Original Article

In vitro study of miRNA-369-3p targeting TCF4 regulating the malignant biological behavior of colon cancer cells

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Background: Colorectal carcinoma (CRC) is a common malignant tumor of the digestive tract. It is characterized by a high degree of malignancy, early metastasis and poor prognosis. Studies have shown the effect of miR-369-3p on the biological function of a variety of tumors. However, the mechanism by which miR-369-3p and its potential target genes participate in the pathogenesis of CRC has not been elucidated. This study aims to study the relationship between miR-369-3p and transcription factor 4 (TCF4), to reveal the mechanism of the occurrence and development of CRC, and to provide a promising target for the treatment of CRC.

Methods: Real-time quantitative polymerase chain reaction (RT-qPCR) was used to detect the miR-369-3p levels in CRC tissues and cells. miR-369-3p mimics and/or TCF4 overexpression vectors were transfected into SW480 cells. The expression of miR-369-3p and *TCF4* mRNA was detected using RT-qPCR. Bioinformatics analysis predicted the binding site of miR-369-3p to the *TCF4* 3'UTR, and the targeting relationship was verified by a dual luciferase reporter gene assay. Cell proliferation and invasion were investigated by labeled immunofluorescence assay using BrdU antibody and Transwell assay. The oxidative stress ability of cells was determined by commercial kits. The levels of proteins related to cell proliferation and invasion were measured by western blotting.

Results: The level of miR-369-3p was significantly down-regulated in CRC tissues and cell lines, especially in SW480 cells ($P<0.05$). The expression of TCF4 was negatively correlated with that of miR-369-3p. High levels of miR-369-3p targeting TCF4 suppressed cell proliferation and downregulated the protein expression of Ki67 and PCNA ($P<0.05$). Overexpressed miR-369-3p binding TCF4 inhibited cell invasion and decreased the protein levels of vascular endothelial growth factor (VEGF) and E-cadherin ($P<0.05$). Furthermore, upregulation of miR-369-3p increased the activity of superoxide dismutase (SOD) while decreasing the content of malondialdehyde (MDA) and activity of lactate dehydrogenase (LDH) by blocking the expression of TCF4 ($P<0.05$).

Conclusions: MiR-369-3p inhibits the proliferation, invasion and oxidative stress of CRC cells by targeting TCF4, thus defining miR-369-3p as a potential target for the diagnosis and treatment of CRC.

Keywords: Colorectal carcinoma (CRC); miR-369-3p; transcription factor 4 (TCF4); cell proliferation; cell invasion

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Introduction

Colorectal carcinoma (CRC) is a common gastrointestinal malignant tumor and the fourth leading cause of cancer-related deaths (1). In the past 10 years, despite the great progress made in diagnosis and treatment, the 5-year survival rate of colon cancer patients is still only 50% to 65% (2). Currently, chemotherapy is still one of the key methods for the treatment of colorectal cancer (3). In recent years, with the emergence of new chemotherapy drugs, targeted drugs and the formulation of chemotherapy regimens, the treatment of colorectal cancer has made great progress, but there are still a large number of patients with poor efficacy and even recurrence or metastasis after treatment (4). Researchers have identified many oncogenes and oncogenes by exploring the biological mechanisms of colon cancer. Studies have shown that alterations in the expression of certain oncogenes or oncogenes suppressors may also contribute to the development of colon cancer. A better understanding of the mechanisms of colon cancer onset, progression, migration and recurrence, and the exploration of new molecular markers for colon cancer will contribute to the early diagnosis and treatment of colon cancer.

MicroRNAs (miRNAs) are a class of endogenous small non-coding RNAs that can lead to protein degradation by directly binding to target mRNAs (5). A study has shown that miRNAs play an important role in promoting or inhibiting tumor cell proliferation, invasion and drug resistance by regulating oncogenes or tumor suppressor genes (6). A study has shown that miR-369-3p plays a tumor suppressor role in several cancers. For example, miR-369-3p inhibits the viability and motility of hepatocellular carcinoma (HCC) cells by binding to paired Box 6 (7). miR-369-3p overexpression inhibits cell proliferation and migration of endometrioid adenocarcinoma (8). Another study showed that miR-369-3p inhibits cell proliferation and induces apoptosis in thyroid cancer (9). Dong *et al.* (10) reported that miR-369-3p played an anticancer role in gastric cancer cells by regulating jun proto-oncogene and v-akt mouse thymoma virus oncogene homolog 1. Hao *et al.* (11) found that inhibition of miR-369-3p sensitizes cisplatin (DDP) to the inhibitory effect of lung cancer cell invasion in the presence of DDP treatment. Interestingly, Ogawa *et al.* found that miRNA-369-3p induced epigenetic reprogramming and inhibited the malignant phenotype of human colon cancer cells (12). However, the regulatory mechanism of miR-369-3p in human CRC has not yet been elucidated. Therefore, this study focuses on the effects of miR-369-3p on the proliferation, invasion and oxidative stress of CRC cells to confirm that miR-369-3p may be a target for the diagnosis and treatment of colon cancer. We present this article in accordance with the MDAR reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-628/rc).

