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ARTICLE

Colon cancer cell proliferation is inhibited by miR-142a-5p via regulation of the PLK1 gene

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The morbidity and mortality of colon cancer are still very high, although medical technology has greatly improved in recent years. This study explored the role of miR-142a-5p in the proliferation of colon cancer cells. The binding between miR-142a-5p and PLK1 was verified by using luciferase assays. Western blotting was employed to determine whether PLK1 is the direct target gene of miR-142a-5p. In addition, a Cell Counting Kit-8 (CCK-8) assay was used to determine whether miR-142a-5p inhibited the proliferation of colon cancer cells by targeting PLK1. Our results show the clear binding between miR-142a-5p and PLK1. Reduced PLK1 expression in colon cancer cells transfected a miR-142a-5p mimic and increased PLK1 expression in colon cancer cells transfected with a miR-142a-5p inhibitor were observed. Moreover, the overexpression of PLK1 promoted cell proliferation, while the miR-142a-5p mimic inhibited cell proliferation. In conclusion, our findings indicate that miR-142a-5p inhibits the proliferation of colon cancer cells by downregulating PLK1.

Keywords: colon cancer; cell proliferation; miR-142a-5p; PLK1; tumor

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Colon cancer is one of the most common malignant tumors in the world, and its morbidity and mortality are very high [1]. In recent years, with the advancement of medical technology, the survival of colon cancer patients has improved, but the incidence of colon cancer is still high [2]. Most patients are diagnosed at the middle to late stages and miss the best period for treatment [3]. Defining the exact molecular mechanisms of colon cancer development and progression is critical for the diagnosis and treatment of colon cancer.

Polo-like kinase 1 (PLK1) is a class of highly conserved serine/threonine kinases that are highly regulated in eukaryotic cells and play a very important regulatory role in the cell cycle [4]. It has been reported that PLK1 is highly expressed in various cancers (such as colon cancer, non-small cell lung cancer, breast cancer, esophageal cancer, and endometrial cancer), and it acts as an oncogenic protein to promote the development of cancer [5-9]. In leukemia cells, the knockdown of PLK1 with a small interfering RNA can significantly inhibit the proliferation of leukemia cells [10]. In colon cancer, high PLK1 expression may promote the metastasis of tumor cells, and PLK1 may be one of the target proteins for colon cancer treatment [11].

MicroRNAs (miRNAs) are a class of noncoding RNAs that are 19-22 nucleotides in length and play an important regulatory role in the development and progression of cancer [12]. Studies have shown that miRNAs may directly bind to the coding sequence of a mRNA or bind to the 3'-untranslated region (UTR) of the target gene mRNA to inhibit gene

