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ORIGINAL ARTICLE

Basic Study

MicroRNA-363-3p inhibits colorectal cancer progression by targeting interferon-induced transmembrane protein 1

Yun Wang, Shao-Kai Bai, Tao Zhang, Cheng-Gong Liao

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Abstract

BACKGROUND

The molecular mechanisms of colorectal cancer development and progression are far from being elucidated.

AIM

To investigate the role of microRNA-363-3p (miR-363-3p) in the progression of colorectal cancer.

METHODS

Real-time polymerase chain reaction was performed to detect miRNA expression in human colorectal cancer tissues and paired normal colorectal tissues. PITA 6 was utilized to predict the targets of miR-363-3p. Dual-luciferase reporter system was used to validate the target of miR-363-3p. Plate colony formation assay and wound-healing assay were performed to evaluate cancer cells' clonogenic survival ability and migration ability, respectively. Cell proliferation was examined by cell counting kit-8 assay. Immunohistochemical staining was used to determine the expression level of interferon-induced transmembrane protein 1 (IFITM1) in colorectal cancer tissues and adjacent tissues. The TCGA and GTEx databases were used to compare the expression levels of *IFITM1* mRNA in colorectal cancer tissues and normal colorectal tissues and analyze the correlation between the expression levels of IFITM1 mRNA and overall survival and disease-free survival of patients. A colorectal cancer cell line with a deficiency of IFITM1 was constructed, and the regulation effect of IFITM1 on the clonogenic growth of colorectal cancer cells was clarified.

RESULTS

MiR-363-3p was decreased in colorectal cancer tissues compared to normal colorectal tissues. IFITM1 was characterized as a direct target of miR-363-3p. Overexpression of miR-363-3p led to decreased clonogenic survival, proliferation, and migration of colorectal cancer cells, which could be reversed by forced

IFITM1 expression.

CONCLUSION
MiR-363-3p can constrain clonogenic survival, proliferation, and migration of colorectal cancer cells *via* targeting IFITM1.

Key Words: MicroRNA-363-3p; Proliferation; Clonogenic survival; Colorectal cancer; Interferon-induced transmembrane protein 1

Core Tip: MicroRNAs (miRNAs) have been implicated in almost all known cancer processes. Although many algorithms can predict target genes for miRNA, the exact regulatory relationships still need to be experimentally verified. In this study, we investigated the role of miR-363-3p in clonogenic survival, proliferation, and migration of colorectal cancer cells and interferon-induced transmembrane protein 1 (IFITM1) was identified as a direct target of miR-363-3p. These findings widen and deepen the understanding of the molecular function of miR-363-3p and IFITM1.

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INTRODUCTION

Colorectal cancer is the third most common cancer type worldwide; in 2020, almost 2 million cases were diagnosed. Colorectal cancer is the second most common cause of cancer death, leading to almost 1 million deaths per year, accounting for approximately 10% of new tumor cases and deaths[1]. In 2019, China (607900), the United States (227242), and Japan (160211) had the highest number of new cases of colorectal cancer, and China (261777), India (79098), and the United States (84026) had the highest number of colorectal cancer deaths[2]. Low-dairy diets (15.6%), smoking (13.3%), low-calcium diets (12.9%), and alcohol consumption (9.9%) are important risk factors for colorectal cancer[2], but the molecular mechanisms of colorectal cancer development and progression are far from being elucidated.

MicroRNAs (miRNAs) are small endogenous non-coding RNAs (ncRNAs) of about 22 nucleotides in size. miRNAs play important roles in gene regulation *via* a posttranscriptional manner, and their dysregulation is implicated in various human diseases including cancer. It is estimated that miRNAs can target more than 60% of human protein-coding genes [3]. Mechanically, miRNAs prevent the translation of target mRNAs that are then sequestered into mRNA-processing bodies (P-bodies) and degraded. MiRNAs also contribute to the degradation of the target mRNAs without sequestration to P-bodies[4]. The specificity of miRNA - mRNA interaction is bestowed mainly by a miRNA's first eight nucleotides (known as seed sequence)[5]. Over the past period, miRNAs have been implicated in almost all known cancer processes. Depending on the target gene and tumor type, some miRNAs typically negatively affect oncogenes encoding proteins, while some other miRNAs can inhibit known tumor suppressors, so miRNAs can act as onco-miRNAs or tumor suppressor miRNAs[6]. For example, miR-100 and miR-125b coordinately repressed five Wnt/β-catenin negative regulators, resulting in increased Wnt signaling in colorectal cancer[7]. MiR-146a targets PTGES2 and suppresses colorectal cancer[8]. Recently, miR-363-3p was reported to participate in the regulation of a variety of diseases. In addition, the downregulation of miR-363-3p is closely correlated with the degree of differentiation, tumor-node-metastasis stage, and lymph node metastasis in gastric cancer[9]. Overexpression of miR-363-3p is a strong predictor of favorable prognosis in adenocarcinoma of the uterine cervix[10]. MiR-363-3p suppresses tumor growth and metastasis of colorectal cancer *via* targeting sphingosine kinase 2[11] and SRY-related high-mobility-group box 4 (SOX4)[12]. In contrast, the expression of miR-363-3p was increased in glioma[13] and pediatric T-cell acute lymphoblastic leukemia [14]. MiR-363-3p functions as onco-miRNA promotes cell proliferation, protects against apoptosis, and enhances invasion by directly targeting PDHB in glioma[13] and PTEN and BIM in leukemic cells[14]. Based on previous studies, we speculated that miR-363-3p might exert an essential effect on colorectal cancer progression.