Highlight box

Key findings

- miR-369-3p inhibits the proliferation, invasion and oxidative stress of colorectal carcinoma (CRC) cells by targeting transcription factor 4.

What is known and what is new?

- miR-369-3p plays a tumor suppressor role in several cancers.
- miR-369-3p was downregulated in CRC tissue and cell lines and miR-369-3p may be a target for the diagnosis and treatment of colon cancer.

What is the implication, and what should change now?

- This might provide light in the miRNA field and targets for the treatment of CRC.

Methods

CRC tissue samples

Twenty pairs of CRC tissues and normal tissues were collected from patients diagnosed in our hospital from January 2021 to September 2022. All patients who provided samples were confirmed by pathological examination and did not receive radiotherapy and chemotherapy before surgery. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was obtained from each patient, and all the experiments were approved by the Ethics Committee of Wuwei Hospital of Traditional Chinese Medicine (No. 2021011). All tissue samples were maintained in liquid nitrogen.

Cell culture

Human CRC cell lines SW480 (cat. no. BFN60800644), HT-29 (cat. no. BFN60800646), HCT-116 (cat. no. BFN60800649) and SW620 (cat. no. BFN60800643) (Bluefbio, China) and human normal colonic epithelial cell CCD-841CoN (cat. BFN60804016) (Bluefbio, China) were cultured in DMEM (Gibco, USA) containing 10%

FBS (Gibco, USA), 100 U/mL lyophilic streptomycin and streptomycin (Gibco, USA) in an incubator with 5% CO_2, and saturated humidity at 37 ℃.

Cell transfection

The miR-369-3p mimics, negative control miRNA mimics (mimic-NC), TCF4-overexpressing vectors (pcDNA-TCF4) and corresponding empty vectors (pcDNA3.1) were all purchased from RiboBio Co., Ltd. (RioBio, China). The cells were randomly divided into the control, mimic, pcDNA-TCF4 and mimic + pcDNA-TCF4 groups. The cells were incubated into 6-well plates and transfected at a density of 50–70%. Lipofectamine 2000 (Invitrogen, USA) was used to transiently transfect the above plasmids in groups, and the transfection procedure was in accordance with the manufacturer's instructions. Approximately 48 h after transfection, cells were harvested and used for subsequent assays.

RNA extraction and real time quantitative PCR (RT-qPCR)

RNA was extracted from cells using TRIzol (Invitrogen, USA). RT-qPCR was performed on a 7900HT RT-PCR system (Applied Biosystems, USA). Complementary DNA (cDNA) was synthesized using the TaqMan MicroRNA RT Reagent Kit (Takara, Japan). The expression of miR-369-3p and transcription factor 4 (*TCF4*) was analyzed with SYBR-Green II (Takara, Japan). U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as endogenous controls for miR-369-3p and *TCF4* mRNA expression, respectively. The primer sequences were as follows: U6 forward, 5'-TGCGGGTGCTCGCTTCGCAGC-3', and reverse, 5'-CCAGTGCAGGGTCCGAGGT-3'; GAPDH forward, 5'-CGGAGTCAACGGATTTGGTCGTAT-3' and reverse, 5'-AGCCTTCTCCATGGTGGT GAAGAC-3'; miR-369-3p forward, 5'-TGACCTAAGGGACTCCCACAA-3', reverse, 5'-TAGCAATATTGCACAGAAGGC-3'; *TCF4* forward, 5'-CCTGGCTATGCAGGAATGTT-3', reverse, 5'-CAGGAGGCGTACAGGAAGAG-3'. Three independent experiments were performed in triplicate.

Western blotting

Total proteins from cells were isolated with radioimmunoprecipitation assay (RIPA) buffer (Sobarbio, China). Protein concentrations were measured using a BCA Protein Assay Kit (Beyotime, China). Equal amounts of protein (30 μg/lane) were fractionated using a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to polyvinyl fluoride (PVDF) membranes (Sobarbio, China). Then, the membranes were completely blocked with 5% nonfat milk in Tris-buffered saline with Tween 20 (TBST) for 2 h at room temperature. Membranes were incubated at 4 ℃ overnight with the following primary antibodies: TCF4 (1/10,000; cat. no. ab217668; Abcam, UK), Ki67 (1/5,000; cat. no. ab92742; Abcam, UK), PCNA (1/1,000; cat. no. ab92552; Abcam, UK), vascular endothelial growth factor (VEGF) (1 μg/mL; cat. no. ab46154; Abcam, UK), E-cadherin (1/500; cat. no. ab40772; Abcam, UK) and GAPDH (1/500–1/10,000; cat. no. ab8245; Abcam, UK). Subsequently, the membranes were further developed with goat anti-rabbit horseradish peroxidase (HRP) conjugated secondary antibody for 1 h at room temperature. Protein expression levels were visualized with enhanced chemiluminescence (ECL) detection solution. Three independent experiments were performed.