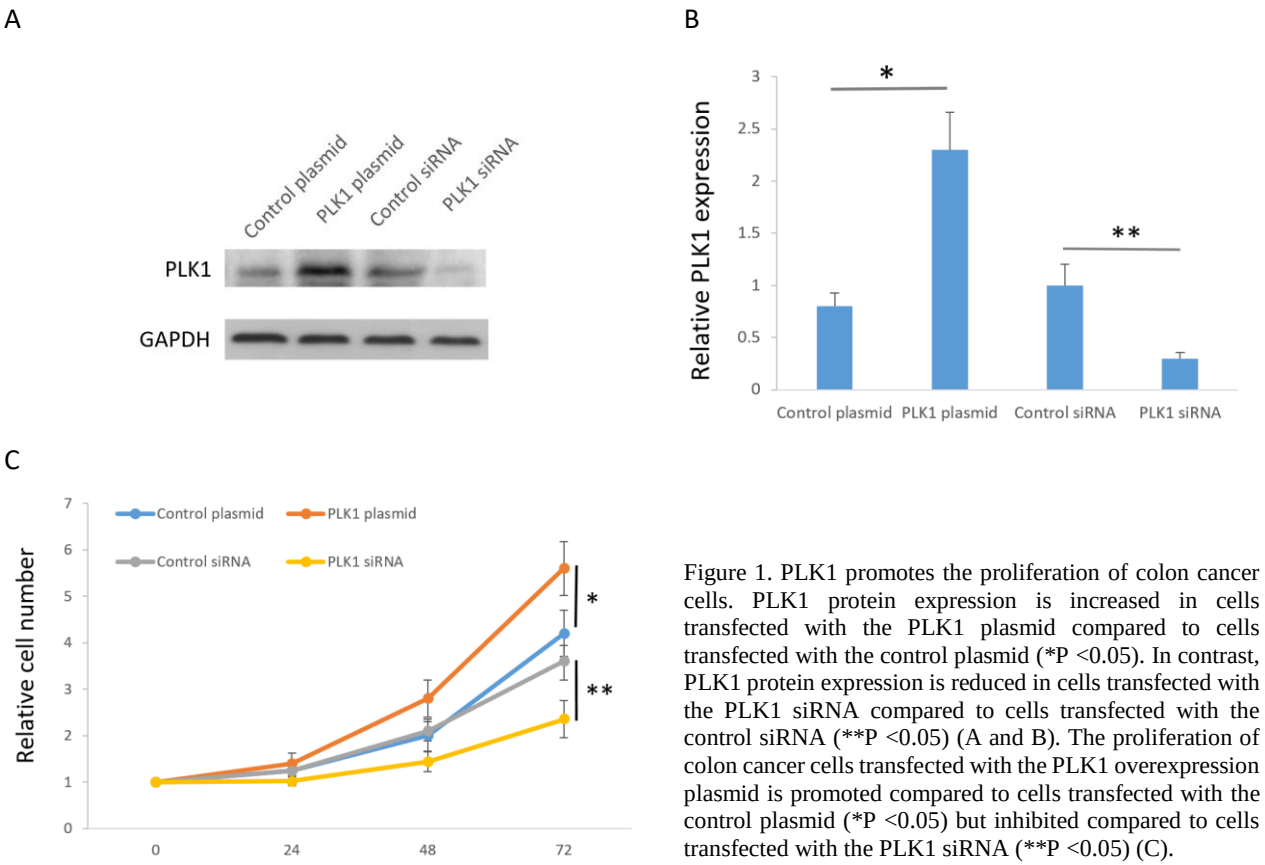


Figure 1. PLK1 promotes the proliferation of colon cancer cells. PLK1 protein expression is increased in cells transfected with the PLK1 plasmid compared to cells transfected with the control plasmid (*P <0.05). In contrast, PLK1 protein expression is reduced in cells transfected with the PLK1 siRNA compared to cells transfected with the control siRNA (**P <0.05) (A and B). The proliferation of colon cancer cells transfected with the PLK1 overexpression plasmid is promoted compared to cells transfected with the control plasmid (*P <0.05) but inhibited compared to cells transfected with the PLK1 siRNA (**P <0.05) (C).

expression [13-14]. miRNAs, as important posttranscriptional regulators, are involved in many physiological and pathological processes, such as hematopoietic cell differentiation [15], cell proliferation [16], organ development [17] and tumorigenesis [18-19]. miRNAs can function as tumor suppressor miRNAs or cancer-promoting miRNAs in the development of tumors [20], and many miRNAs are also involved in the development of colon cancer [21]. Studies have shown that miR-142a-5p plays important roles in the pathophysiology of many diseases, such as hyperlipidemia [22] and myocardial infarction [23], but little is known regarding miR-142a-5p in colon cancer. In this study, we investigated the effects and regulatory mechanisms of miR-142a-5p on colon cancer proliferation.

