

MicroRNA-375 regulates proliferation and apoptosis of colon cancer by suppressing CTGF-EGFR signaling pathway

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Abstract: Aim: The aim of this study was to explain the effect and mechanism of microRNA-375 in colon cancer. Methods: The HT29 and SW620 cells were respectively divided into 3 groups: NC group, BL group and miRNA group. We measured the cell proliferation, apoptosis and cell cycle of difference groups. The relative proteins expressions were evaluated by WB assay. Results: Compared with NC groups, the cell proliferation of miRNA groups were significantly suppressed ($P < 0.05$, respectively), the cell apoptosis of miRNA groups were significantly enhanced ($P < 0.05$, respectively) and the G1 phased rate of miRNA groups were significantly increased ($P < 0.05$, respectively) in HT29 and SW620 cells. To explain the mechanism of miRNA-375 in colon cancer, the relative proteins expressions were measured by WB assay. The CTGF, EGFR, PI3K, AKT and ERK proteins expressions of miRNA-375 groups were significantly suppressed compared with NC groups ($P < 0.05$, respectively). Conclusion: miRNA-375 overexpression had anti-tumor effects to colon cancer via CTGF-EGFR signaling pathway.

Key words: miRNA-375, CTGF, EGFR, PI3K, colon cancer

Introduction

Colon cancer is one of the high incidence tumors in digestive system. The mechanism of colon cancer is complex, and it is still not clear. Previous studies have found that it is related to a series of oncogene activation and inactivation of tumor suppressor genes (1); among them, microRNA (miRNA) also plays an important role in the development and progression of tumor, including cell proliferation, differentiation, angiogenesis, apoptosis, invasion and metastasis (2, 3). Abnormal expression of miRNAs may lead to abnormal expression of corresponding proteins, which can play a role in tumor suppression or carcinogenesis (4, 5). Recent studies have found that miRNA-375 has a significant association with the biological activity of a variety of tumor cells (6-10), but the correlation between miRNA-375 and colon cancer is relatively limited. In our present study, we evaluated the effects and mechanisms of miRNA-375 over-expression in HT-29 and SW620 cells which were two kinds of colon cancer cell lines in cell apoptosis and proliferation by vitro study.

Materials and Methods

Materials and reagents

DMEM culture medium and trypsin were purchased from Gibco (USA); Human colon cancer cell lines HT-29 and SW620 (ATCC, USA), miRNA-375 were purchased from Nanjing Kingsy biotechnology Co. (China), Lipofectamine 2000 and TRIzol reagent (Invitrogen, USA), Methylthiazolyldiphenyl-tetrazolium bromide (MTT), Dimethyl sulphoxide (DMSO) and phosphate buffered saline (PBS) (Beijing Ding Ting Biotechnology Co., Ltd. , China). Mice anti-human CTGF, EGFR, PI3K, AKT and Erk anti-body (Abcam, USA).

Cell culture and transfection

Colon cancer cell lines HT29 and SW620 were cultured in DMEM contained 10% fetal bovine serum (FBS), the cells were cultured in incubator (37°C, 5% CO₂). When cells grow well, they were used in experiments. The colon cancer cells were respectively divided into 3 groups: Normal Control (NC) group, Blank (BL) group

and miRNA-375 (miRNA) group. miRNA-375 and BL respectively transfected miRNA-375 or empty-vector into HT29 and SW620 cells.

The cell proliferation by MTT assay

The cells of 3 groups were respectively cultured for 48 h, the HT-29 and SW620 of difference groups were digested by trypsin, the cells were inoculated into 96-hole-plate as 2×10^3 cells/hole, and cultured in CO₂ incubator for 48 h, adding the 20 μ l MTT (5 mg/ml) solution, reaction at 37 °C for 4 h, The cell culture medium was added with 100 μ l DMSO solution. After shaking reaction, the optical density (OD) of each hole at 490 nm was measured by enzyme analyzer, and the cell proliferation rate of the cell was calculated.