Labeled immunofluorescence assay using BrdU antibody

The cells were inoculated into 96-well plates and cultured to a cell density of 50–70%. After adding 20 μL BrdU Solution (MultiSciences, China) for 4 h, the cells were fixed with 4% paraformaldehyde for 20 min, permeated with 0.2% Triton X-100 for 10 min, and incubated with BrdU antibody (ab6326, Abam, USA) overnight at 4 ℃. Finally, the cells were covered with Prolong Gold Antifade Reagent with DAPI (#9071, Cell Signaling Technology, USA) and photographed with an IX73 fluorescence microscope (Olympus, Japan). Three independent experiments were performed in triplicate.

Flow cytometry analysis

The transfected SW480 cells were harvested and re-suspended to detect the ability of apoptosis. Subsequently, cells were stained with 5 μL Annexin V-FITC (Sigma-Aldrich, USA) and 10 μL propidium iodide (PI) (Sigma, USA) in the dark for 15 minutes. Flow cytometry (Beckman, USA) was used to evaluate the results.

Transwell assay

The dissolved Matrigel (Corning, USA) was mixed with

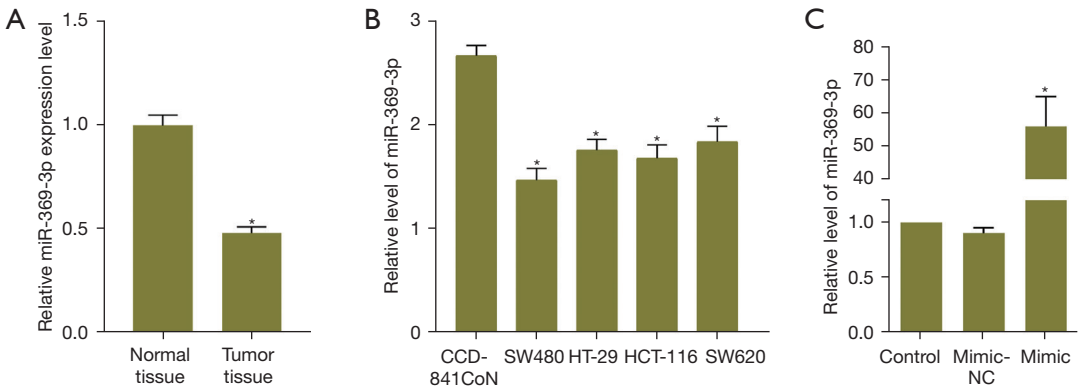

Figure 1 Expression of miR-369-3p in colon cell lines by RT-qPCR. (A) Expression of miR-369-3p in CRC samples was compared with that in adjacent normal tissues; determination via RT-qPCR. *, P<0.05 *vs.* the normal tissue. (B) Determination of miR-369-3p expression in a variety of colon cell lines. *, P<0.05 *vs.* the CCD-841CoN cell line. (C) Determination of miR-369-3p expression in SW480 cells transfected with miR-369-3p mimic. *, P<0.05 *vs.* control group. NC, negative control; RT-qPCR, real time quantitative polymerase chain reaction; CRC, colorectal carcinoma.

serum-free medium (1:8 dilution) and evenly spread to the bottom of the Transwell chamber (upper chamber surface). The Matrigel was solidified in a 37 ℃ incubator for approximately 2 h. 1×10^5 cells were cultured in the serum-free medium, and the cells were uniformly cultivated on the upper of the Transwell chamber. Then, the Transwell chamber was placed into the pore plate with 10% fetal bovine serum (FBS) medium for culture. After 24 h of cell culture, the Transwell chamber was removed, and the cells in the chamber and the residual Matrigel were wiped with cotton swabs and washed with PBS 3 times. The cells that passed through the bottom of the chamber were fixed with paraformaldehyde, stained with crystal violet (Solarbio, China, cat. no. G1063), counted under the microscope and analyzed for drawing. Images of three random fields were collected to calculate the number of invading cells.

Detection of superoxide dismutase (SOD) and LDH activities and malondialdehyde (MDA) content

The SOD activity detection kit, MDA content detection kit, and lactate dehydrogenase (LDH) activity detection kit were all purchased from Solarbio Co., Ltd. (Solarbio, China). The cells were treated and tested according to the kit manufacturer's instructions. The absorbance was measured at 450 nm. Three independent experiments were performed in triplicate.