Interferon-induced transmembrane protein 1 (IFITM1), also known as DSPA2a and CD225, is a member of the interferon-induced transmembrane protein family. Friedman *et al*[15] first identified the IFITM1 gene in neuroblastoma cells. The IFITM1-coding gene is located at 11p15.5, and the IFITM1 protein consists of 125 amino acid residues with a molecular weight of about 13.96 kDa, including the C-terminal extracellular domain, two transmembrane domains, and N-terminal intracellular domains. Li *et al*[16] found higher levels of IFITM1 expression in gallbladder adenocarcinoma and adenosquamous cell carcinoma tissues with high tumor-node-metastasis (TNM) stage and with lymph node metastasis and invasion. In estrogen receptor (ER)-positive breast cancer, IFITM1 expression levels are associated with TNM staging and poor prognosis[17]. Therefore, IFITM1 is closely related to the occurrence and development of tumors, but the regulation and clinical significance of IFITM1 in colorectal cancer tissues still need to be studied in depth. In this

study, we investigated the role and the underlying mechanisms of miR-363-3p in the clonogenic survival, proliferation, and migration of colorectal cancer cells. To our knowledge, this is the first study to identify IFITM1 as a direct target of miR-363-3p.

MATERIALS AND METHODS

Cell culture

Human colorectal cancer cell lines SW480, SW1116, Colo320, and Caco-2 were obtained from American Type Culture Collection and cultured in RPMI 1640 medium (Thermo Fisher Scientific, Waltham, MA, United States) containing 10% fetal bovine serum (Thermo Fisher Scientific) and Penicillin-Streptomycin (Thermo Fisher Scientific). IFITM1 knockout SW480 cell line (SW480KO) was generated using the CRISPR/Cas9 system. gRNA targeting sequence was 5′-CCGCTGT-GGTGTCCGGATGC-3′. SW480 cells were transfected with PX459 V2.0 containing the gRNA sequence using Lipofectamine 2000 (Invitrogen, Waltham, MA). After 48 h of transfection, the positive cells were selected with puromycin at 2 μg/mL for 5 d. The puromycin-resistant cells were seeded into a 96-well plate at one cell per well using CytoFLEX SRT (Beckman, Brea, CA). The knockout cells were confirmed by western blotting. All cultures were maintained at 5% CO_2 and 37 °C.

Tissue samples

Colorectal cancer tissues and adjacent normal tissues were obtained from patients at Tangdu Hospital, Air Force Medical University. All human individuals provided written informed consent. The study was approved by the Hospital Ethics Committee (202203-116). All participants (aged 42-76 years, 60% males, stages ranging from I to IVA) did not receive chemotherapy or radiation therapy before resection. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Agomir/antagomir transfection

Agomir-363-3p, agomir-NC, antagomir-363-3p and antagomir-NC were obtained from RiboBio (Guangzhou, China). Cells in the logarithmic growth phase were trypsinized, resuspended, and seeded into 6-well plates. After being cultured overnight, cells were transfected with 75 pmol agomir or antagomir using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. After 48 h, the cells were collected for subsequent analysis.

RNA isolation, cDNA transcription, and quantitative polymerase chain reaction

Total RNA was extracted using a Trizol reagent (Life Technologies, Carlsbad, CA) based on the supplier's instruction. MiRNA was reversely transcribed using the miRNA 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China). mRNA was reversely transcribed using the SuperScript™ IV First-Strand Synthesis System with ezDNase™ Enzyme (Thermo Fisher Scientific). Quantitative polymerase chain reaction (qPCR) with specific primers was performed with the SYBR kit (TaKaRa, Shiga, Japan). Samples were normalized to housekeeping expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or snRNA U6 using the $2^{-\Delta\Delta Ct}$ method. The sequences of primers were as follows: IFITM1 sense, 5′-CCAAGGTCCACCGTGATTAAC-3′; antisense, 5′-ACCAGTTCAAGAAGAGGGTGTT-3′; GAPDH sense, 5′-GCACCGT-CAAGGCTGAGAAC-3′; antisense, 5′-TGGTGAAGACGCCAGTGGA-3′; miRNA363-3p sense, 5′-AATTGCACG-GTATCCA-3′; antisense, 5′-AGTGCAGGGTCCGAGGTATT-3′; snRNA U6 sense, 5′-CTCGCTTCGGCAGCACA-3′; antisense, 5′-AACGCTTCACGAATTTGCGT-3′.

Western blotting

Cells were lysed with RIPA lysis buffer containing protease inhibitors. Protein concentration was determined using a BCA kit (Thermo Fisher Scientific). The proteins were separated using standard gel electrophoresis and blotted onto polyvinylidene fluoride membranes. Membranes were blocked with 5% nonfat milk in poly(butylene succinate-butylene terephthalate) (PBST) and incubated in primary antibody solution at 4 °C overnight. After being washed with PBST three times, the membranes were incubated with a secondary antibody at 24 °C for 45 min. Immunoblots were developed using an ECL-chemiluminescence Kit (Merck Millipore, Watford, United Kingdom), according to the manufacturer's instructions. The primary antibody against IFITM1 (5B5E2) was obtained from Proteintech (Wuhan, China). α-tubulin was used as loading control and its antibody was purchased from Cell Signaling Technology (Danvers, MA). HRP-linked secondary antibodies were obtained from Thermo Fisher Scientific. The raw blots have been included in Supplementary Figure 1.