The cell apoptosis by flow cytometry

The cells of difference groups were respectively cultured for 48 h, after that, the cells were collected as 10^6 cells. The cells were washed 2 times with ice pre cooled PBS, adding 200 μ l buffer solution to mix, Adding 5 μ l propidium iodide (PI) and 5 μ l AnnexinV-FITC, culture in light avoidance condition for 20 min, adding 400 μ l binding buffer, the cell apoptosis rate were measured by flow cytometry.

The cell cycle by flow cytometry

The cells of difference groups were respectively cultured for 48 h. The cells were washed 2 times with ice pre cooled PBS, centrifuge removal by PBS, adding 70% pre-cooled EtOH to fix the cell for 24 h, Centrifugal discard fixative, after PBS washing once, the cells were collected in flow tube, adding 1 ml propidium iodide (PI) staining solution contained 100 mg/L RNase A and 5 mg/L PI. The cells were slowly and fully suspended, incubated for 30 min at room temperature, and then detected red fluorescence and light scattering by flow cytometry at 488 nm excitation wavelength. The cell cycle was analyzed by Flowjo software.

The relative proteins by WB assay

When the cells of difference groups grew to 80% fusions, the cells were collected and cleaned with PBS for 2 times. After that, RIPA Buffer was applied to cleavage 15 min on ice, then centrifugation 15 min at 4 C, and the concentration of

protein was detected by Bradford reagent, then take the same amount of protein sample, add 6×SDS-PAGE sample buffer, and boil 5 min. After denaturation, carry out 10% PAGE electrophoresis, adding the relative primary antibody (CTGF, EGFR, PI3K, AKT and Erk), turn film, adding the second anti-body (HRP labeled rat anti-IgG) and color. GADPH was as reference in this study.

Statistical analysis

SPSS 19.0 statistical software was used for statistical analysis. The experimental data were expressed by mean \pm standard deviation (Mean \pm SD). One way ANOVA method was used to compare between the groups, $P \leq 0.05$ regard as statistically significant differences.

Results

miRNA-375 has effects to cell proliferation in colon cancer cells

To explain the effects of miRNA-375 in the cell proliferation of colon cancer cells (HT-29 and SW 620), we measured the cell proliferation of HT-29 and SW 620 by MTT assay, the results were shown that the cell proliferation rate of miRNA group was significantly suppressed in HT-29 ($P < 0.05$, Figure 1) and SW 620 ($P < 0.05$, Figure 2) compared with NC groups.