Materials and methods

Colon cancer cell lines

Human colon cancer cell lines SW480 and HCT116 were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. SW480 and HCT116 cells were cultured in 5% CO_2 at 37 °C and in complete DMEM (HyClone) supplemented with antibiotics (100 U/mL penicillin and 100 mg/L streptomycin) and 10% fetal bovine serum (HyClone). The cells were used for subsequent experiments after reaching the logarithmic growth phase.

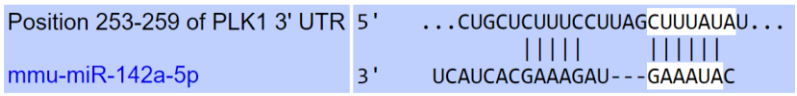
miR-142a-5p transfection

The miR-142a-5p mimic and the miR-142a-5p inhibitor were purchased from Guangzhou Ruibo Biological Co., Ltd. Cell transfection experiments were performed with Lipofectamine 3000 reagent (Invitrogen) according to the manufacturer's instructions. A small interfering RNA (siRNA) targeting PLK1 was purchased from Guangzhou Ruibo Biotechnology Co., Ltd., and a PLK1 overexpression plasmid (including the open reading frame only) was purchased from Shanghai Jima Biotechnology Co., Ltd. Cell transfection experiments were performed with Lipofectamine 3000 reagent according to the manufacturer's instructions.

Detection of PLK1 expression

After transfection for 24 h, the cell lysates of the transfected group were collected and centrifuged at 12,000 g at 5 °C for 5 min, and the supernatant was aspirated. Next, 5× protein loading buffer was added and incubated at 100 °C for 5 min. After electrophoresis by SDS-PAGE (10%), the membrane was transferred (ice bath, 0.3 A, 1.5 h) and blocked (5% skim

A



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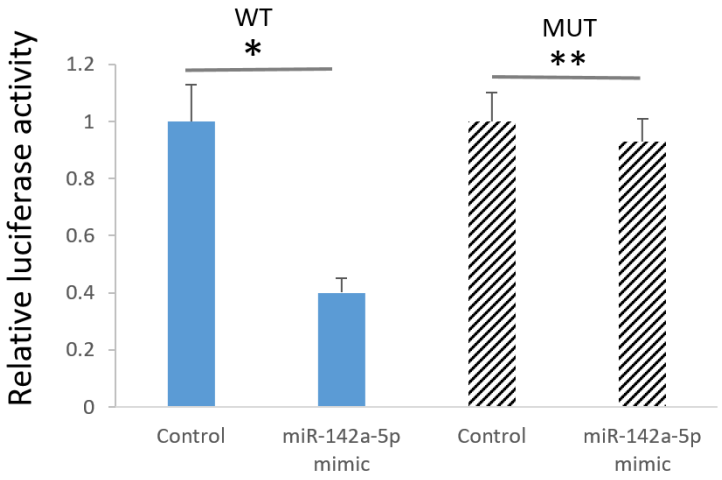

Figure 2. Prediction and dual luciferase reporter plasmid assay to confirm the binding of miR-142a-5p with the PLK1 gene. TargetScan was used to predict whether miR-142a-5p has a binding site for the 3'-UTR of PLK1 (A); Luciferase reporter assay shows that the fluorescence value of the 3'-UTR of the PLK1 gene was significantly decreased after adding the miR-142a-5p mimic (*P < 0.05), while after the sequence mutation and transfection with the miR-142a-5p mimic, the fluorescence value was not significantly different from that of the control group (**P > 0.05).

milk powder, room temperature for 1 h). Then, a PLK1 I antibody (ABclonal; diluted 1:1000) was added and incubated overnight at 4 °C. The membrane was washed with TBST for 5 min for a total of 6 times. The diluted secondary antibody was added and incubated for 1 h at room temperature. The membrane was washed with TBST for 5 min for a total of 6 times. GAPDH (Abcam) was used as the control.

