Original Paper

miR-142-3p Suppresses Cell Growth by Targeting CDK4 in Colorectal Cancer

Xiangyu Zhu^a Si-ping Ma^a Dongxiang Yang^b Yanlong Liu^c Yong-peng Wang^a
Tao Lin^a Yan-xi Li^a Shi-hua Yang^a Wan-chuan Zhang^a Xin-ling Wang^d

^aDepartment of Colorectal Surgery, Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute, Liaoning, ^bDepartment of Orthopedics, The Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, Liaoning, ^cDepartment of Colorectal Surgery, Harbin Medical University Cancer Hospital, Heilongjiang, ^dDepartment of Ophthalmology, the Fourth Affiliated Hospital, China Medical University, Liaoning, China

Key Words

Colorectal cancer • miR-142-3p • CDK4 • Cell cycle

Abstract

Background/Aims: Deregulation of microRNAs (miRNAs) has been associated with a variety of cancers, including colorectal cancer (CRC). Here, we investigated anomalous miR-142-3p expression and its possible functional consequences in primary CRC samples. **Methods:** The expression of miR-142-3p was measured by quantitative RT-PCR in 116 primary CRC tissues and adjacent non-tumor tissues. The effect of miR-142-3p up- or down-regulation in CRC-derived cells was evaluated *in vitro* by cell viability and colony formation assays and *in vivo* by growth assays in xenografted nude mice. **Results:** Using quantitative RT-PCR, we found that miR-142-3p was down-regulated in 78.4 % (91/116) of the primary CRC tissues tested when compared to the adjacent non-tumor tissues. We also found that the miR-142-3p mimic reduced *in vitro* cell viability and colony formation by inducing cell cycle arrest in CRC-derived cells, and inhibited *in vivo* tumor cell growth in xenografted nude mice. Inversely, we found that the miR-142-3p inhibitor increased the viability and colony forming capacity of CRC-derived cells and tumor cell growth in xenografted nude mice. In addition, we identified CDK4 as a potential target of miR-142-3p by predictions and dual-luciferase reporter assays. Concordantly, we found that miR-142-3p mimics and inhibitors could decrease and increase CDK4 protein levels in CRC-derived cells, respectively. **Conclusion:** From our results we conclude that miR-142-3p may act as a tumor suppressor in CRC and may serve as a tool for miRNA-based CRC therapy.

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Introduction

Colorectal cancer (CRC) is one of the leading causes of death worldwide [1]. Development of CRC is a stepwise process comprising genetic changes in tumor-suppressor genes in most of the cases. In addition, a multitude of molecular alterations of genes have been described in the literature as being associated with development and prognosis of CRC [2]. However, the mechanisms that drive the process of neoplastic transformation are not well understood. Thus, there is an urgent need to elucidate the underlying molecular mechanisms of CRC and find new molecular targets for treatment against this disease.

MiRNAs are endogenous non-coding 20-22 nucleotide long RNAs that may act as post-transcriptional regulators of gene expression [3]. They regulate gene expression at the transcriptional and post-transcriptional level by completely or incompletely binding to the 3'-UTR of their target gene messenger RNA (mRNA) and by repressing the translation or promoting the degradation of the target gene to exert biological functions [4, 5]. MiRNA expression deregulation has been observed in a wide range of human diseases, including cancer [4], and it has been shown that this deregulation may play a pivotal role in processes such as cellular differentiation, proliferation, angiogenesis, apoptosis and invasion [5]. To date, deregulated miRNAs and their roles in CRC development have attracted much attention. A series of miRNAs have been reported that miR-19 [6], miR-140-5p [7], miR-149 [8], miR-153 [9], miR-195-5p [10] and miR-301a [11], play important roles in the development of CRC.

Recently, we identified several significantly deregulated miRNAs in CRC, including miR-138, miR-206, miR-214, miR-498 and miR-142-3p, using a miRNA expression microarray approach (unpublished data). Most of these miRNAs have previously been reported to be involved in several types of cancers, including CRC [12-16]. In the present study, we investigated the role of miR-142-3p in CRC. Our results showed that the expression of miR-142-3p was down-regulated in CRC tumor tissues compared to paired adjacent non-tumor tissues. Moreover, *in vitro* experiments proved that miR-142-3p inhibited cell proliferation in the CRC cell lines. In addition, CDK4 was identified as a novel direct target gene of miR-142-3p. Our findings suggested that miR-142-3p has a tumor suppressive effect in CRC by inhibiting cell proliferation.

