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Original Research Article

MiR-145 inhibits proliferation of primary colon adenocarcinoma cells via induction of apoptosis, cell cycle arrest and inhibition of cell migration

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Abstract

Purpose: *To investigate the role and potential of miR-145 as a therapeutic target for the treatment of primary colon adenocarcinoma in cell lines*
Methods: *The expression of miR145 was determined by quantitative real time-polymerase chain reaction (RT-PCR), while cell viability was determined by MTT assay. Apoptosis was assessed by 4',6-diamidino-2-phenylindole (DAPI), acridine orange/ethidium bromide (AO/EB), and annexin V/PI double staining. Cell cycle analysis was performed by flow cytometry, while immunoblotting was used to determine protein expression.*
Results: *The expression of miR-145 was significantly enhanced in all the colon adenocarcinoma cell lines investigated. On the other hand, suppression of miR-145 expression led to significant decrease in cell viability, activation of apoptosis, G2/M cell cycle arrest, and inhibition of migration of colon adenocarcinoma cells.*
Conclusion: *These results indicate that miR-145 regulates the proliferation and metastasis of colon adenocarcinoma cells. Thus, it may be a prospective drug target for the treatment of this disease.*

Keywords: *MicroRNA, Apoptosis, Cell migration, Proliferation*

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INTRODUCTION

Primary colon adenocarcinoma is one of the lethal cancers, and is responsible for significant level of mortality across the globe [1]. The current treatment for colon adenocarcinoma involves surgery and chemotherapy. However, the success rate of these treatments is low. Moreover, a number of adverse effects are associated with currently available drugs [2,3]. Therefore there is need to identify and study newer and more efficient drugs with minimum side effects, as well as novel therapeutic targets.

MicroRNAs are small molecules and they are not translated into proteins [4,5]. Diverse roles have been ascribed to microRNAs. For example, it is believed that the expressions of thousands of genes in humans are controlled by microRNAs. In addition, cell proliferation is regulated by microRNAs from several types of cells [6]. The

miRNAs have also been implicated in the development and progression of some deadly diseases, including cancer [7]. Therefore, they are considered as potential therapeutic targets [8].

In the present study, the expressions of MiR-145 in four different colon adenocarcinoma cell lines were determined. In addition, the effect of miR-145 on the proliferation, cell viability and migration of the human colon adenocarcinoma cells were investigated.

EXPERIMENTAL

Chemicals, reagents and cell cultures

All chemicals and reagents were procured from Sigma-Aldrich Co. (St. Louis, MO, USA), unless otherwise indicated. Four human primary colon adenocarcinoma cell lines (WiDr, SW48, LS123, LS180), and one normal colon cell line (CCD-18Co) were obtained from Type Culture Collection of Chinese Academy of Sciences, Shanghai, China. The cells were grown in RPMI-1640 medium and maintained at standard culturing conditions.

RNA isolation, cDNA synthesis and analysis of expression

The total RNA was extracted from the colon adenocarcinoma cells with RNeasy Kits (Qiagen), and Omniscript RT (Qiagen) was employed to reverse-transcribe the cDNA using 1 µg of the extracted RNA. The cDNA was then used as template for quantitative real-time PCR (qRT-PCR), with Taq PCR Master Mix Kit (Qiagen) in line with the guidelines of the instrument manufacturer.

Inhibition of miR-145

The inhibitor of human miR-145 (MiR145-In, 107 units/mL), and its non-specific miRNA lentivirus (MiR-C, 107 units/mL) were procured from GenePharma (Shanghai, China). The SW48 cells were then incubated with lentiviral particles and polybrene (8 mg/mL) for 48 h. Thereafter, the cells were harvested, cultured in a fresh medium, and incubated further for 3 –7 days for stable transduction. The cells were then passaged and preserved *in vitro* for use in subsequent experimentation.

Cell viability assay

The cell viability of the lung cancer cells was assessed by MTT assay. The lung cancer cells were seeded in 96-well plates at a density of 2 x 10^5 cells/well. The cells were then incubated with MTT at 37 ^oC for 4 h. Thereafter, absorbance was read at 570 nm in a microplate reader at different time intervals (24 and 48 h) to determine the viability of the cells.

Apoptotic assays

Colon adenocarcinoma cells (SW48) were cultured in 6-well plates at a density of 1×10^6 cells per well, and incubated for 24 h. This was immediately followed by DAPI staining. Then, the cell samples were examined and photographed with a fluorescence microscope. To determine populations of apoptotic SW48 cells, the cells were platted in 6-well plates and treated with Annexin V/FITC and PI for 20 min. The percentage of apoptotic cells was determined by flow cytometry (BDBiosciences, San Jose, CA, USA).