Dual-luciferase reporter assay

The predicted binding region of miR-363-3p in IFITM1 3′ untranslated region (UTR) (pGL3-wt) or mutated targeting sequence (pGL3-mt) was ligated into the pGL3-Basic vector. SW480 cells were transfected with pGL3 construct and pRL-TK (ratio of 50 to 1) using Lipofectamine 2000 (Invitrogen). Luciferase activity was measured using Dual-Luciferase Reporter Assay System (Promega, Madison, WI) according to the manufacturer's instructions. The relative luciferase activity was calculated by dividing the results from the Firefly luciferase assay over the Renilla luciferase assay.

Plate colony formation assay

Cells were digested to prepare single-cell suspensions and seeded at 300 cells/2 mL in 6-well plates. Cells were cultured

for about 2 wk until visible clones formed. The clones were fixed with 4% paraformaldehyde at 24 °C for 15 min. After being rinsed with phosphate buffered saline (PBS) three times, the clones were stained with Coomassie brilliant blue R250 at 24 °C for 20 min. The plates were washed with PBS several times to remove the residual dye. The clones in each well were counted.

Wound-healing assay

Cells were seeded at 3 × 10^5 cells per well in 24-well plates. After the cells became confluent, a 10 μL pipette tip was used to wound the monolayer by scratching and the cells in suspension were removed by changing the medium. With a cell-free gap prepared, a series of time-lapse images were acquired as cells migrated into the cell-free gap. The change in the wound width (the average distance between the two margins of the scratch) was measured using ImageJ.

Cell proliferation assay

Cell proliferation was determined using cell counting kit-8 (CCK-8, Solarbio, Beijing, China). Cells were digested to prepare single-cell suspensions and seeded at a density of 2 × 10^3 cells/100 μL in 96-well plates. 10 μL of the CCK-8 solution was added to each well of the plate. After incubating the plate for 2 h in the incubator, the absorbance at 450 nm was measured using a microplate reader (Fluoroskan FL, Thermo Fisher Scientific). Subtraction of the blank well absorbance (absorbance of wells containing medium and CCK-8) was performed before analysis.

Immunohistochemical staining

Sections 5 μm in thickness were prepared from formalin-fixed, paraffin-embedded tissues. Paraffin sections were deparaffinized, hydrated followed by a Tris-EDTA-based antigen retrieval step, and blocked against non-specific binding using normal goat serum (Cell Signaling Technology) followed by incubation using an anti-IFITM1 antibody (5B5E2, Proteintech) at 4 °C overnight. After being washed for 20 min, the sections were treated with universal biotinylated anti-mouse/rabbit/goat IgG derived from the horse (Vector Laboratories, Burlingame, CA) at 24 °C for 30 min. Signal development was performed using the NovaRED kit (Vector Laboratories) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 (San Diego, CA, United States). Student's *t*-test was used to compare the two groups. The One-Way ANOVA was used to compare the means across three or more groups. Survival analysis was performed using the Kaplan-Meier method and compared using the log-rank test. Immunohistochemical (IHC) score was analyzed using a χ^2 test. Statistical significance was considered at $P < 0.05$.

RESULTS

MiR-363-3p is downregulated in human colorectal cancer tissues

We first determined the expression pattern of miR-363-3p in 50 colorectal cancer tissues and paired normal colorectal tissues using real-time PCR. As shown in Figure 1A, we found that the expression of miR-363-3p was decreased in colorectal cancer tissues compared to the paired normal colorectal tissues.

MiR-363-3p inhibits clonogenic survival, proliferation, and migration of colorectal cancer cells

To determine the effect of miR-363-3p on colorectal cancer progression, we first determined the expression level of miR-363-3p in human colorectal cancer cell lines (Figure 1B). As SW480 and SW1116 had the highest and lowest expression of miR-363-3p, respectively, the two cell lines were used in the subsequent experiments. We transfected SW1116 cells with agomir-363-3p and found that overexpression of miR-363-3p led to decreased clonogenic survival (Figure 1C), proliferation (Figure 1D), and migration (Figure 1E). In contrast, transfection of SW480 cells with antagomir-363-3p resulted in enhanced clonogenic survival (Figure 1F), proliferation (Figure 1G), and migration (Figure 1H). All these data suggest that miR-363-3p is a suppressive player involved in colorectal cancer progression.

MiR-363-3p directly targets IFITM1

MiRNAs are supposed to regulate various cellular behaviors by targeting specific sites in mammalian mRNAs. Using PITA 6[18], IFITM1 was predicted as a promising target for miR-363-3p (Figure 2A). We found that overexpression of agomir-363-3p in SW1116 cells significantly decreased IFITM1 expression at both mRNA and protein levels (Figures 2B and C). As expected, inhibiting miR-363-3p *via* transfecting SW480 cells with antagomir-363-3p increased IFITM1 expression (Figures 2D and E). To examine the interaction between miR-363-3p and its targeting site in IFITM1 mRNA, luciferase reporter gene assays using constructs containing the predicted targeting sequence (pGL3-wt) and mutated targeting sequence (pGL3-mt) were performed. We found that co-transfection of agomir-363-3p and pGL3-wt in SW1116 cells led to decreased luciferase activity compared with the scramble control (Figure 2F), while co-transfection of agomir-363-3p and pGL3-mt in SW1116 cells showed luciferase activity comparable to that of the scramble control (Figure 2G). All these results demonstrate that IFITM1 is a direct target of miR-363-3p.

