitor performance. E. quantitative real-time polymerase chain reaction to analyze SPTNB2 expression following miR-214-3p inhibition.

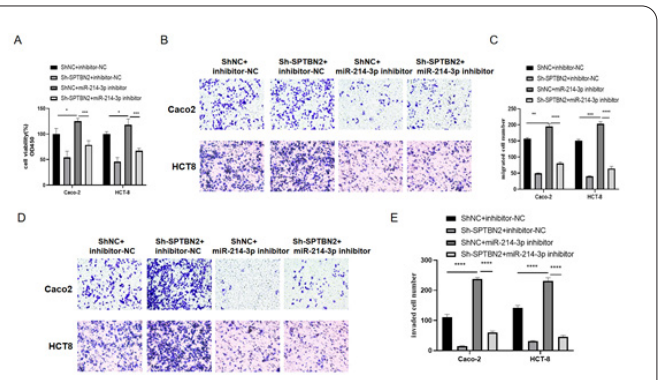

Figure 4. miR-214-5p inhibits CRC cell growth. However, this effect may be partially reversed by downregulating SPTBN2. Reducing SPTBN2 expression can counteract miR-214-3p's inhibitory effect on cell growth in a CCK-8 experiment. The effects of miR-214-3p on cell migration and invasion are attenuated when SPTBN2 is downregulated, as shown by the Transwell experiment.

to predict who may develop CRC. Family history of CRC, inflammatory bowel illness, obesity, diabetes, smoking, excessive intake of red and processed meat, and excessive alcohol consumption are all risk factors for developing CRC, according to epidemiological research (2-5). First-degree relatives had the highest risk of developing CRC, and those with inflammatory bowel illness had the highest chance of developing CRC if they had a first-degree relative with the disease, especially if the condition was diagnosed at a young age or if numerous first-degree relatives had CRC. The relevance of genetic variables in CRC's etiology is becoming clearer. A large sample of twins suggests that the hereditary component of CRC risk accounts for 34.35 percent of the disease. In addition to being a common cause of familial adenomatous polyposis and hereditary non-polyposis colon cancer, genetic factors account for fewer than 5% of CRC occurrences overall, making it unclear whether genes have a role in predicting disease risk. The etiology and progression of CRC are clearly influenced by both environmental and genetic factors. As a result, learning more about CRC might benefit from determining risk factors and locating biomarkers.

Current research on SPTBN2 has focused on aspects in neurodegenerative diseases (11, 22). Spinal cerebellar ataxia type 5 is caused by mutations in this gene (26). More than 90% of malignant peripheral nerve sheath tumors (MPNST) have been reported to express SPTBN2, in contrast to normal peripheral nerves and benign neurofibromas. Therefore, SPTBN2 may serve as a biomarker for the diagnosis of MPNST (8). In addition, SPTBN2 is highly expressed in patients with lymph node metastases and advanced colorectal cancer, both of which are associated with a dismal prognosis (9). Recent research has revealed that SPTBN2 expression is increased in ovarian cancer, plays a significant role in a number of biological processes connected to carcinogenesis, and is strongly correlated with a bad prognosis (10). SPTBN2 was discovered to be significantly expressed in colon cancer cells in our study, and its knockdown prevented cell proliferation, migration, and invasion. Colorectal cancer patients have SPTBN2 proposed as a possible therapeutic target.

Although there are many potential influences on gene expression, post-transcriptional gene regulation involving microRNAs is still mostly understood (27). This is because their interactions with target genes are made possible by their synergistic combinatorial associations with microRNAs. The importance of microRNAs (miRNAs) in development and cellular homeostasis control, among other biological processes, is becoming increasingly clear (28). miRNAs control target mRNAs and fine-tune the production of proteins. As a result, dysregulation of miRNA function can cause a number of human disorders, including Alzheimer's disease and malignancies of the head and neck, breast, lung, prostate, colon, and other organs (29-33). To comprehend the normal biological activities of miRNAs and their significance in the emergence of disease, it is essential to identify miRNA targets and their functional regulatory networks. In this study, we used Starbase for target analysis and selected miR-214-3p as a possible upstream regulator of SPTBN2 for validation. Targeting the mediator complex subunit 19 (MED19) has been linked to tumor suppressive effects of miR-214 in colorectal cancer (34). Furthermore, inhibiting the PLAGL2-MYH9 axis with microRNA-214-3p reduces human colorectal cancer growth and metastasis (35-39). In both COAD and CRC cells, we identified high levels of miR-214-3p expression. A quantitative real-time polymerase chain reaction investigation found that silencing miR-214-3p significantly boosted SPTBN2 expression. Inhibition of miR-214-3p also aided in the growth, migration, and invasion of colorectal cancer cells. However, miR-214-3p inhibitor effects were considerably recovered by SPTBN2 deletion.

Conclusion

All things considered, our research indicates. Inhibiting SPTBN2 expression reduces colorectal cancer cell growth, migration, and invasion. miR-214-3p is responsible for controlling this impact. These results might lead to novel hypotheses on the processes behind the dysregulation that causes colorectal cancer.

Consent for publications

The author read and proved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

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Conflict of Interests

The authors have declared no conflict of interest exists.

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