Cell viability detection

The number of cells at 4 time points (0, 24, 48, and 72 h) after transfection was determined using the Cell Counting Kit-8 (CCK-8) kit (Japan Tongren) according to the manufacturer's instructions. The absorbance at 450 nm was monitored using a microplate reader. All experiments were repeated three times.

Luciferase assay

The dual luciferase reporter gene was used to detect whether miR-142a-5p directly targets the PLK1 gene. The 3'-UTRs for the wild-type and mutant PLK1 luciferase reporter plasmids were synthesized by Shanghai Tongke Biotechnology Co., Ltd., and the sequence of the 3'-UTR of PLK1 was inserted into the pmirGLO vector (Promega, E1330) to form the PLK1 3'-UTR plasmid. An equal amount of the luciferase reporter plasmid described above and a β-galactosidase (β-gal) expression plasmid (Ambion) and 20 pmol of the miR-142a-5p mimic or control were transfected into colon cancer cells with Lipofectamine 3000 reagent. The β-gal plasmid was used as a control to determine transfection efficiency. After 24 h, cells were detected using a luciferase assay kit.

Statistics

SPSS 22.0 software was utilized to perform the data analysis. The data are expressed as the mean ± standard deviation (SD). One-way analysis of variance was used for comparison between groups. $P < 0.05$ was considered statistically significant.