IFITM1 mediates the regulatory effects of miR-363-3p on colorectal cancer progression

As miR-363-3p modulates IFITM1 expression, next we determined whether IFITM1 contributed to the regulatory effects of miR-363-3p on colorectal cancer progression. We generated IFITM1 knockout SW480 cell line (SW480KO) using the

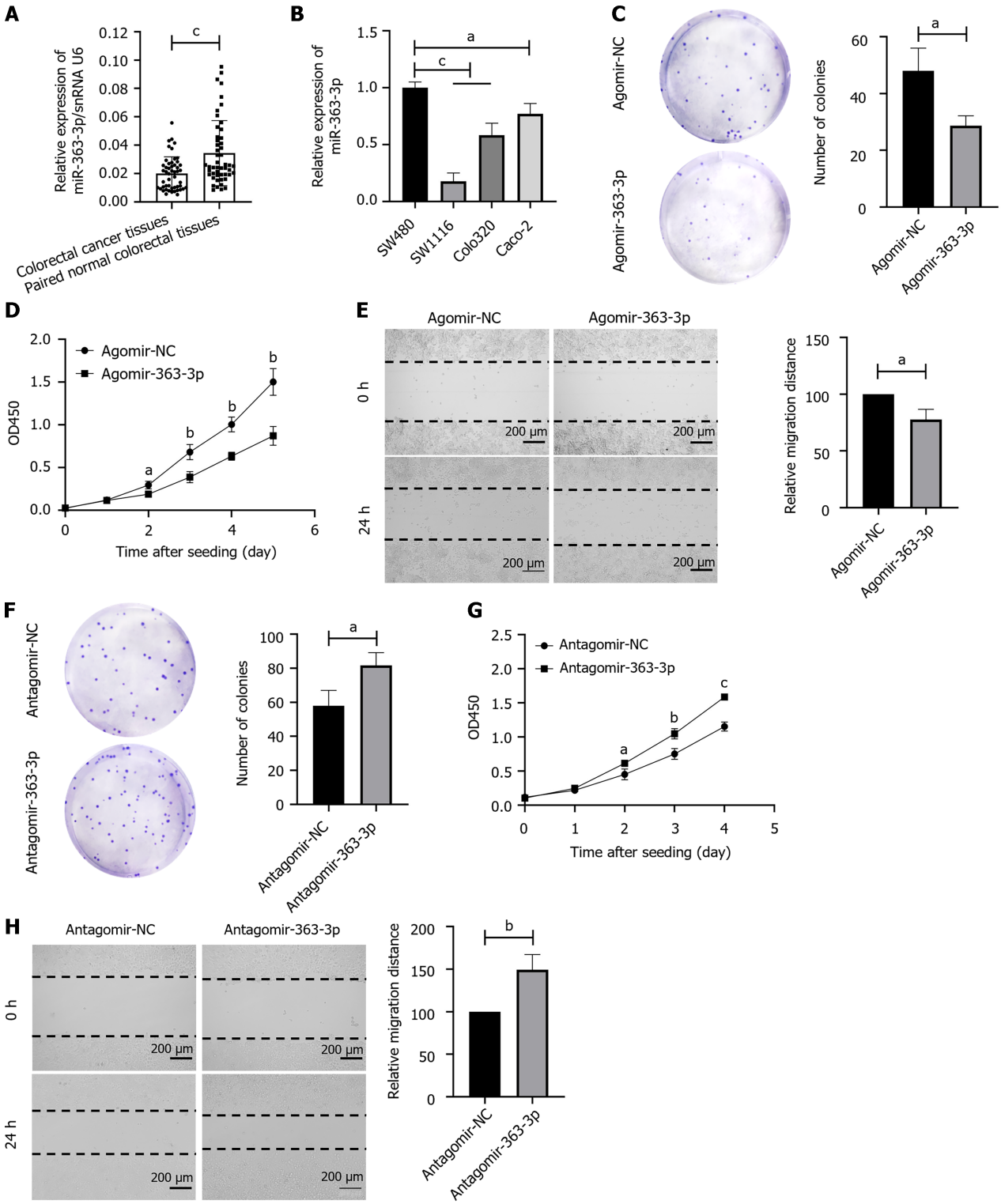

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Figure 1 MicroRNA-363-3p is downregulated in colorectal cancer tissues and inhibits clonogenic survival, proliferation and migration of colorectal cancer cells. A: Quantitative polymerase chain reaction (qPCR) analysis of microRNA-363-3p (miR-363-3p) expression in colorectal cancer tissues and paired normal colorectal tissues; B: qPCR analysis of miR-363-3p expression in human colorectal cancer cell lines; C: Representative images of clonogenic survival of SW1116 cells transfected with agomir-363-3p or agomir-NC. The graph shows the number of colonies; D: Proliferation curve of SW1116 cells transfected with agomir-363-3p or agomir-NC; E: Representative images of the gaps at 0 and 24 h after scratching. SW1116 cells were transfected with agomir-363-3p or agomir-NC. The graph shows the relative migration distance. The scale bar is 200 μm; F: Representative images of clonogenic survival of SW480 cells transfected with antagomir-363-3p or antagomir-NC. The graph shows the number of colonies; G: Proliferation curve of SW480 cells transfected with antagomir-363-3p or antagomir-NC; H: Representative images of the gaps at 0 and 24 h after scratching. SW480 cells were transfected with antagomir-363-3p or antagomir-NC. The graph shows the relative migration distance. The scale bar is 200 μm. The results were shown as the mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. miR-363-3p: MicroRNA-363-3p; NC: Negative control.