Materials and Methods

Human tissue specimens

Fifty six pairs of human CRC and adjacent non-tumor tissue samples were obtained immediately after resection from patients undergoing primary surgical treatment in Department of colorectal surgery, Cancer Hospital of China Medical University, LiaoNing, China. None of the patients had undergone preoperative irradiation or chemotherapy. The tissue samples were frozen in liquid nitrogen and stored at -80°C until use. The tissue samples were obtained from 86 male and 30 female patients (Average age: 63.2 years; range: 53 to 68 years) and their clinicopathological features are listed in Table 1. Written consent for research purposes was obtained from the patients before tissue collection and the protocol was approved by the Institutional Review Board of China Medical University.

Table 1. The data of patients (n=116). * Diameter of the biggest nodule, ** TNM: tumor-node-metastasis

Variable	Value
Age(years)	63.2±10.1
Gender(male)	86(74.1%)
Tumor size*(cm)	5.46
Tumor grade	
Well-differentiated (G1-2)	53
Moderately-differentiated (G3)	51
Poorly-differentiated (G4)	12
TNM stage**	
I	31
II	37
III	36
IV	12

Cell cultures

The human embryonic kidney-derived cell line HEK293T and the human CRC-derived cell lines HT29 and SW116 were maintained in Dulbecco's modified eagle's medium (DMEM, Hyclone, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 100 U penicillin/ml, 100 mg streptomycin/ml and 10% fetal bovine serum (FBS, Gibco) at 37°C in a humidified atmosphere of 5% CO₂.

Transfections

The transfections were carried out using the FuGene HD transfection reagent (Roche, Indianapolis, IN, USA) according to the manufacturer's protocol. In brief, 2×10⁴ HT29 and SW116 cells or 5×10⁴ HEK293T cells, seeded in 24-well plates, were transfected with the indicated plasmid DNAs, miRNA duplexes (GenePharma, Shanghai, China) or siRNAs (GenePharma) and collected 24-48 hours after transfection for further assessment.

Quantitative real time PCR

cDNA synthesis and qRT-PCR-based miRNA expression analyses were carried out using TaqMan microRNA assay kits (Applied Biosystems) according to the manufacturer's protocol. Briefly, total RNA was extracted using TRIzol Reagent (Invitrogen) from the clinical samples or CRC-derived cell lines, and used to synthesize cDNA with gene-specific primers. Reverse transcription reactions were carried out using 100 ng RNA, 50 nM/L stem-loop RT primers, 1×RT buffer, 0.25 mM/L of each dNTP, 3.33 U/μl MultiScribe reverse transcriptase and 0.25 U/μl RNase inhibitor. 15 μl mixtures were incubated for 30 min at 16°C, 30 min at 42°C, 5 min at 85°C, and then held at 4°C. The resulting cDNA product was subsequently used for qRT-PCR analysis. The PCR reaction mixtures (20 μl) included 1.33 μl RT product, 1×TaqMan universal PCR master mix and 1 μl primers and probe mix from the TaqMan microRNA assay kit. The reaction mixtures were incubated in 96-well optical plates at 95°C for 5 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. The PCR reactions were run on a StepOne Plus real time PCR machine (Applied Biosystems) and the data were analyzed using SDS v2.3 software. The Ct value was defined as the fractional cycle number at which the fluorescence passed a fixed threshold. The fold change was calculated using the 2^{-ΔΔCt} method and presented as the fold-expression change in tumor tissues relative to their corresponding non-tumor tissues after normalization to the endogenous control.

Luciferase reporter constructs

The 3'-UTR fragment of CDK4 (Genbank accession no. NM_000075) containing the putative miR-142-3p binding sequence (1334-1344nt) was amplified using the primers 5'-GGCTGCCATGGAAGGAAGAAAAGC-3' (forward) and 5'-GTCTTGCTCTGTTGCCAGGCTGGAG-3' (reverse). The resulting PCR product was cloned into a *firefly* luciferase reporter vector (pGL3; Promega Corporation, Madison, WI, USA), and termed pGL3-CDK4-3'UTR. A plasmid that carried mutations in the complementary sites for the seed region of miR-142-3p was generated based on the pGL3-CDK4-3'UTR plasmid using a MutanBEST Kit (Takara Bio Inc., Shiga, JP), and termed pGL3-CDK4-3'UTR-mut. The correctness of the plasmids was confirmed by sequence analysis.