Statistical analysis

All data were analyzed by GraphPad Prism 8.0 software (GraphPad Software, Inc., La Jolla, CA, USA), and the data are expressed as the mean ± standard deviation. All experiments were repeated three times. The Kolmogorov-Smirnov and Shapiro-Wilk method normality tests were used to determine whether the data conformed to a normal distribution. If the data were normally distributed, an analysis of variance (ANOVA) was performed, followed by a post hoc Tukey test for multiple comparisons. Bilateral P<0.05 was considered statistically significant.

Results

The expression of miR-369-3p was downregulated in CRC cell lines

We examined the expression of miR-369-3p in CRC tissues and normal tissues, normal osteoblasts (CCD-841CoN) and in a variety of CRC cell lines (SW480, HT-29, HCT-116 and SW620). The results showed that the levels of miR-369-3p were downregulated in CRC tissues and all CRC cell lines compared with normal tissues and CCD-841CoN cells (*Figure 1A*,*1B*). The lowest expression of miR-369-3p was detected in SW480 cells. Then, SW480 cells were selected for subsequent study. To reveal the biological function of miR-369-3p in CRC cells, gain-of-function

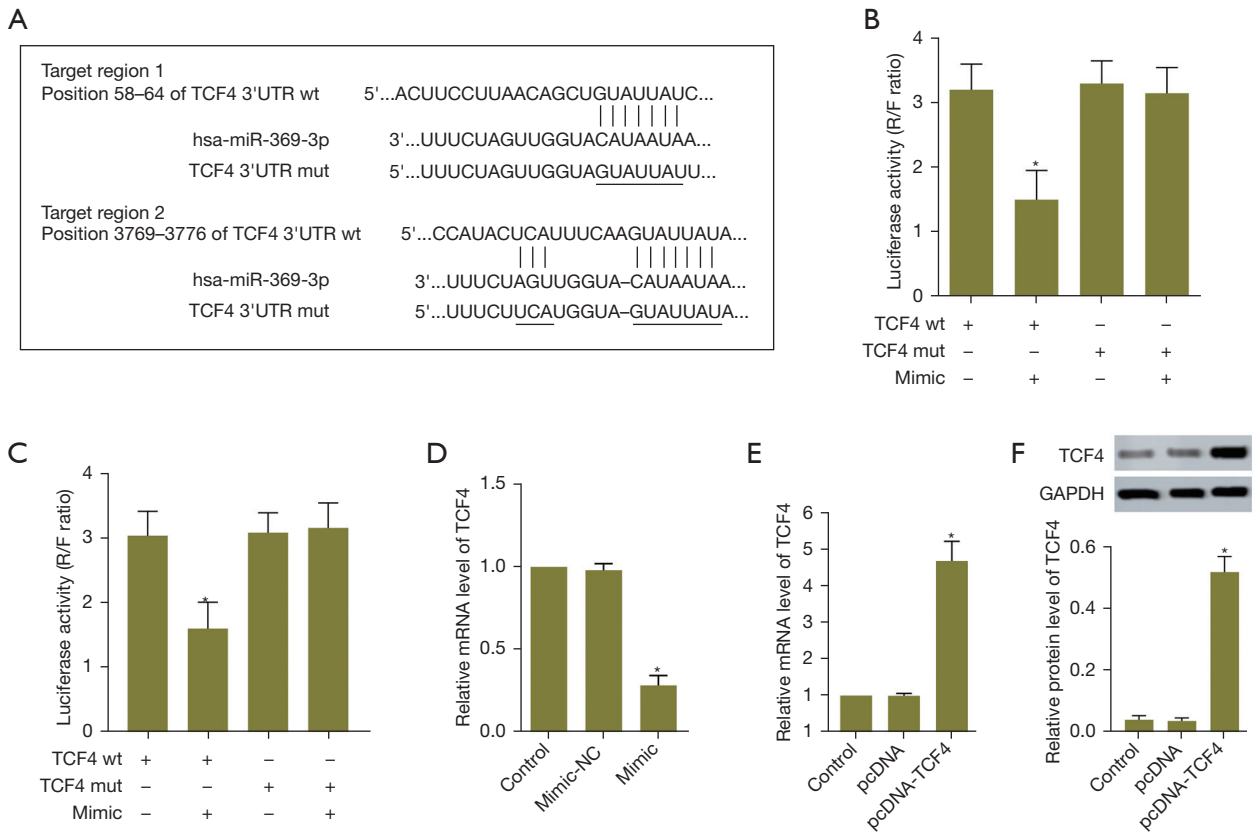

Figure 2 Regulation of TCF4 at mRNA and protein level by miR-369-3p binding. (A) The putative sites of miR-369-3p targeting TCF4 predicted by TargetScan are shown. (B) Analysis of miR-369-3p binding target region 1 of 3'UTR of TCF4 mRNA by Dual luciferase reporter gene assay. (C) Analysis of miR-369-3p binding target region 2 of 3'UTR of TCF4 mRNA by Dual luciferase reporter gene assay. (D) Analysis of relative mRNA expression of TCF4 in SW480 cells transfected with miR-359-3p mimic by RT-qPCR. (E) Analysis of relative mRNA expression of TCF4 in SW480 cells transfected with pcDNA-TCF4 vectors by RT-qPCR. (F) Analysis of relative protein expression of TCF4 in SW480 cells transfected with pcDNA-TCF4 vectors by western blotting. *, P<0.05 *vs.* control group. R/F, Renilla/Firefly; wt, wild type; mut, mutant; TCF4, transcription factor 4; NC, negative control; pcDNA, pcDNA3.1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-qPCR, real time quantitative polymerase chain reaction.