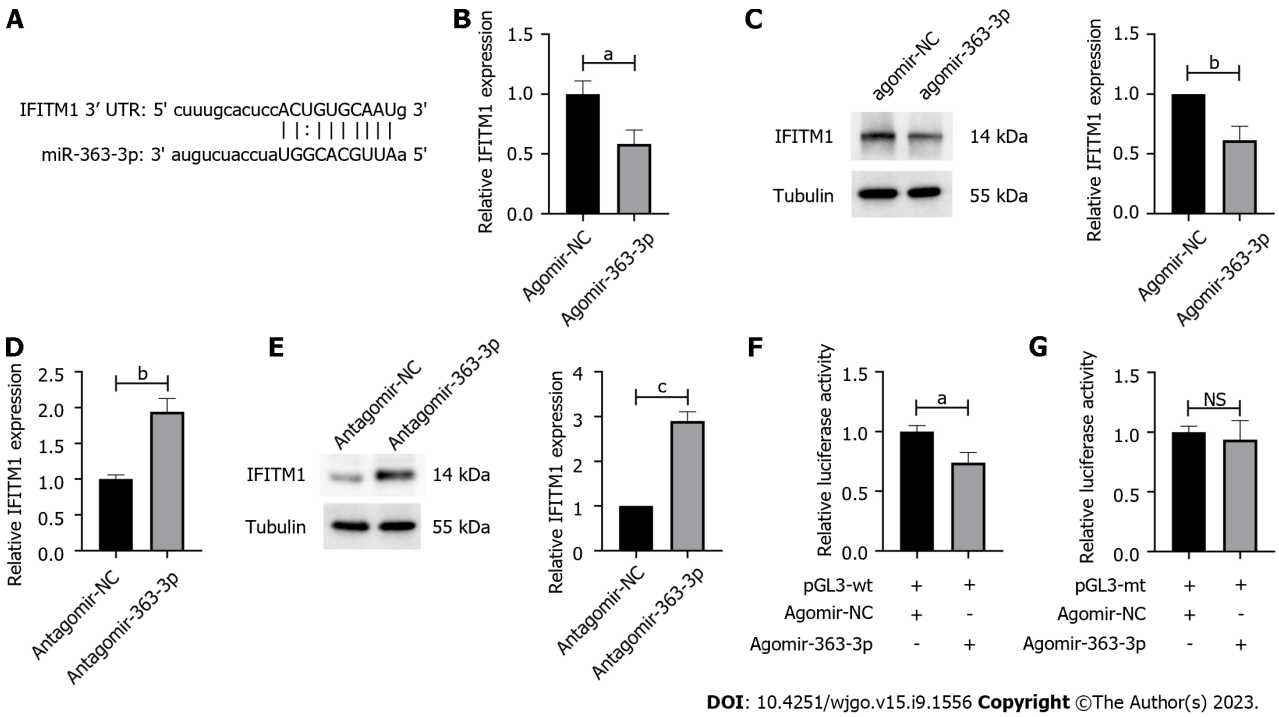

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Figure 2 Interferon-induced transmembrane protein 1 is a direct target of microRNA-363-3p. A: The complementary sequences of microRNA-363-3p were identified in 3' untranslated region of interferon-induced transmembrane protein 1 (*IFITM1*) mRNA; B: Quantitative polymerase chain reaction (qPCR) analysis of *IFITM1* mRNA expression in SW1116 cells transfected with agomir-363-3p or agomir-NC; C: Western blot analysis of IFITM1 expression in SW1116 cells transfected with agomir-363-3p or agomir-NC. Graph shows quantification of relative levels of IFITM1 expression; D: qPCR analysis of *IFITM1* mRNA expression in SW480 cells transfected with antagomir-363-3p or antagomir-NC; E: Western blot analysis of IFITM1 expression in SW480 cells transfected with antagomir-363-3p or antagomir-NC. Graph shows quantification of relative levels of IFITM1 expression; F and G: Luciferase reporter gene assays using constructs containing the predicted targeting sequence (pGL3-wt) and mutated targeting sequence (pGL3-mt). SW1116 cells were transfected with the indicated constructs. The results were shown as the mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. UTR: Untranslated region; IFITM1: Interferon-induced transmembrane protein 1; miR-363-3p: MicroRNA-363-3p; NC: Negative control; NS: Not significant.

CRISPR-Cas9 system (Figure 3A) and inhibited miR-363-3p *via* transfecting antagomir-363-3p. As mentioned above, inhibiting miR-363-3p resulted in elevated clonogenic survival, proliferation, and migration in SW480 cells, in contrast, these effects became marginal in SW480KO cells (Figures 3B-D). These results suggest that IFITM1 contributes, at least partially, to the regulatory effects of miR-363-3p on clonogenic survival, proliferation, and migration of colorectal cancer cells.

The abundance of miR-363-3p displays the negative correlation with IFITM1 expression in human colorectal cancer tissues

We identified IFITM1 as a direct target of miR-363-3p using colorectal cancer cell lines. To make this conclusion more solid, we evaluated the expression of miR-363-3p and IFITM1 in human colorectal cancer tissues. We found that the mRNA and protein expression levels of IFITM1 in colorectal cancer tissues were significantly higher than those in normal colorectal tissues (Figures 4A and B, Supplementary Table 1); TCGA data also showed that *IFITM1* mRNA expression was increased in colorectal cancer tissues (Figure 4C) and was not associated with overall survival (OS) in patients with colorectal cancer (Figure 4D), but was positively correlated with disease-free survival (DFS) in patients with rectal cancer (Figure 4E). We also evaluated miR-363-3p and *IFITM1* mRNA expression in 24 colorectal cancer tissues using qPCR. We found that *IFITM1* mRNA expression was inversely correlated to miR-363-3p expression ($R^2 = 0.2216$, Figure 4F). These results indicate that miR-363-3p negatively modulates IFITM1 expression in colorectal cancer tissues.