Cell cycle analysis

The distribution of the SW48 cells in different phases of cell cycle was investigated by seeding approximately$1x10^5$ cells in each well of a 6-well plate. The cells were transfected with miR-145-containing vector, or with empty vector, and kept at 37 °C overnight to allow for adherence. Then, the distribution of the SW48 cells in various cell cycle phases was determined by flow cytometry.

Wound healing assay

The capacity of the SW48 cells to migrate was determined by wound healing assay. The cells were seeded at a density of 5×10^4 cells/well and cultured in 96-well plates. The plates were then incubated overnight at 37 $^\circ$C to achieve adherence. A sterile pipette tip was then used to scratch the cell culture, and the cells were washed with phosphate-buffered saline to remove the detached cells. The SW48 cells were then photographed after 48 to assess their migration.

Western blot analysis

The SW 48 cells were lysed in ice-cold hypotonic buffer. Protein concentration was estimated in each cell extract, the protein samples were subjected to electrophoresis (SDS–PAGE). This was followed by transference to a nitrocellulose membrane and incubation with the primary antibody (1:1000) for 24 h at 4 $^\circ$C. Thereafter the membrane was incubated with HRP-conjugated secondary antibody (1:1000) at 24°C for about 1 h, and the protein bands were visualised by enhanced chemi-luminescence.

Statistical analysis

Each experiment was repeated three times and the results presented as mean ± SD. One way ANOVA and Tukey's test were used for statistical analysis. Difference between the samples were considered statistically significant at $p < 0.05$.

RESULTS

Expression of miR-145 in colon adenocarcinoma cell lines

The results revealed that the transcripts of miR-145 were significantly upregulated in all the colon adenocarcinoma cell lines (WiDr, SW48, LS123, and LS180), when compared to the normal colon cell line (CCD-18Co) ($p < 0.05$, Figure 1). The WiDr cell line exhibited the lowest expression of miR-145, while SW48 cell line showed the highest expression of miR-145. Thus, SW48 cell line was selected for further experimentation.

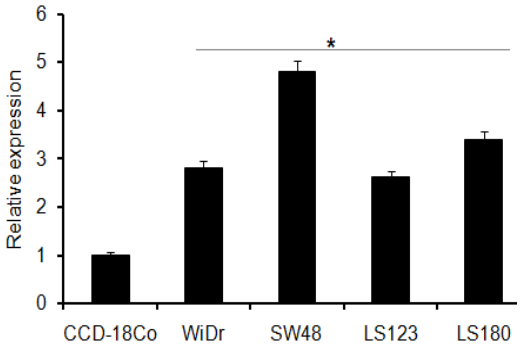

Figure 1: Expression of miR-145 in different primary colon adenocarcinoma cell lines (WiDr, SW48, LS123 and LS180) compared to miR-145 expression in normal colon cell line (CCD-18Co). Values are mean ± SD; *$p < 0.05$)

Downregulation of miR-145 promoted apoptosis

The expression of miR-145 was suppressed in SW48 cells through lentiviral transfection, in order to find out its role in colon adenocarcinoma cell line SW48. The results revealed that cells in which miR-145 expression was inhibited had less cell viability than the control SW48 cells without miR-145 inhibition (Figure 2). These findings suggest that down-regulation of miR-145 in SW48 cells suppressed their viability. Results from DAPI, EB/OR and Annexin-V/PI staining revealed that the suppression of miR-145 in SW48 cells triggered apoptotic cell death (Figure 3). Thus, miR-145 suppression decreased cell viability via inducement of apoptosis.

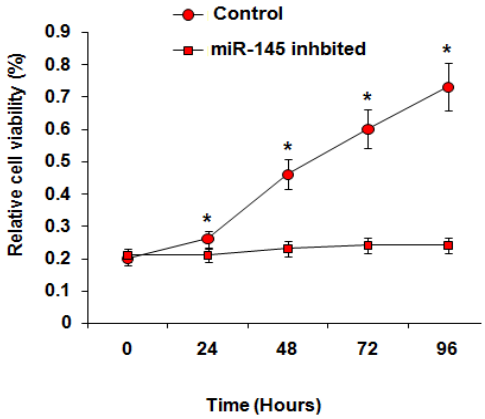

Figure 2: Effect of miR-145 inhibition of cell viability. The experiments were carried out in triplicates and expressed as mean ± SD (*$p < 0.05$ vs control)