assays were carried out by transfecting SW480 cells with miR-369-3p mimic. According to the qRT-PCR assay, the miR-369-3p mimic obviously increased the level of miR-369-3p compared with normal cells (*Figure 1C*). This indicated that the transfection was successful.

miR-369-3p targets the TCF4 gene

A putative target of miR-369-3p was predicted to be *TCF4* by TargetScan. The binding sites between miR-369-3p were the 58–64 and 3769–3776 positions of *TCF4* mRNA 3'UTR (*Figure 2A*). To confirm that TCF4 was a direct target gene of miR-369-3p, SW480 cells were cotransfected with wild type (wt) or mutation (mut) TCF4-3'UTR vectors and miR-369-3p mimics. The dual luciferase assay data demonstrated that the overexpression of miR-369-3p significantly reduced the luciferase activity of TCF4 wt but did not change the luciferase activity of TCF4 mut (*Figure 2B,2C*). RT-qPCR results indicated that upregulation of miR-369-3p reduced the mRNA levels of *TCF4* in SW480 cells (*Figure 2D*). To determine that the effect of miR-369-3p on CRC cells was mediated through TCF4, we transfected SW480 cells with pcDNA-TCF4 vectors for rescue assays. The mRNA and protein levels of TCF4 in SW480 cells were upregulated