DISCUSSION

MiR-363-3p has been reported to be dysregulated and exert a promoting or inhibiting effect on tumor development and progression in many types of cancers. MiR-363-3p was significantly decreased in hepatocellular carcinoma (HCC)[19], papillary thyroid carcinoma[20], lung cancer[21], osteosarcoma[22], gastric cancer[9], $CD133^+$ larynx cancer stem-like cells [23] and colorectal cancer[11]. However, the underlying mechanisms behind this dysregulation are far from clear. Li *et al* [24] reported that miR-363-3p is activated by its upstream transcription activator MYB in osteoporosis pathogenesis. MiR-363-3p could be sponged by circCTNNA1[25], circ_0002111[26], lncRNA NR2F1-AS1[27], lncRNA SNHG5[28], and lncRNA MALAT1[29] in colorectal cancer, papillary thyroid carcinoma, non-small cell lung cancer, and clear cell renal cell carcinoma.

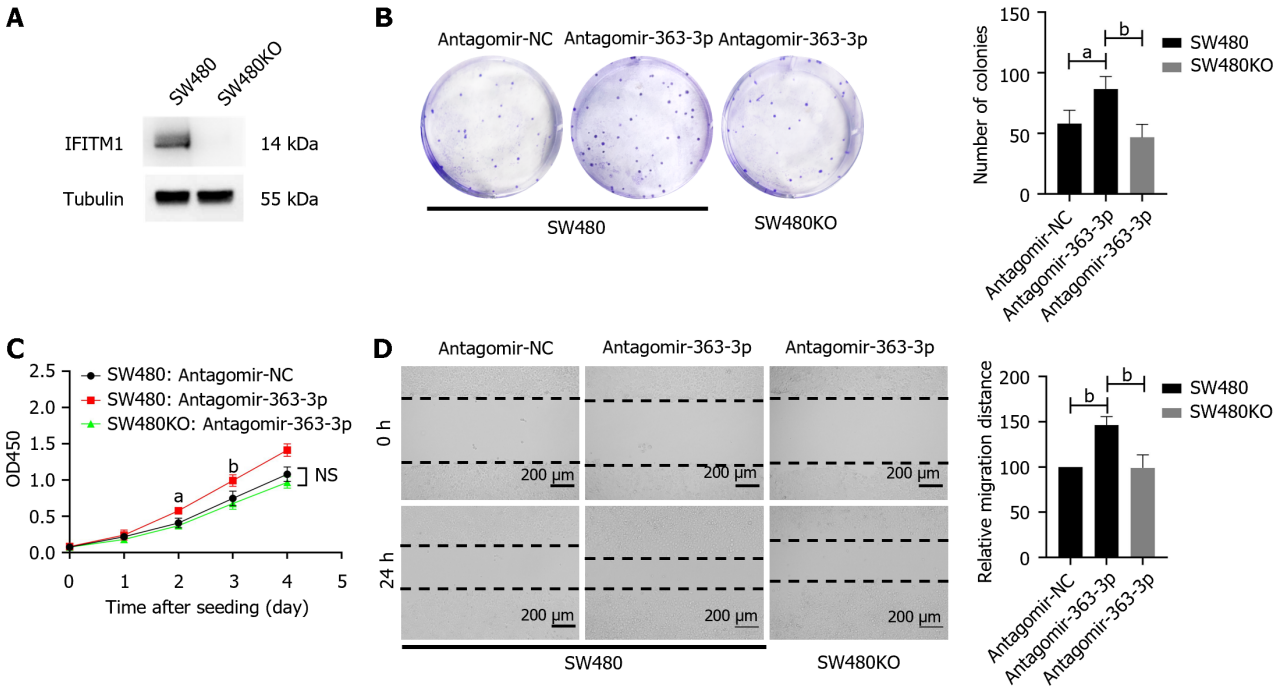

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Figure 3 Interferon-induced transmembrane protein 1 mediates the regulatory effects of microRNA-363-3p on clonogenic survival, proliferation and migration of colorectal cancer cells. A: Western blot analysis of interferon-induced transmembrane protein 1 expression in SW480 and SW480KO cells; B: Representative images of clonogenic survival of SW480 or SW480KO cells. Cells were transfected with antagomir-363-3p or antagomir-NC. The graph shows the number of colonies; C: Proliferation curve of SW480 or SW480KO cells. Cells were transfected with antagomir-363-3p or antagomir-NC; D: Representative images of the gaps at 0 and 24 h after scratching. SW480 or SW480KO cells were transfected with antagomir-363-3p or antagomir-NC. The graph shows the relative migration distance. The scale bar is 200 μm. The results were shown as the mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$. NS: No significance; IFITM1: Interferon-induced transmembrane protein 1; NC: Negative control.