Figure 1

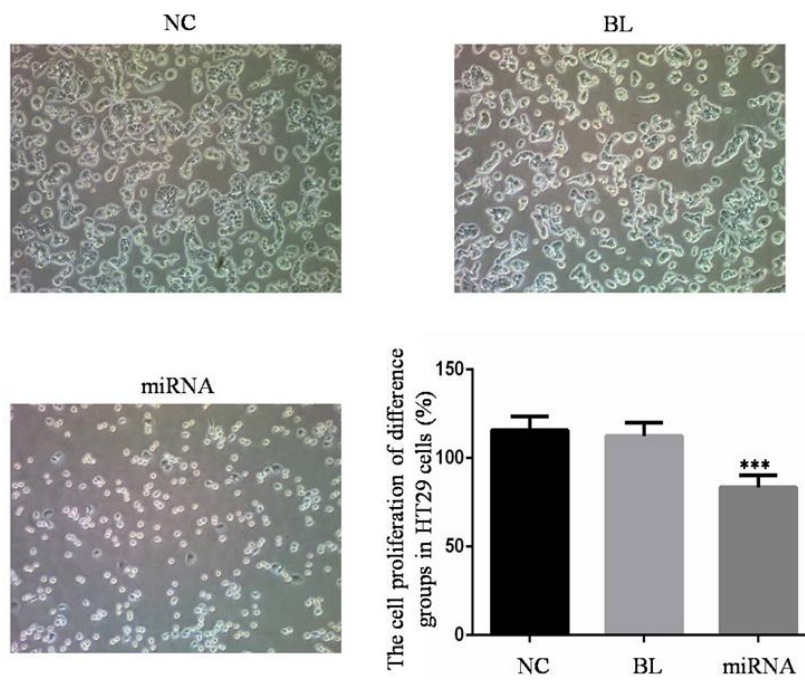


Figure 1. The cell proliferation rate of difference groups in HT-29 cell

***: $P < 0.05$, compared with NC group

Figure 2

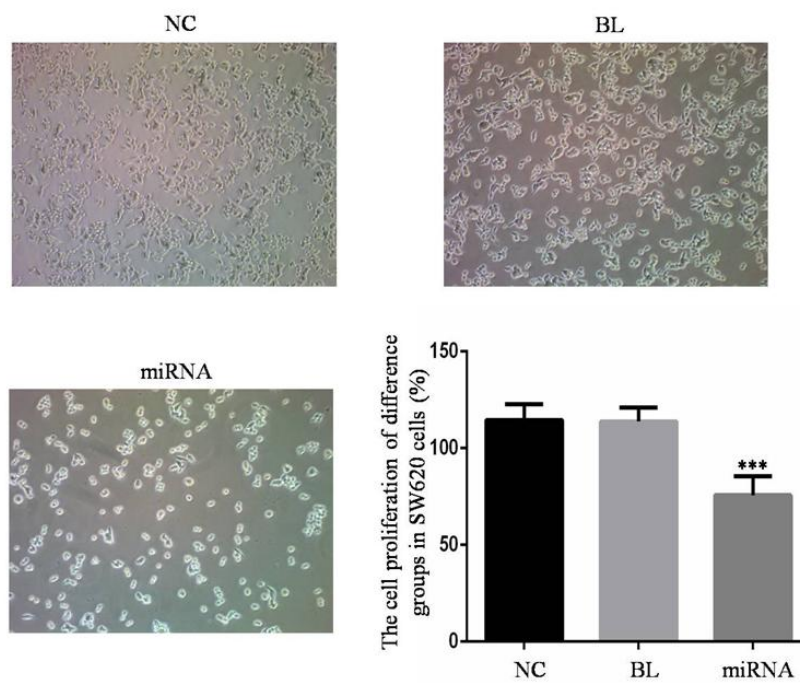


Figure 2. The cell proliferation rate of difference groups in SW 620 cell

***: $P < 0.05$, compared with NC group

miRNA-375 has effects to cell apoptosis in colon cancer cells

To investigation the effects of miRNA-375 to cell apoptosis in HT-29 and SW 620 cells, the cell apoptosis rates of difference groups were measured by flow cytometry, the results were shown that the cell apoptosis rate of miRNA group which were transfected with miRNA-375 were significantly enhanced in HT-29 ($P < 0.05$, Figure 3) cell and in SW 620 ($P < 0.05$, Figure 4) compared with NC groups.