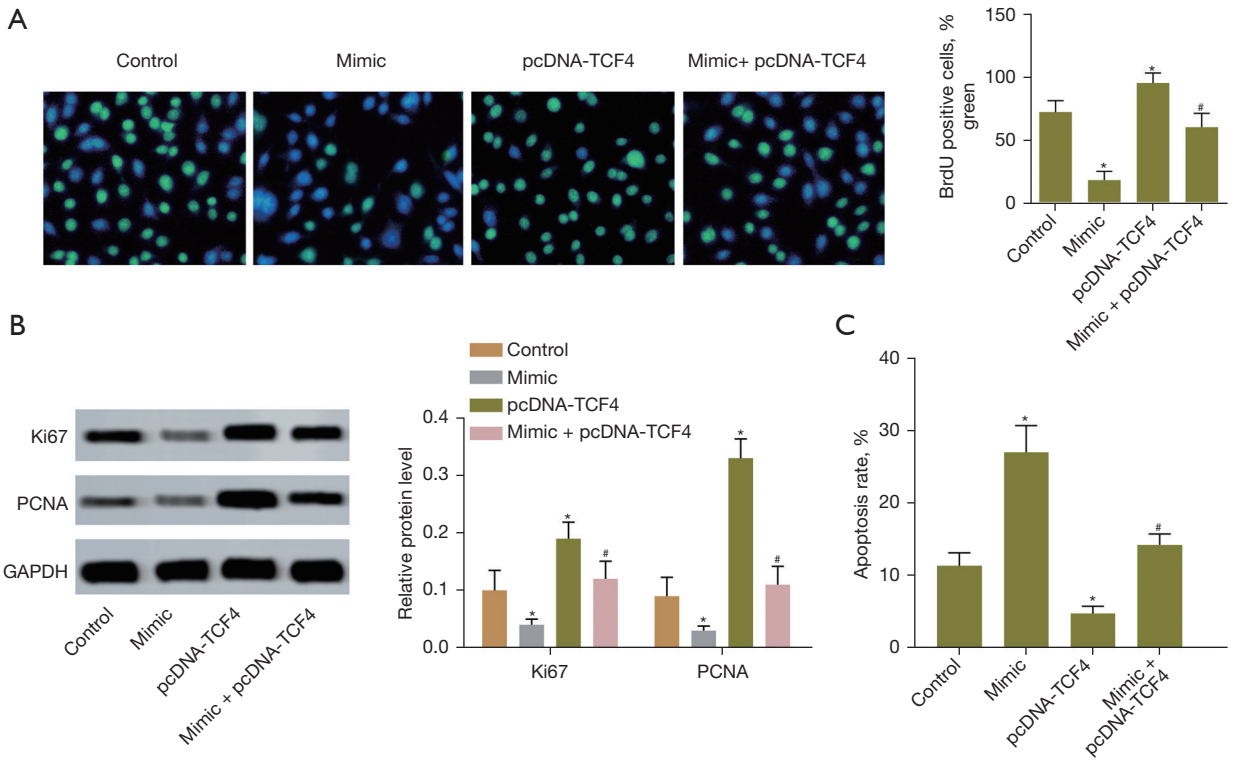

Figure 3 miR-369-3p inhibits cell proliferation by modulating Ki67 and PCNA protein expression. (A) Cell proliferation analysis was evaluated in SW480 cells treated with control mimic, miR-369-3p mimic, pcDNA-TCF4, and miR-369-3p mimic + pcDNA-TCF4 by BrdU assay. Photograph showed at 200×. (B) Evaluation of relative protein levels of Ki67 and PCNA by western blotting in SW480 cells treated with control mimic, miR-369-3p mimic, pcDNA-TCF4, and miR-369-3p mimic + pcDNA-TCF4. GAPDH protein was used as loading control. (C) The apoptotic cells were determined using flow cytometry analysis. *, P<0.05 *vs.* control group; #, P<0.05 *vs.* mimic group. pcDNA, pcDNA3.1; TCF4, transcription factor 4; BrdU, 5-bromo-2'-deoxyuridine; PCNA, proliferating cell nuclear antigen; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

after overexpression of TCF4 (*Figure 2E,2F*).

miR-369-3p targeting TCF4 inhibits CRC cell proliferation

Next, the effect of miR-369-3p and/or TCF4 overexpression on cell proliferation was assessed by BrdU assay. Overexpression of miR-369-3p decreased the number of BrdU-positive cells, while TCF4 upregulation increased the number of BrdU-positive cells compared with normal cells. The BrdU-positive cells were reduced in cells cotransfected with miR-369-3p mimic and pcDNA-TCF4 compared with cells separately transfected with pcDNA-TCF4 (*Figure 3A*). Moreover, the expression of Ki67 and PCNA associated with proliferation was determined by western blotting. The results indicated that high levels of miR-369-3p downregulated the protein expression of Ki67 and PCNA. TCF4 overexpression led to the upregulation of the levels of Ki67 and PCNA. miR-369-3p counteracted the enhanced expression of Ki67 and PCNA by TCF4 (*Figure 3B*). Overexpression of miR-369-3p increased the apoptosis rate of SW480 cell, while TCF4 upregulation decreased the apoptosis rate compared with control group. The apoptosis rate were induced in cells cotransfected with miR-369-3p mimic and pcDNA-TCF4 compared with cells separately transfected with pcDNA-TCF4 (*Figure 3C*). The above experiments demonstrated that miR-369-3p could target TCF4 to inhibit CRC cell proliferation and promote apoptosis.

miR-369-3p targeting TCF4 inhibits CRC cell invasion

To investigate the effect of miR-369-3p on the invasive ability of CRC cells, a Transwell assay was performed.