Results

PLK1 promotes the proliferation of colon cancer cells

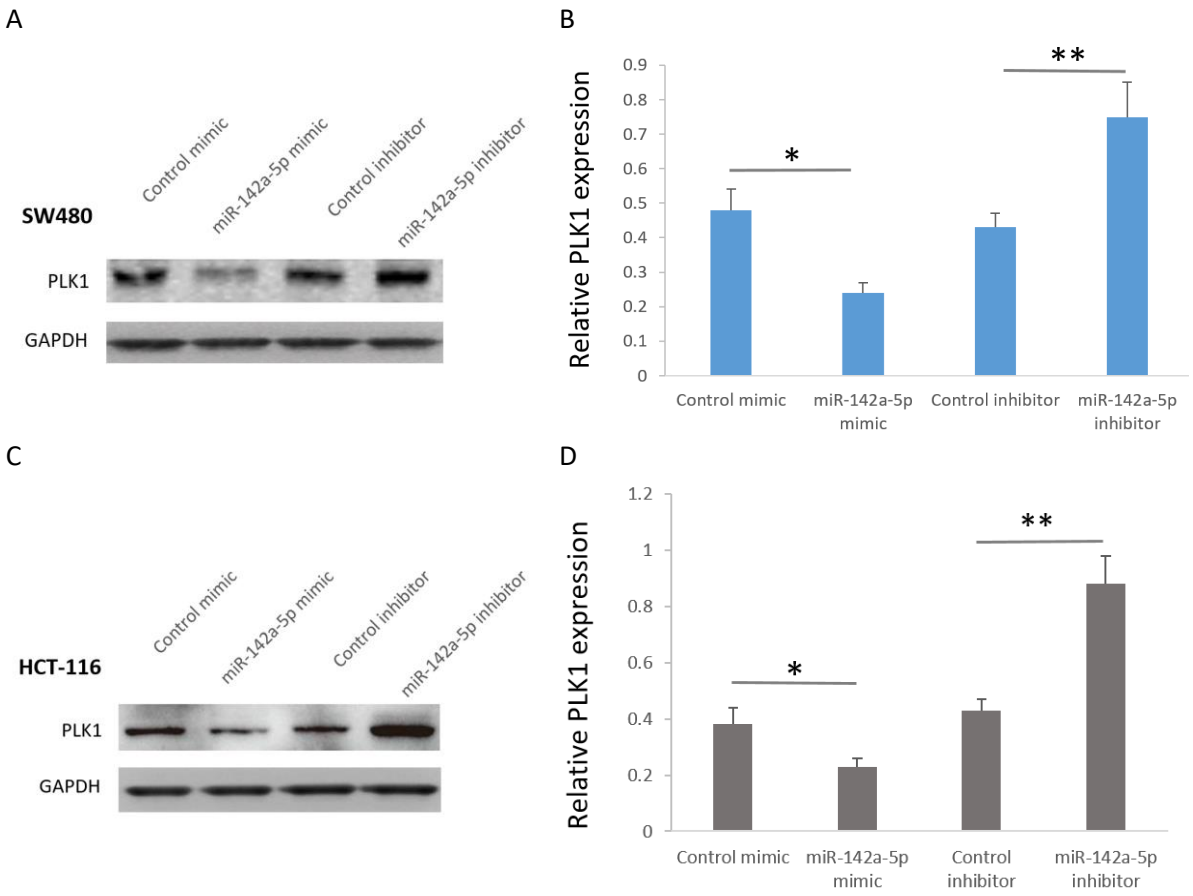

Figure 3. PLK1 expression is regulated by miR-142a-5p in colon cancer cells. The expression of the PLK1 protein was decreased in the miR-142a-5p-overexpressing cell lines compared to that in the control group (A-D). After inhibiting the expression of miR-142a-5p by transfection with the miR-142a-5p inhibitor, the expression of the PLK1 protein was significantly increased compared to that in the control group (A-D). *P < 0.05; **P < 0.05.

The PLK1 overexpression plasmid and the siRNA were transfected into cells for 24 h, and cell proliferation was assessed with the CCK-8 kit. PLK1 expression was increased significantly in cells transfected with the PLK1 overexpression plasmid when compared to cells transfected with the control plasmid (Figure 1A and B). However, PLK1 expression was reduced significantly in cells transfected with the PLK1 siRNA when compared to cells transfected with the control plasmid (Figure 1A and B). In addition, the proliferation of colon cancer cells transfected with the PLK1 overexpression plasmid was promoted significantly compared to cells transfected with the control siRNA (Figure 1C). In contrast, proliferation was reduced in cells transfected with the PLK1 siRNA when compared to cells transfected with the control siRNA, which suggests that PLK1 is involved in the proliferation of colon cancer cells (Figure 1C).

Prediction and validation of PLK1 as a target gene of miR-142a-5p

This study used TargetScan [24-26] to find miRNAs that may bind to PLK1. Among these candidates, miR-142a-5p was predicted by TargetScan as a PLK1-bound miRNA. miR-142a-5p has a binding site for the 3'-UTR of PLK1 (Figure 2A). In addition, the region where the 3'-UTR of PLK1 binds complementarily to the miR-142a-5p sequence is highly conserved between species. To verify the above prediction, this study conducted a dual luciferase reporter gene assay (Figure 2B). The fluorescence value of the 3'-UTR of the PLK1 gene was significantly decreased after adding the miR-142a-5p mimic (*P < 0.05). However, after the sequence mutation and transfection with the miR-142a-5p mimic, the fluorescence value was not significantly different from that of the control group (**P > 0.05).

miR-142a-5p regulates the expression of PLK1 in colon cancer cells

To further demonstrate the effect of miR-142a-5p on PLK1 expression, a miR-142a-5p mimic and a miR-142a-5p

inhibitor were transfected into two colon cancer cells and detected by Western blot 24 h later. After the overexpression of miR-142a-5p in both cell lines, the expression level of the PLK1 protein was decreased compared to that in the control group. After inhibiting the expression of miR-142a-5p by transfection with a miR-142a-5p inhibitor, the expression level of the PLK1 protein was significantly increased (Figure 3A, B).

miR-142a-5p inhibits the proliferation of colon cancer cells

After transfecting colon cancer cells with the miR-142a-5p mimic and the miR-142a-5p inhibitor for 24 h, cell proliferation ability was assessed with the CCK-8 kit. After the overexpression of miR-142a-5p, cell proliferation was significantly inhibited ($P < 0.05$, Figure 4). However, when miR-142a-5p was inhibited by transfection with the miR-142a-5p inhibitor, cell proliferation was significantly higher than that of the control group ($P < 0.05$, Figure 4).