MiR-363-3p inhibits tumorigenesis by directly targeting SOX4[19], high mobility group protein 2 (HMGA2)[30], USP28 [31], and specificity protein 1[32] in HCC. MiR-363-3p inhibits tumor growth by targeting mouse double minute 2[33], proliferating cell nuclear antigen[34], HMGA2[35], neural precursor cell-expressed developmentally down-regulated 9 and SOX4[36] in lung cancer. miR-363-3p suppresses anoikis resistance *via* targeting integrin alpha 6 in papillary thyroid carcinoma[20]. MiR-363-3p is induced by hypoxia-inducible factor 2alpha to promote the stemness of melanoma cells *via* inhibiting p21[37]. MiR-363-3p markedly inhibits the proliferation, migration, and invasion of osteosarcoma cells *via* targeting SOX4[22]. This study provides another piece of evidence supporting miR-363-3p as a tumor suppressor. We confirmed that miR-363-3p is downregulated in human colorectal cancer tissues and inhibits clonogenic survival, proliferation, and migration of colorectal cancer cells.

IFITM1 belongs to a family of small homologous proteins, localized in the plasma and endolysosomal membranes, which regulate T cell differentiation and function and confer cellular resistance to many viruses[38]. There is mounting evidence that IFITM1 is an oncogene. IFITM1 expression is upregulated in gastric cancer[7], aromatase inhibitor-resistant breast cancer[8], triple-negative breast cancer[9], oral squamous cell carcinoma[10], and non-small cell lung cancer[11]. Furthermore, IFITM1 expression levels are closely related to patient outcomes[7,12]. IFITM1 regulates diverse aspects of tumorigenesis and progression, such as tumor cell proliferation, invasion, angiogenesis, metastasis, and therapeutic resistance, indicating that IFITM1 is a promising therapeutic target, and inhibiting IFITM1 (*e.g.*, blocking IFITIM1 by antibody, suppressing IFITM1 expression by oligonucleotides, targeted IFITIM1 degradation using bifunctional small molecules) may be a promising strategy for cancer treatment. In colorectal cancer, the elevated IFITM1 expression significantly correlates with colorectal cancer lymph node and distance metastasis, a more advanced clinical stage as well as a shorter OS[39]. However, TCGA data showed that increased *IFITM1* mRNA expression was not associated with OS in patients with colorectal cancer (Figure 4D), but was positively correlated with DFS in patients with rectal cancer (Figure 4E). He *et al*[40] reported that high expression of IFITM1 is associated with poor prognosis of rectal cancer, and no association was found between IFITM1 expression and the prognostic significance with patients with colon cancer. This discrepancy may be due to several factors. First, analysis using TCGA data focuses on *IFITM1* mRNA expression, however, the level of mRNA expression is not exactly the same as the level of protein expression. Secondly, the antibodies used for IFITM1 detection and the scoring criteria for IHC staining are not exactly the same. Finally, patient survival is associated with many factors, and tumors are highly heterogeneous.

In vitro assays revealed that IFITIM1 promotes migration[41] and invasion[39] of human colorectal cancer *via* caveolin-1. Apc mutation induces the expression of IFITM1 and high expression of IFITM1 reduces the uptake of fibroblast extracellular vesicles[42]. In this study, we identified miR-363-3p as a new epigenetic modulator of IFITM1. We revealed the binding site of miR-363-3p in IFITM1 3′ UTR region and proved that the expression of IFITM1 can be efficiently