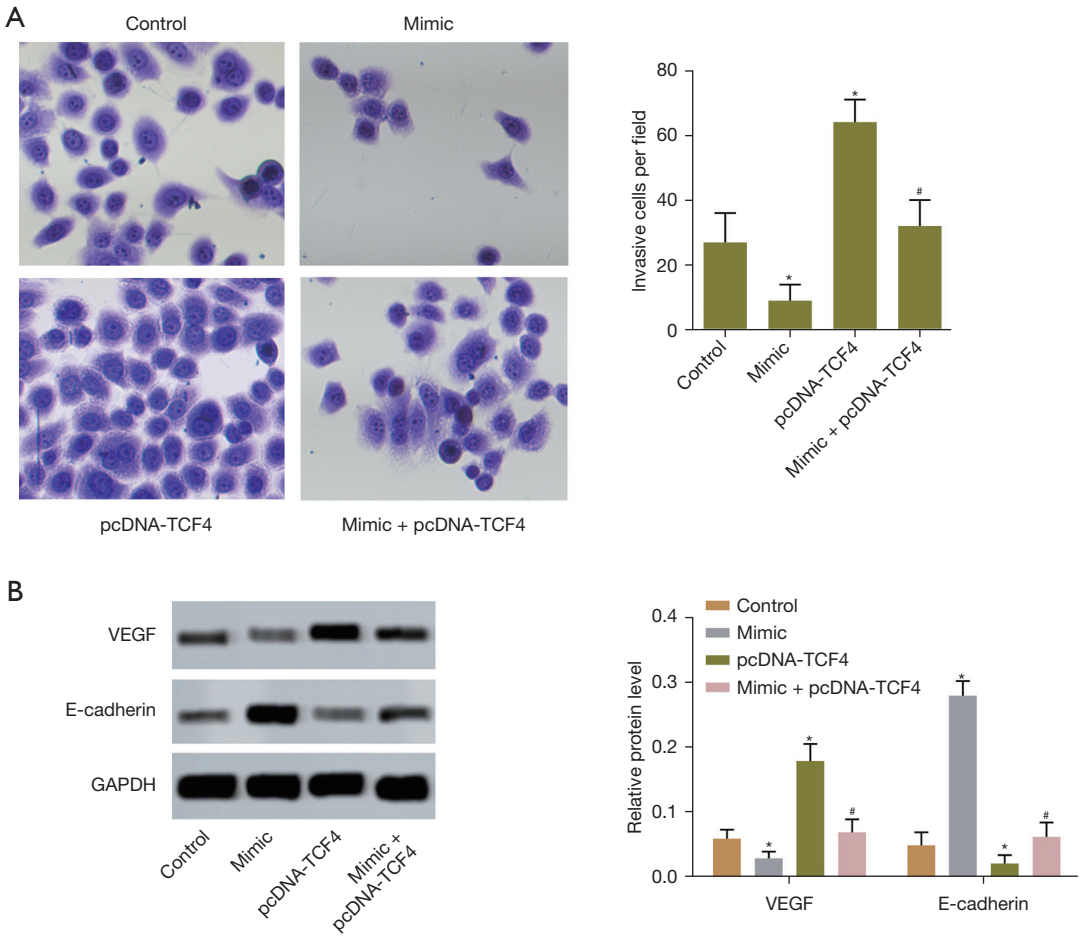

Figure 4 miR-369-3p inhibits cell invasion by regulating VEGF and E-cadherin protein expression. (A) Cell invasion capacity was determined in SW480 cells treated with control mimic, miR-369-3p mimic, pcDNA-TCF4, and miR-369-3p mimic + pcDNA-TCF4 by Transwell assay (crystal violet staining, 200×). (B) Relative protein levels of VEGF and E-cadherin were evaluated in SW480 cells treated with control mimic, miR-369-3p mimic, pcDNA-TCF4, and miR-369-3p mimic + pcDNA-TCF4 by western blotting. *, P<0.05 *vs.* control group; #, P<0.05 *vs.* mimic group. pcDNA, pcDNA3.1; TCF4, transcription factor 4; VEGF, vascular endothelial growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

According to the results, upregulation of miR-369-3p reduced the number of invasive cells and overexpression of TCF4 increased the number of invasive cells. miR-369-3p neutralized the effect of overexpressed TCF4 (*Figure 4A*). Furthermore, the results of western blotting suggested that the levels of invasion-related proteins such as VEGF and E-cadherin in cells transfected with miR-369-3p mimic were lower than those in normal cells. The expression of VEGF and E-cadherin protein was upregulated in cells transfected with pcDNA-TCF4 (*Figure 4B*). MiR-369-3p could reverse the strengthening of TCF4 on cell invasion. These data indicated that miR-369-3p could inhibit CRC cell invasion by binding to *TCF4* mRNA and downregulating mRNA and protein expression.

miR-369-3p targeting of TCF4 attenuates the oxidative stress of CRC cells

In addition, we also measured the levels of SOD, MDA and LDH related to the oxidative stress system. Overexpression of miR-369-3p increased the activity of SOD while reducing the content of MDA and activity of LDH. Upregulated TCF4 decreased the activity of SOD while increasing the level of MDA and activity of LDH. MiR-369-3p counteracted the influence of TCF4 on SOD, MDA and LDH (*Figure 5A-5C*). These results demonstrated that

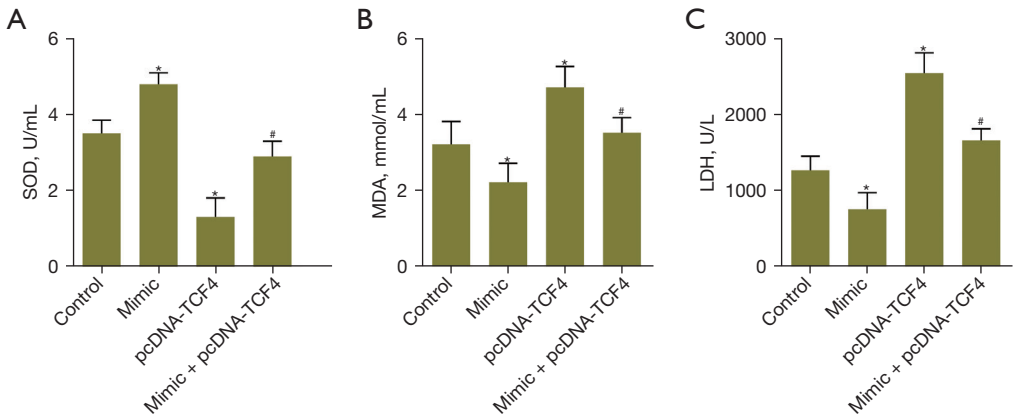

Figure 5 miR-369-3p inhibits oxidative stress by regulating enzymatic activity of SOD and LDH. (A) The activity of SOD was evaluated in cells SW480 treated with miR-369-3p. (B) The content of MDA was analyzed in cells SW480 transfected with miR-369-3p. (C) The activity of LDH was evaluated in cells SW480 treated with miR-369-3p. *, P<0.05 *vs*. control group; #, P<0.05 *vs*. mimic group. SOD, superoxide; pcDNA, pcDNA3.1; TCF4, transcription factor 4; MDA, malondialdehyde; LDH, lactate dehydrogenase.

miR-369-3p binding to TCF4 could alleviate the oxidative stress of CRC cells.