Figure 3

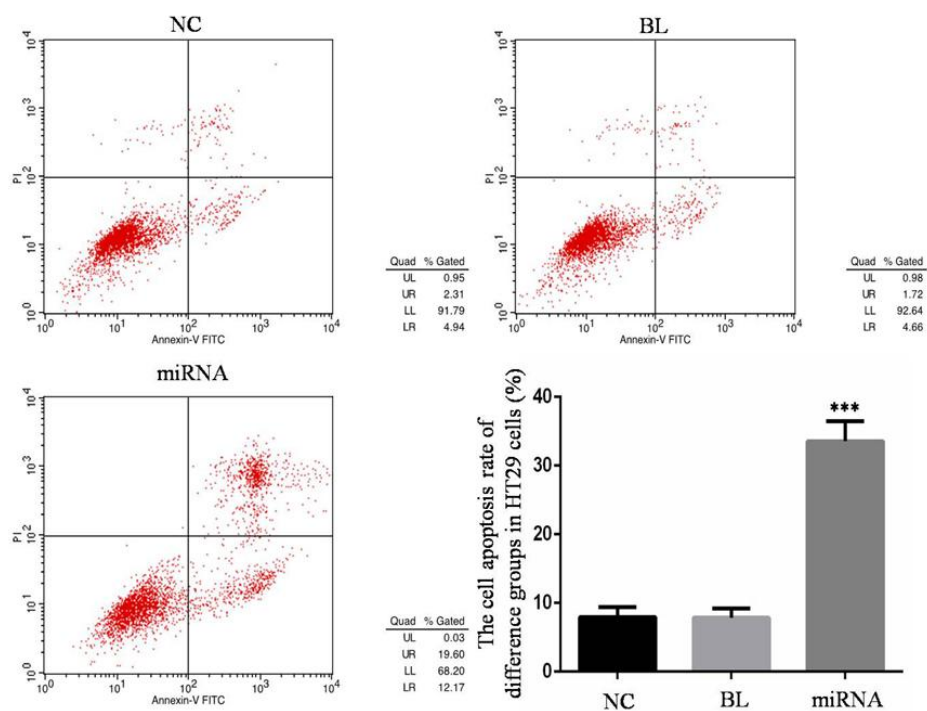


Figure 3. The cell apoptosis rate of difference groups in HT-29 cell

***: $P < 0.05$, compared with NC group

Figure 4

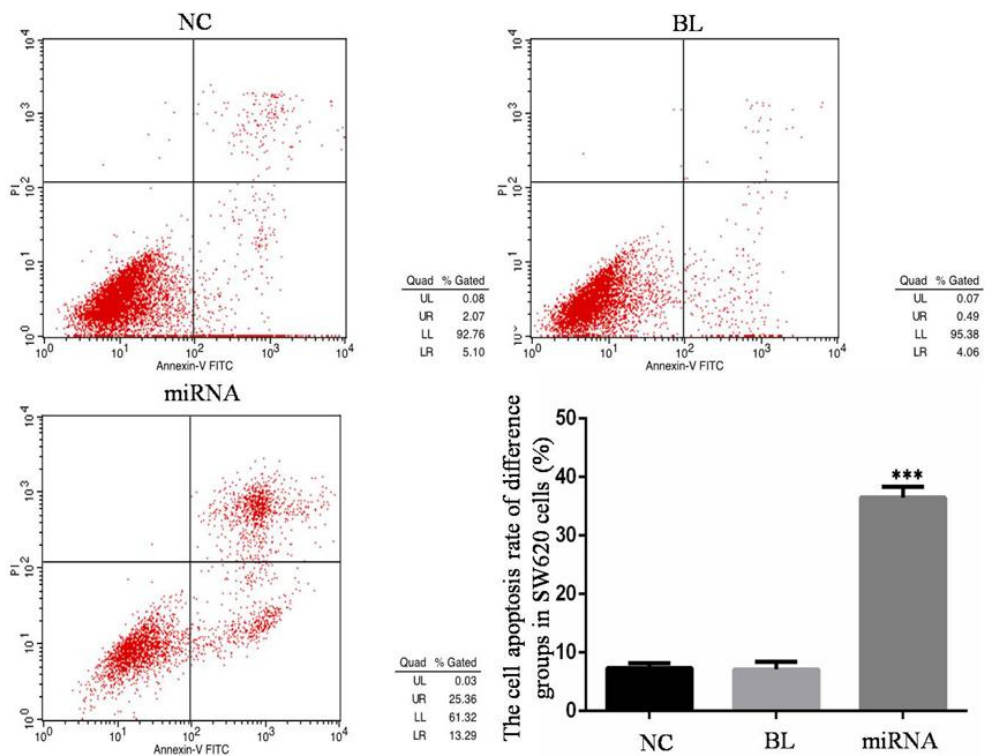


Figure 4. The cell apoptosis rate of difference groups in SW 620 cell

***: $P < 0.05$, compared with NC group

miRNA-375 has effects to cell cycle in colon cancer cells

To evaluate the effects of miRNA-375 to cell cycle in HT-29 and SW 620 cells, we measured the cell cycle of difference groups by flow cytometry. The results were shown that G1 phase rate of miRNA groups were significantly increased in HT-29 ($P < 0.05$, Figure 5) and SW 620 cells ($P < 0.05$, Figure 6).