Cell viability and colony formation assays

Twenty four hours after transfection, 1000 HT29 or SW116 cells were seeded in fresh 96-well plates in triplicate and maintained in DMEM containing 10% FBS for 5 days. Next, the cells were tested for proliferation per 24 hours using a Cell Titer-Blue cell viability assay (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions, and the fluorescence ratios were recorded using a multi-plate reader (Synergy 2, BioTek, Winooski, VT, USA). Additionally, 24 hours after transfection, 2000 HT29 or SW116 cells were seeded in fresh 6-well plates in triplicate and maintained in DMEM containing 10% FBS for 2 weeks. Next, cell colonies were fixed in 20% methanol and stained with 0.1% coomassie brilliant blue R250 at room temperature for 15 min. Finally, the colonies were counted using an ELIspot Bioreader 5000 (BIO-SYS, Karben, GE).

Cell cycle and apoptosis assays

Forty eight hours after transfection, 1×10^5 HT29 and SW116 cells were harvested, washed once in phosphate buffer saline (PBS), and fixed in 70% ethanol at 4°C overnight. Next, DNA staining was performed with 50 mg/ml propidium iodide and 1 mg/ml RNase A at room temperature for 30 min. The cell populations in the G0-G1, S and G2-M phases of the cell cycle were measured using Cell Lab Quanta SC flow cytometry (Beckman Coulter, Fullerton, CA, USA) and the data were analyzed using the FlowJo v7.6 software package.

Tumorigenicity assays in nude mice

Male BALB/c nude mice (5-6 weeks old) were obtained from the ShenYang Experimental Animal Center (ShenYang, China). All animal handling and experimental procedures were approved by the Animal Experiments Ethics Committee of the China Medical University. For the *in vivo* tumorigenicity assays, all pyrimidine nucleotides in the miR-142-3p mimic, miR-142-3p inhibitor or the NC duplex were substituted by their 2'-O-methyl analogues to improve the RNA stability. MiR-142-3p mimic or miR-142-3p inhibitor transfected HT29 cells (1×10^6) were suspended in 100 μ l PBS and injected subcutaneously into left side of the posterior flank of 6 BALB/c nude mice, respectively. Nonrelated control (NC) transfected or non-transfected HT29 cells (1×10^5) were injected subcutaneously into right side of same 12 mice. Tumor growth was examined daily and the tumor volumes were calculated every week using the formula for hemi-ellipsoids: $V = \text{length (cm)} \times \text{width (cm)} \times \text{height (cm)} \times 0.5236$. After 5 weeks, the mice were sacrificed and the tumors were dissected and photographed.

Dual-luciferase reporter assay

HEK293T cells, seeded in 24-well plates in triplicate, were co-transfected with pGL3-CDK4-3'UTR or pGL3-CDK4-3'UTR-mut and the miR-142-3p mimic or nonrelated control RNA duplex (NC duplex, GenePharma) using the FuGene HD transfection reagent. The pRL-TK construct (Promega Corporation, Madison, WI, USA) was also transfected as a normalization control. The cells were collected 48 hrs after transfection, and luciferase activity was measured using a dual-luciferase reporter assay kit (Promega Corporation) and recorded using the multi-plate reader (Synergy 2, BioTek).