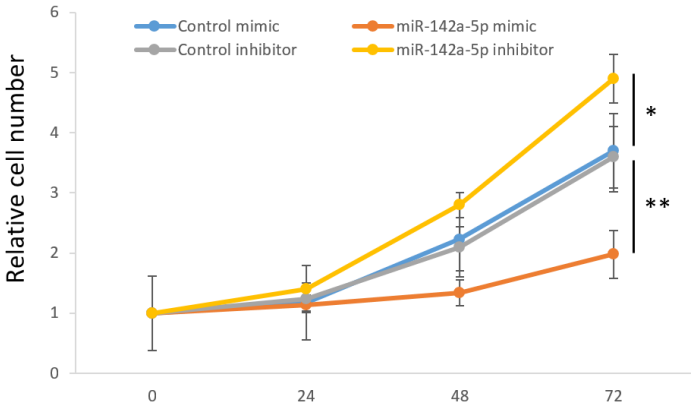

Figure 4. miR-142a-5p inhibits the proliferation of colon cancer cells. The proliferation of colon cancer cells transfected with the miR-142a-5p mimic is reduced while the proliferation of cells transfected with the miR-142a-5p inhibitor is increased compared to the corresponding control group (*$P < 0.05$).

Discussion

As important posttranscriptional regulators, miRNAs play an important role in the development of tumors [27]. Existing studies have shown that miRNAs have both inhibitory and stimulatory effects on the development of colon cancer [28]. Studies have shown that miR-593 affects the proliferation of colon cancer by regulating the expression of the PLK1 gene [29]. PLK1 was originally identified in Drosophila as a protein involved in spindle formation, and further studies indicated that this kinase plays a key role in centrosome biology, spindle dynamics, chromosome segregation, and cytokinesis [30]. Numerous studies have shown that the early stages of PLK1 cancer development play a role, and its overexpression is negatively correlated with the survival rate of non-small cell lung cancer, head and neck cancer and esophageal cancer patients [31-32]. The use of small molecule inhibitors of PLK1 to treat tumors such as leukemia and non-small cell lung cancer is currently a clinical treatment strategy [33]. However, in colon cancer, the regulation of the presence or absence of miRNAs in PLK1 has not been studied.

To identify miRNAs that may regulate the expression of the PLK1 gene, the bioinformatics program TargetScan was used to predict whether miR-142a-5p might bind to PLK1 miRNA [26-28]. We found that PLK1 promoted the proliferation of colon cancer cells (as assessed by CCK-8 cell proliferation assays), which is consistent with the results of other studies [34], and after interfering with the expression of miR-142a-5p in colon cancer cells, we found that miR-142a-5p inhibited the proliferation of colon cancer cells, which indicates that the effect of miR-142a-5p is contrary to that of PLK1 on colon cancer cell proliferation. These results also suggest that miR-142a-5p acts as a tumor suppressor miRNA in colon cancer cells, which provides an experimental basis for clarifying the function of miR-142a-5p in colon cancer.

To verify the binding site between miR-142a-5p and the PLK1 gene, we performed dual luciferase reporter gene assays and found that the fluorescent value of PLK1 wild-type cells transfected with miR-142a-5p mimic decreased, but that of the mutant did not change. These results suggest that the 3'-UTR of the PLK1 gene is present at the site of miR-142a-5p binding. This study also found that the expression level of the PLK1 gene was significantly decreased after the overexpression of miR-142a-5p in two colon cancer cell lines. However, the expression level of the PLK1 gene was significantly increased when miR-142a-5p was inhibited. In conclusion, this study suggests that miR-142a-5p inhibits the proliferation of colon cancer cells by downregulating PLK1.

Conflicting interests

The authors have declared that no conflict of interests exist.

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