Figure 5

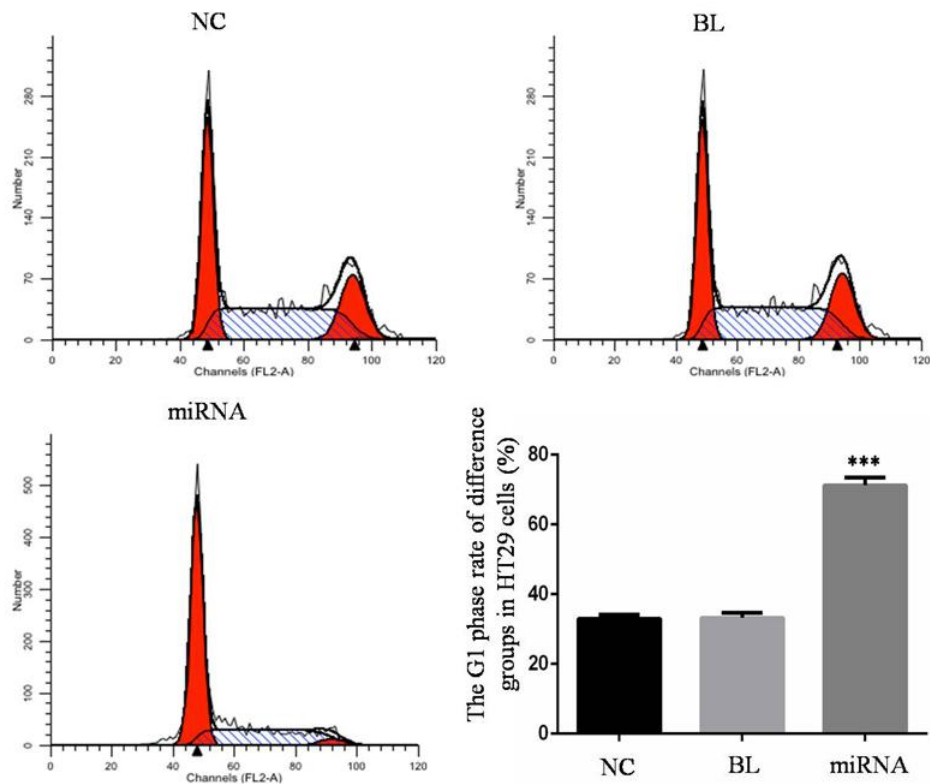


Figure 5. The G1 phase rate of difference groups in HT-29 cell

***: $P < 0.05$, compared with NC group

Figure 6

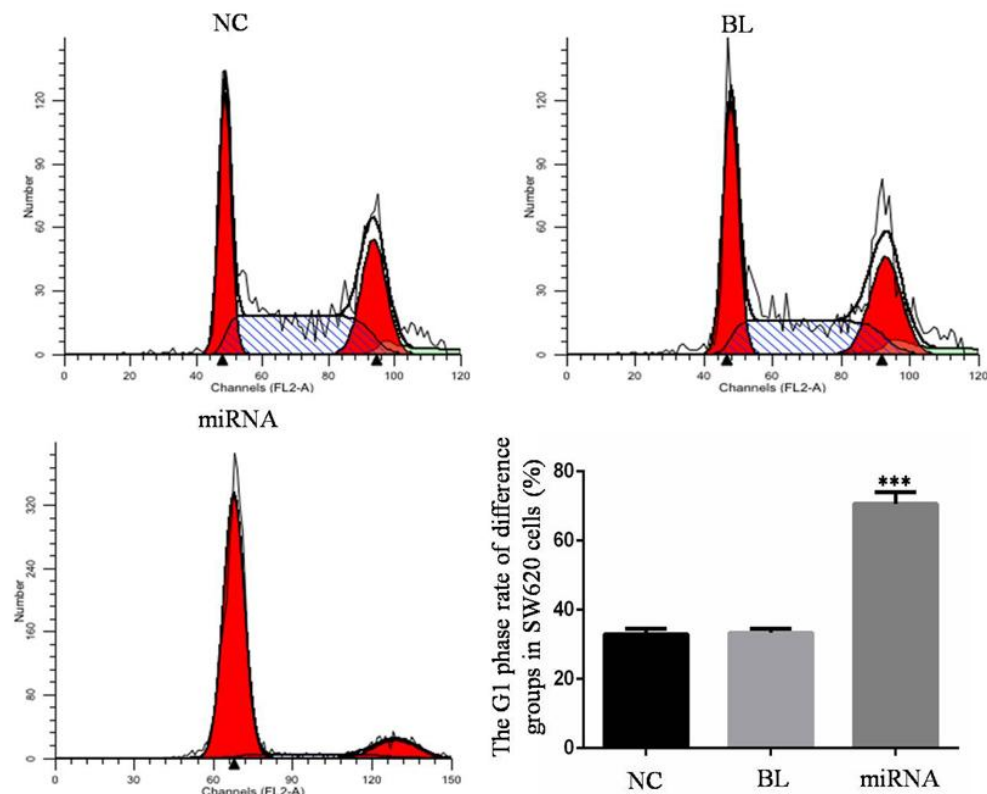


Figure 6. The G1 phase rate of difference groups in SW 620 cell

***: $P < 0.05$, compared with NC group

miRNA-375 has effects to relative protein in colon cancer cells

To explain the mechanisms of miRNA-375 in colon cancer development, we measured the relative proteins expression of difference groups by WB assay. The results were shown that CTGF, EGFR, PI3K, AKT and ERK proteins expressions of miRNA groups were significantly suppressed in HT-29 ($P < 0.05$, Figure 7) and SW 620 ($P < 0.05$, Figure 8) compared those of NC groups.