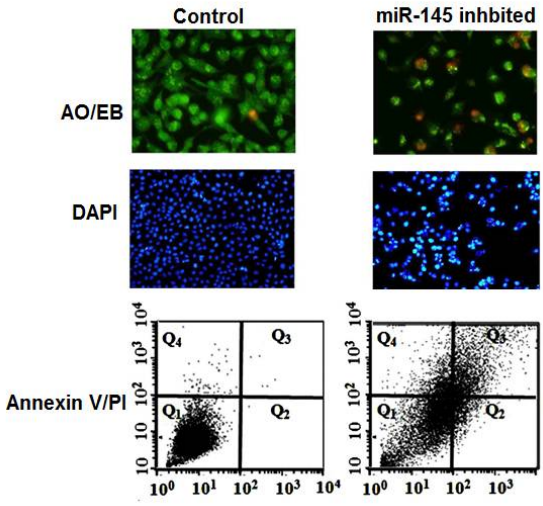

Figure 3: Inhibition of miR-145 promotes apotosis in SW48 colon adecarcinoma cells as indicated by AO/EB, DAPI and annexin V/PI staining. The experiments were carried out in triplicates

Down-regulation of miR-145 caused G2/M cell cycle arrest

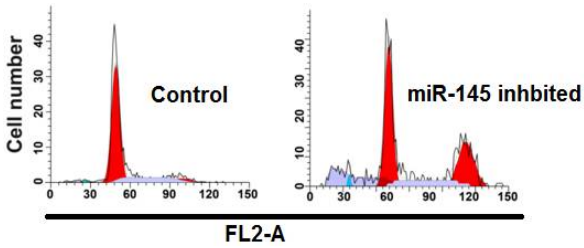

Figure 4: Inhibition of miR-145 triggers G2/M cell cycle arrest in SW8 colon adenocarcinoma cell lines. The experiments were carried out in triplicate

It was revealed that the cells treated with miR-145 mimics accumulated in G2/M phase of the cell cycle, in contrast to the control SW48 cells (Figure 4). Thus miR-145 exerted anti-proliferative effects on the SW48 colon

adenocarcinoma cells partly through G2/M cell arrest.

Suppression of miRNA-145 inhibited cell invasion

Results from wound healing assay revealed that suppression of miR-145 significantly decreased the cell migration capacity of SW48 cells after 20 h. Relative cell migration in the SW8 cells treated with mimics-NC was 34 %, while 17 % migration was obtained in SW48 treated with miR-145 mimics (Figures 5A - 5B).

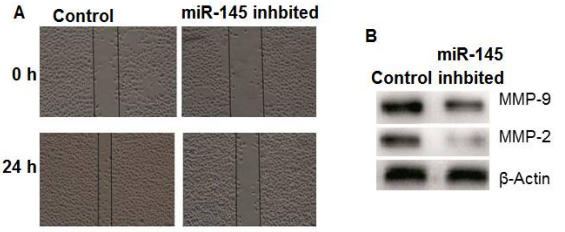

Figure 5: Effect of miR-145 on (A) Cell migration as determined by wound healing assay (B) Expression of MMP-9 and MMP-2 as determined by western blotting. The experiments were carried out in three biological replicates

DISCUSSION

Colon adenocarcinoma contributes to the high rate of cancer-related mortality world-wide [9,10]. The current treatments for colon adenocarcinoma have lots of adverse side effects and could be toxic to the patients. These shortcomings have necessitated interest in the screening and identification of novel and safer therapeutic targets [11]. In the last few decades, miRNAs have emerged as strong candidates with potential as prospective therapeutic targets for the treatment of cancers [12]. The expressions of a number of miRNAs are altered in cancerous tissues. In the present study, the expressions of miR-145 in colon adenocarcinoma and normal cell lines were investigated, and the results showed that the expression of miR-145 was significantly upregulated in all adenocarcinoma cell lines studied. These findings are in agreement with previous investigations which reported upregulation of the expression of miR-145 in breast and colorectal cancer cells [13,14].

The silencing of miR-145 expression with lentiviral transduction of miR-145 inhibitor triggered time-dependent decreases in cell viability of adenocarcinoma SW48 cells. In previous studies, it was also observed that suppression of mIR-145 inhibited the proliferation of several types of colon cancer cells. Interestingly, the suppression of miR-145 expression induced apoptosis and G2/M cell cycle arrest in SW48 colon adenocarcinoma cells. It is has been reported that apoptosis and cell cycle arrest are two important mechanisms for assessing the growth and proliferation of cancer cells [15,16]. Thus, miR-145 can be considered an important target for anticancer drugs. Cell migration also plays an essential role in the metastasis of cancer cells [17]. In the present study, suppression of miR-145 expression also inhibited the migration of colon adenocarcinoma SW48 cells. This finding reinforces the potential of miR-145 as a therapeutic target.

CONCLUSION

The results of the present study indicate that the expression of miR-145 is upregulated in colon adenocarcinoma cells, while its suppression promotes apoptosis, triggers cell cycle arrest and inhibits cell migration. Therefore, miR-145 is most likely to be a potential therapeutic target for the treatment of colon adenocarcinoma.

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