Western blotting

Total protein was extracted using a modified RIPA buffer with 0.5% SDS and the proteinase inhibitor cocktail (Complete Mini, Roche). Equal amounts of protein from CRC tissues and their adjacent non-tumor tissues were electrophoresed in 10% SDS-PAGE mini gels and transferred to PVDF membranes (Immobilon P[®], Millipore, Billerica, MA, USA). After blocking with 5% nonfat milk, the membranes were incubated with the rabbit anti-CDK4 antibody (1:1000 dilution, Epitomics, Inc., Burlingame, CA, USA) or mouse anti-GAPDH antibody (1:5000 dilution, Epitomics, Inc.) at 4°C overnight, followed by incubation with HRP-conjugated goat anti-rabbit or goat anti-mouse antibody (1:10000 dilution, KPL, Gaithersburg, MA, USA) for 1 hr at room temperature. Finally, signals were developed using Super Signal West Pico chemoluminescent substrate (Pierce, Rockford, Ill, USA), visualized by the Gene Gnome HR Image Capture System (Syngene, Frederick, MD, USA) and analyzed by Gene tools (Syngene).

miRNA target prediction

MiRanda (www.microrna.org) was used to predict miRNA targets and conserved sites bound by the seed region of miR-142-3p.

Statistical analysis

Data are presented as mean $\pm$ SD. Comparisons were made by using a two-tailed t test or one-way ANOVA for experiments with more than two subgroups. Correlation analyses were performed using Spearman correlation coefficient. $P < 0.01$ was considered statistically significant. Associations between miR-142-3p expression and cancer-specific survival rates were assessed using the Kaplan-Meier method.

Results

miR-142-3p is down-regulated in CRC

To investigate the relevance of miR-142-3p in CRC, we first set out to measure its expression in 18 pairs of tumor versus adjacent non-tumor tissues by qRT-PCR. miR-142-3p was found to be down-regulated in more than 70% of the tumor tissues compared to the non-tumor tissues tested (Fig. 1A). To validate these initial qRT-PCR data, the expression of miR-142-3p was measured in another series of 38 paired CRC tissues. Again, miR-142-3p showed a significant down-regulation in the tumor tissues compared to the adjacent non-tumor tissues (116 pairs in total, Fig. 1B). In addition, we found that patients whose primary tumors did not exhibit miR-142-3p down-regulation showed a trend towards a better survival (Fig. 1C). The mean survival in the poor survival group was 57.4 months (low miR-142-3p expression, n=82), whereas the mean survival in the better survival group was 93.8 months (high miR-142-3p expression, n=34). No statistical significant correlations were observed between miR-142-3p expression and the other clinicopathological features tested (age, sex, stage and tumor size; data not shown).

miR-142-3p induces cell cycle arrest

The significant reduction in miR-142-3p expression observed in the primary tumor tissues versus the non-tumor tissues suggests a possible role in CRC development. To test this hypothesis, we first set out to assess the effect of miR-142-3p on the growth of HT29 and SW116 CRC-derived cells transfected or not with the miR-142-3p mimic, miR-142-3p inhibitor or NC duplex. We found that the expression of miR-142-3p was increased 27.5-fold (HT29) and 26.0-fold (SW116) in cells transfected with 20 nM miR-142-3p mimic, and was decreased 32.5-fold (HT29) and

Fig. 1. MiR-142-3p is downregulated in CRC. A. Expression of miR-142-3p in 18 CRC tumor tissues (T) and adjacent non-tumor tissues (N). B. Expression of miR-142-3p in 116 CRC tumor tissues (T) and adjacent non-tumor tissues (N). The fold change was calculated using the $2^{-\Delta\Delta C_t}$ method and presented as the fold-expression change in tumor tissues (T) relative to their adjacent non-tumor tissues (N) after normalization to the endogenous control (U6). *P<0.05; **P<0.01. C. Probability of cancer-specific survival by miR-142-3p expression levels in CRC.

Fig. 2. MiR-142-3p suppresses cellular growth. A. Expression of miR-142-3p in HT29 and SW116 cells after transfection with miR-142-3p mimic, miR-142-3p inhibitor or NC duplex. B. Effect of miR-142-3p on the viability of CRC-derived cells. C. Effect of miR-142-3p on colony formation of CRC-derived cells. D. Effect of miR-142-3p on tumor growth in xenografted nude mice. Representative results (B–D) of HT29 and SW116 cells transfected with miR-142-3p mimic, miR-142-3p inhibitor or NC duplex. Columns, means of three independent experiments; bars, $\pm$ SD; * P < 0.01; ** P < 0.001.