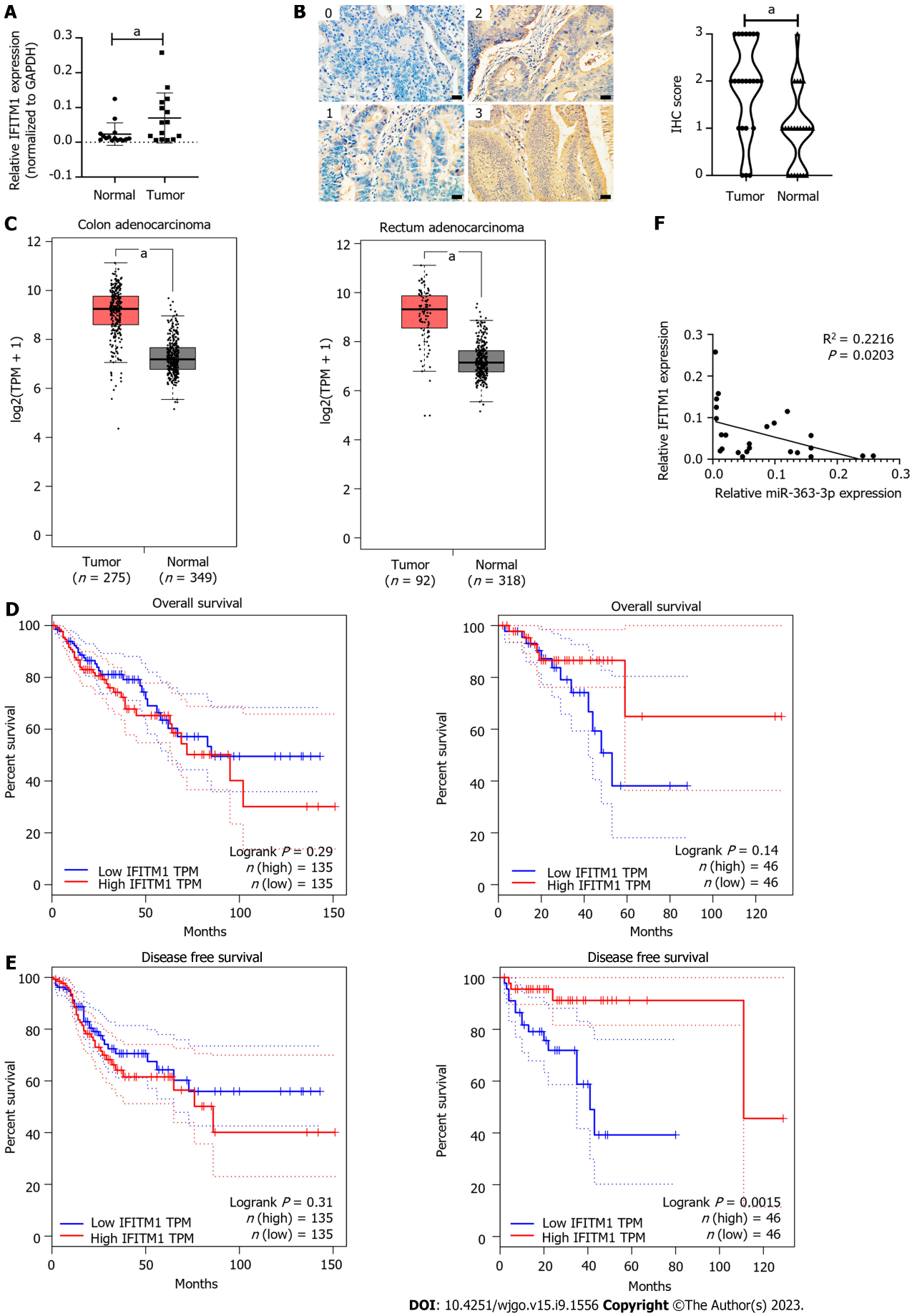

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Figure 4 Expression levels of significance and interferon-induced transmembrane protein 1 are negatively correlated in human colorectal

cancer tissues. A: Quantitative polymerase chain reaction analysis of interferon-induced transmembrane protein 1 (*IFITM1*) mRNA expression in colorectal cancer tissues and paired normal colorectal tissues; B: Representative images of IFITM1 expression detected by immunohistochemical staining. Immunohistochemical (IHC) scores are shown in the upper left corner, and the scale bar is 50 μm. Violin plots show statistical analysis of IHC scores; C: *IFITM1* mRNA expression levels in colon cancer and normal colon tissues (left panel), rectal cancer, and normal rectal tissues (right panel) were analyzed using TCGA and GTEx databases; D: Correlation analysis of *IFITM1* mRNA expression level and overall survival in patients with colon cancer (left panel) or rectal cancer (right panel); E: Correlation analysis of *IFITM1* mRNA expression level and disease-free survival in patients with colon cancer (left panel) or rectal cancer (right panel); F: Correlation analysis of *IFITM1* mRNA expression level and microRNA-363-3p expression level. The results were shown as the mean ± SD. $^{a}P < 0.05$. IHC: Immunohistochemical; IFITM1: Interferon-induced transmembrane protein 1; miR-363-3p: MicroRNA-363-3p; NC: Negative control.

inhibited by miR-363-3p and that the negative regulatory relationship exists in human colorectal cancer tissues. Moreover, a deficiency of IFITM1 can abolish the regulatory effects of miR-363-3p on clonogenic survival, proliferation, and migration of colorectal cancer cells. It would be interesting to further investigate whether the regulatory relationship between miR-363-3p and IFITM1 is common to other types of cancer, and the related lncRNA or circRNA.

CONCLUSION

Taken together, we identified that the expression of miR-363-3p and IFITM1 was downregulated and upregulated in colorectal cancer, respectively. Furthermore, IFITM1 is a direct target of miR-363-3p and the inhibitory effect of miR-363-3p on colorectal cancer progression is, at least partially, attributed to IFITM1 downregulation. We acknowledge several limitations in the present study. First, we didn't determine the contribution of the miR-363-3p/IFITM1 axis to colorectal cancer progression using *in vivo* models. Second, whether the negative regulatory relationship between miR-363-3p and IFITM1 is prevalent in different kinds of tumors has to be further studied. Last, miR-363-3p is dysregulated in numerous tumors including colorectal cancer, however, the underlying mechanism was not further explored in the current study.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer is the second most common cause of cancer death, however, the molecular mechanisms of tumorigenesis and development of colorectal cancer are far from being elucidated.

Research motivation

MicroRNAs play important roles in gene regulation and modulate numerous physical and pathological processes. The motivation of this study is to reveal the role of microRNA-363-3p (miR-363-3p) in the development of colorectal cancer and the underlying mechanisms.

Research objectives

Compare the expression of miR-363-3p between colorectal cancer tissues and adjacent normal tissues; clarify the role of miR-363-3p in clonogenic survival, migration, and proliferation of colorectal cancer cells; identify the direct target of miR-363-3p in colorectal cancer cells.

Research methods

Real-time polymerase chain reaction was performed to detect miRNA expression. PITA 6 was utilized to predict the targets of miR-363-3p. Dual-luciferase reporter system was used to validate the target of miR-363-3p. Plate colony formation and wound-healing assays were performed to evaluate cancer cells' clonogenic survival and migration ability, respectively. Cell proliferation was examined by cell counting kit-8 assay. Immunohistochemical staining was used to determine the expression level of interferon-induced transmembrane protein 1 (IFITM1).

Research results

MiR-363-3p was decreased in colorectal cancer tissues. IFITM1 was characterized as a direct target of miR-363-3p.

Research conclusions

MiR-363-3p inhibits clonogenic survival, proliferation, and migration of colorectal cancer cells *via* targeting IFITM1.

Research perspectives

MiR-363-3p/IFITM1 axis may represent a therapeutic target in colorectal cancer.

FOOTNOTES

Author contributions: Wang Y and Bai SK designed and performed the assay; Zhang T analyzed the data; Liao CG designed the study and prepared the manuscript.

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