Discussion

A study has confirmed that the increase in morbidity and mortality of patients with colon cancer is related to changes in the interaction of lifestyle, diet, age, obesity, genetic and environmental factors (exposure to carcinogens and smoking, etc.) (13). Due to the high incidence of colon cancer and poor prognosis, colon cancer is a major public health problem and clinical challenge worldwide (14). According to reports, many signal transduction pathways and molecules are related to the occurrence and development of colon cancer (15,16). However, the molecular mechanism of colon carcinogenesis remains largely unknown. Therefore, it is necessary to study the therapeutic targets of colon cancer more deeply.

A large number of studies have shown that miR-369-3p plays a tumor suppressor role in a variety of tumors (11,17,18). Compared with the normal control group, the expression of miR-369-3p was downregulated in HCC tissues and cell lines. Overexpression of miR-369-3p inhibited the proliferation, migration and invasion of HCC cells (19). miR-369-3p inhibits the proliferation and promotes the apoptosis of papillary thyroid carcinoma (PTC) cells by downregulating the expression of Tetraspanin 13 (TSPAN 13) (20). miR-369-3p is downregulated in HCC. Overexpressed miR-369-3p inhibited cell viability and motility in HCC by targeting PAX6 (7). In this research, we found that miR-369-3p was downregulated in CRC tissue and cell lines.

Cumulative studies have shown that multiple miRNAs can regulate CRC cells by targeting target genes. It was reported that overexpression of miRNA-143 inhibited colon cancer cell proliferation by suppressing glucose uptake (21). The data from Zeng *et al.* indicated that miR-378 inhibited the proliferation, migration and invasion of colon cancer cells by targeting SDAD1 (22). Another study showed that miR-185 inhibited colon cancer cell proliferation and invasion by targeting Wnt1 (23). Li *et al.* (24) also demonstrated that miR-195 suppressed colon cancer proliferation and metastasis by targeting WNT3A. Similarly, the target gene of miR-369-3p in CRC cells was predicted and confirmed to be TCF4 by bioinformatics software and experiments in the present study.

TCF4 is the key Wnt signaling molecule that can interact with β-catenin (25). In some cell types, TCF4 gradually becomes an important regulator of epithelial-mesenchymal transformation (EMT) and plays an important role in embryonic development, tissue repair and cancer metastasis. Various convergent lines of evidence support the role of TCF4 as an EMT regulator in different cells (26). Liu *et al.* demonstrated that TCF4 might play a tumorigenic role in epithelial ovarian cancer and is a useful independent prognostic indicator (27). A study from Lee *et al.* indicated that TCF4 induced enzalutamide resistance via neuroendocrine differentiation in prostate cancer (28). Another study has shown that miRNA-495 targeting TCF4 inhibits the activation of the Wnt/β-Catenin pathway and

hinders the progression of non-small cell lung cancer (29). In our study, TCF4 played a cancer-promoting role in CRC cells by inducing proliferation and invasion.

In addition, we also studied the effect of the miR-369-3p/TCF4 axis on oxidative stress in colorectal cancer cells. The normal function of mitochondria plays a key role in cell proliferation and apoptosis, and reactive oxygen species (ROS) are normal byproducts of mitochondrial metabolism and protein folding (30). Excessive ROS are eliminated by the antioxidant system in normal cells, while cancer cells seem to benefit from increased levels of ROS. Higher ROS in cancer cells may activate proliferation and survival pathways (31). Similar to normal cells, antioxidant proteins such as SOD, catalase, and nuclear factor erythroid 2-related factor 2 (Nrf2) can inhibit excessive ROS levels. Cancer cells also show increased levels of antioxidant proteins to eliminate ROS (32). Our research indicated that overexpression of TCF4 accelerated oxidative stress, while miR-369-3p reversed the effect of TCF4 on oxidative stress in CRC cells by binding to *TCF4* mRNA. However, this study has not been confirmed by *in vivo* experiments, which was a limitation of this study. Therefore, the next study should be conducted *in vivo* to verify the inhibitory effect of miR-369-3p on CRC.

Conclusions

In conclusion, miR-369-3p could inhibit human CRC cell proliferation and invasion and attenuate oxidative stress by targeting TCF4. This might provide light in the miRNA field and targets for the treatment of CRC.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-628/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-628/dss

Peer Review File: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-628/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-628/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was obtained from each patient, and all the experiments were approved by the Ethics Committee of Wuwei Hospital of Traditional Chinese Medicine (No. 2021011).

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