41.5-fold (SW116) in cells transfected with 20 nM miR-142-3p inhibitor (Fig. 2A). From day 2 (HT29) or day 3 (SW116) onwards after transfection, the viability of the cells transfected with the miR-142-3p mimic significantly decreased compared to that of the NC duplex transfected or non-transfected cells, whereas the viability of the cells transfected with the miR-142-3p inhibitor significantly increased (Fig. 2B). These results indicate miR-142-3p can inhibit the growth of CRC-derived cells.

To validate the inhibitory effect of miR-142-3p on cellular growth, colony forming assays were performed on HT29 and SW116 cells transfected or not with the miR-142-3p mimic, miR-142-3p inhibitor or NC duplex. As shown in Fig. 2C, HT29 and SW116 cells transfected with 20 nM miR-142-3p mimic exhibited fewer and smaller colonies (341 and 328 colonies, respectively) compared to the NC duplex transfected (587 and 794 colonies, respectively) and non-transfected cells (573 and 782 colonies, respectively), whereas cells transfected with 20 nM miR-142-3p inhibitor exhibited more and larger colonies (1334 and 1258 colonies, respectively).

To further confirm the above findings, an *in vivo* mouse model was used. After treatment with the miR-142-3p mimic or miR-142-3p inhibitor for 5 weeks, the tumor volume curves revealed significant decreases in growth rates during the 3rd, 4th and 5th weeks after treatment with the miR-142-3p mimic and significant increases in growth rates during the 4th and 5th weeks after treatment with the miR-142-3p inhibitor, whereas no significant differences in growth rates were observed in the NC group and the non-transfected control group (Fig. 2D). These results indicate that miR-142-3p significantly inhibits the growth of CRC-derived HT29 cells in the xenograft mouse model.

To investigate the mechanism underlying the growth inhibitory effect of miR-142-3p, flow cytometry was carried out. We found that the percentages of the miR-142-3p mimic transfected HT29 and SW116 cells in the G0-G1 phase were 17.5% (HT29) and 13.2% (SW116) higher than that of the NC duplex transfected or non-transfected cells, which coincided with a 42.3% (HT29) and a 36.5% (SW116) decrease in S phase cells (Fig. 3A). In the miR-142-3p inhibitor transfected cells, the percentages of cells in the G0-G1 phase were 9.6% (HT29) and 7.4% (SW116) lower than that of the NC duplex transfected or non-transfected cells, which coincided with a 15.2% (HT29) and a 18.6% (SW116) increase in S phase cells (Fig. 3B). These results indicate that miR-142-3p may inhibit CRC-derived HT29 and SW116 proliferation by inducing cell cycle arrest in the G1/S phase.

CDK4 is a direct target of miR-142-3p

It is generally accepted that miRNAs may exert their functions through regulating the expression of downstream target genes. CDK4 was predicted to be a potential target of miR-142-3p by TargetScan and PicTar, since the 3'-UTR of the CDK4 mRNA contains a complementary site for the seed region of miR-142-3p (Fig. 4A). To validate whether CDK4 acts as a direct target of miR-142-3p, human CDK4 3'-UTR fragments containing wild-type or mutant miR-142-3p binding sequences (Fig. 4A) were cloned downstream of the firefly luciferase reporter gene in pGL3.

In HEK293 cells co-transfected with the reporter plasmids and the miR-142-3p mimic or NC duplex, the luciferase activity of the reporter that contained the wild-type 3'-UTR was found to be significantly suppressed by the miR-142-3p mimic, whereas the luciferase activity of the mutant reporter was unaffected (Fig. 4B), indicating that miR-142-3p may suppress CDK4

Fig. 3. miR-142-3p induces cell cycle arrest and enhances cell apoptosis. A. Effect of miR-142-3p on the cell cycle. B. Effect of miR-142-3p on apoptosis. Representative results of HT29 and SW116 cells transfected with miR-142-3p mimic, miR-142-3p inhibitor, NC duplex. Columns, means of three independent experiments; bars, $\pm$ SD; * P <0.01; ** P <0.001.

Fig. 4. CDK4 is a direct target of miR-142-3p. **A.** Putative miR-142-3p binding sequence in the 3'-UTR of CDK4 mRNA. The mutation was generated in the CDK4 3'-UTR sequence in the complementary site of the miR-142-3p seed region. **B.** Suppressed luciferase activity of wild-type CDK4 3'UTR by miR-142-3p mimic. HEK293T cells were co-transfected with pGL3-CDK4-3'UTR or pGL3-CDK4-3'UTR-mut, and miR-142-3p mimic or NC duplex. Firefly luciferase activity of each sample was measured 48 h after transfection and normalized to Renilla luciferase activity. **C.** Expression of endogenous CDK4 is regulated by miR-142-3p. The expression level of endogenous CDK4 in HT29 cells was assessed 48 h after transfection with miR-142-3p mimic, miR-142-3p inhibitor or NC duplex by Western blotting. GAPDH was used as an internal control. Columns, means of three independent experiments; bars, $\pm$ SD; * P <0.01.

expression through the miR-142-3p binding sequence in its 3'-UTR. Furthermore, we found that transfection of the miR-142-3p mimic decreased CDK4 expression and that transfection of the miR-142-3p inhibitor increased CDK4 expression in HT29 cells at the protein (Fig. 4C), but not the mRNA, level (data not shown) suggesting that CDK4 expression may be inhibited by miR-142-3p at the posttranscriptional level. Together, these results show that miR-142-3p may regulate the expression of endogenous CDK4 by directly targeting the 3'-UTR of its mRNA and, thus, that human CDK4 may be a new target of miR-142-3p.

CDK4 knockdown induces cell cycle arrest

To assess whether CDK4 expression down-regulation, like miR-142-3p expression up-regulation, results in CRC growth cessation, the effect CDK4 expression knockdown on cellular growth was examined. First, HT29 cells were transfected or not with the CDK4 siRNA or control siRNA. After 72 hours a dose-dependent knockdown of CDK4 was observed in the siRNA transfected cells (Fig. 5A). Through cell viability and cell cycle analyses, we subsequently found that the *in vitro* knockdown of CDK4 resulted in a repression of cell viability (Fig. 5B) and an induction of cell cycle arrest in HT29 cells (Fig. 5C). In order to confirm the effect of miR-142-3p on CDK4 expression, rescue experiments were performed for the cell viability and cell cycle assays. We found that exogenous over-expression of CDK4 in miR-142-3p mimic-transfected HT29 cells reversed the effect of the miR-142-3p mimic (Fig. 5B and 5C). Similar data were obtained with CDK4 siRNA transfected SW116 cells (data not shown). These results indicate that CDK4 is most likely involved in the induction of CRC-derived cell cycle arrest by miR-142-3p.

Fig. 5. CDK4 induces cell cycle arrest. A. Efficient siRNA-mediated inhibition of CDK4 expression. The expression of endogenous CDK4 was assessed 48 h after transfection with CDK4 siRNA or NC siRNA by Western blotting. GAPDH was used as an internal control. B. CDK4 knockdown reduces the viability of CRC-derived cells. Representative cell viability results are shown of HT29 cells transfected with CDK4 siRNA and NC siRNA, or not transfected cells. C. CDK4 knockdown induces cell cycle arrest in CRC-derived cells. Representative cell cycle results are shown of HT29 cells transfected with CDK4 siRNA, NC siRNA and, or not transfected cells. Columns, means of three independent experiments; bars, $\pm$ SD; * P <0.01, ** P <0.001.

Discussion

In the past, miRNAs have been found to be frequently deregulated in CRC, and some miRNAs were found to be associated with particular clinicopathological features of CRC, such as metastasis, recurrence and prognosis [17-19]. Moreover, compelling evidence indicates that miRNAs may play important roles in CRC progression and may directly contribute to the proliferation, avoidance of apoptosis and metastasis of CRC [20, 21]. MiR-103 is an oncogene miRNA that promotes colorectal cancer proliferation and migration through down-regulation of the tumor suppressor genes DICER and PTEN [22]; miR-204-5p acts as a tumor suppressor in colorectal cancer through inhibiting RAB22A [23]; miR-144 was markedly down-regulated in colorectal cancer and can inhibit the proliferation and migration of

colorectal cancer HCT116 cells [24]; the IL-6R/STAT3/miR-34a loop was necessary for EMT, invasion, and metastasis of CRC cell lines [25]; miR-378 expression was low in colon cancer tissues and cell lines and miR-378 not only inhibits the proliferation of colon cancer cells *in vitro* by inducing apoptosis, but also inhibits migration and invasion by inhibiting the EMT of colon cancer cells [26]; miR-153 was highly expressed in a cellular model of advanced stage colorectal cancer and increased colorectal cancer invasiveness and resistance to oxaliplatin and cisplatin [27]; miR-451 was over-expressed in multiple colorectal cancer tissues and might inhibit AMPK to activate mTORC1, which mediates FSCN1 expression and cancer cell progression [28].

miR-142 is expressed in many other tissues and displays a functional role in cancer, virus infection, inflammation and immune tolerance [29]. Most recent, miR-142-3p has been reported to be associated with several types of cancer. MiR-142-3p was significantly upregulated in renal cell carcinoma and miR-142-3p inhibitor could significantly suppressed cell migration and proliferation, and promoted cell apoptosis in 786-O and ACHN cells [30]; miR-142-3p was down-regulated in both cancer cell lines and cancer specimens and miR-142-3p overexpression suppressed proliferation by leading cell cycle arrest in G2/M[31]; miR-142-3p was downregulated in hepatocellular carcinoma (HCC) tissues and the overexpression of miR-142-3p inhibited aerobic glycolysis and thus proliferation of HCC cells by targeting lactate dehydrogenase A (LDHA) [32]; miR-142-3p was significantly lower in ovarian cancer tissues and cell lines and Ectopic expression of miR-142-3p significantly inhibited the proliferation of ovarian cancer cells by targeting sirtuin 1 (SIRT1) and increased the sensitivity of SKOV3/DDP cells to cisplatin [33]; miR-142-3p was markedly downregulated in gastric cancer tissues and could inhibit the proliferation, invasion and migration of gastric cancer cells [34]; miR-142-3p inhibited proliferation and invasion in HeLa and SiHa cells by targeting frizzled 7 receptor [FZD7] [35]; miR-142-3p downregulated MGMT expression and also sensitized Glioblastoma multiforme [GBM] cells to alkylating drugs [36]; miR-142-3p overexpression increased PI3K, Akt, and mTOR phosphorylation by targeting High mobility group box-1 [HMGB1] in NSCLS cells [37]. According to CRC, three studies have suggested that miR-142-3p is involved in CRC development. On one hand, miR-142-3p promotes cellular invasion by activating RAC1 in colorectal cancer cells [38]. On another hand, miR-142-3p functions as a tumor suppressor by targeting CD133, ABCG2, and Lgr5 in colon cancer cells [39]. Moreover, downregulation of plasma miR-142-3p was reported in CRC patients [40]. Thus, the role of miR-142-3p on CRC progression is unclear. In this study, miR-142-3p was indentified to play a role in cell growth and apoptosis in CRC.

Based on an *in silico* MiRanda search, CDK4 was predicted to be a target of miR-142-3p. CDK4 is known to be expressed in nearly all proliferating cells and to be able to promote cell cycle progression [41]. CDK4 has been reported to be regulated by several miRNAs, including miR-206, miR-506, miR-138, miR-124, miR-545 and miR-302 [42-47]. We, for the first time, identified CDK4 as a target of miR-142-3p in CRC, which may provide new insights into the mechanisms underlying its tumorigenesis. In addition, cell cycle progression is known to be regulated by a family of cyclin-dependent kinases (CDKs) and their activating partners (Cyclins). The G1/S phase cell cycle transition is regulated primarily by D-type Cyclins (D1, D2 or D3) in complex with CDK4/CDK6, and E-type Cyclins (E1 or E2) in complex with CDK2. These complexes cooperate in phosphorylating and in preventing Rb binding to E2F, thus activating E2F-mediated transcription and driving cells from the G1 into the S phase of the cell cycle [48]. Whether the CDK/pRb/E2F pathway is also involved in the miR-142-3p-mediated induction of cell cycle arrest in CRC cells requires further investigation.

Conclusion

In conclusion, we here provide evidence that low miR-142-3p expression contributes to cell viability and cell cycle progression in CRC via direct binding to the CDK4 3'-UTR. As such, miR-142-3p may act as a tumor suppressor and as a miRNA-based CRC therapeutic agent.

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Disclosure Statement

The authors declare that they have no competing interests.

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