Figure 7

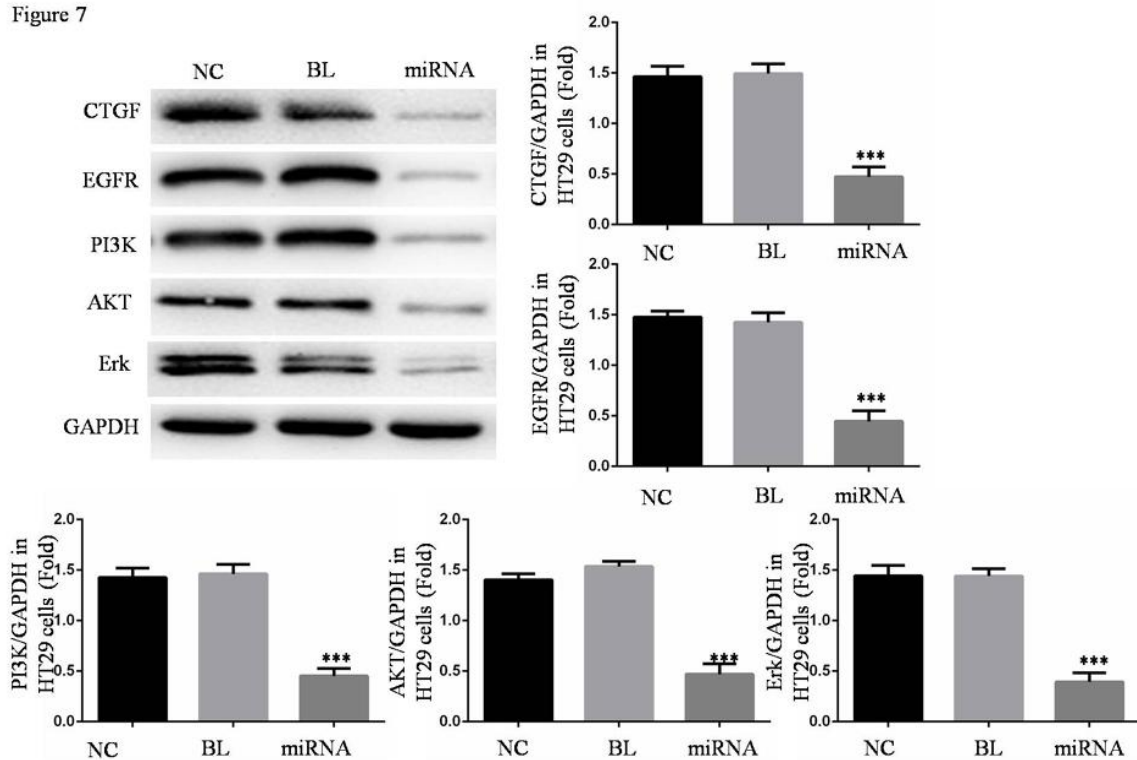


Figure 7. The relative proteins expressions of difference group in HT-29 cell

***: $P < 0.05$, compared with NC group

Figure 8

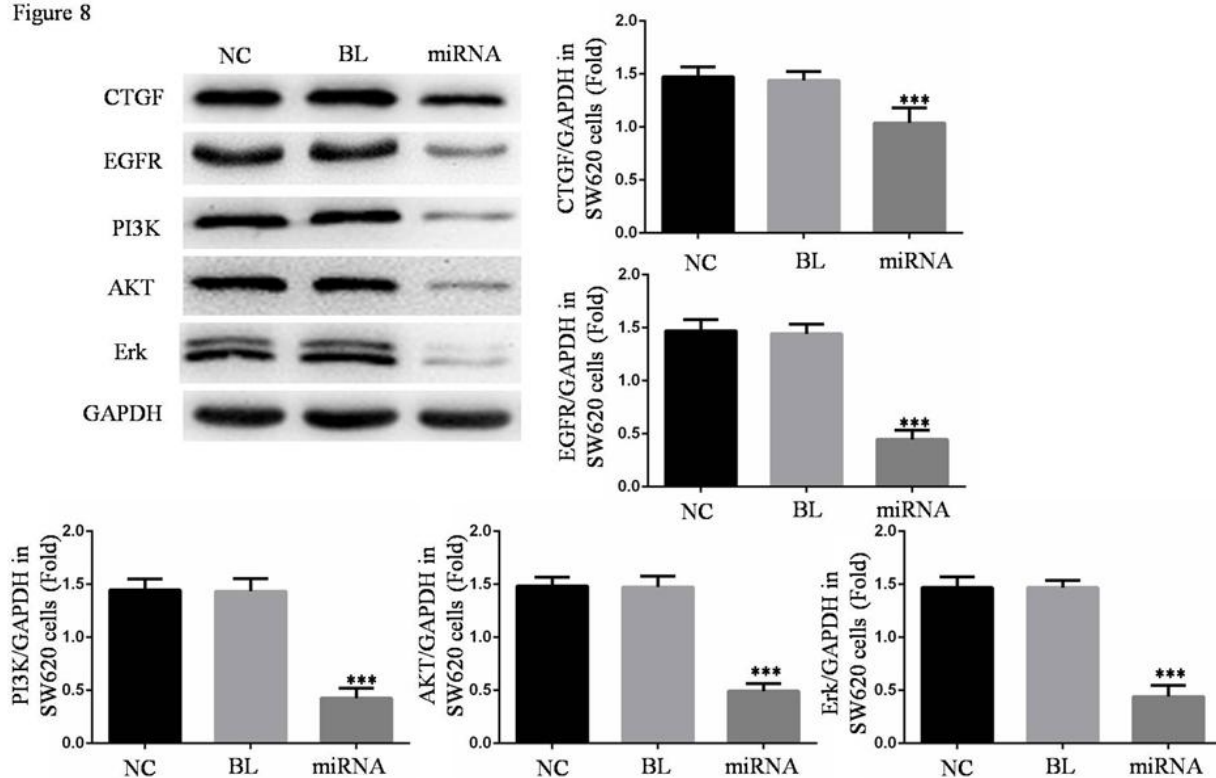


Figure 8. The relative proteins expressions of difference group in SW 620 cell

***: $P < 0.05$, compared with NC group

Discussion

The incidence of colon cancer in China is relatively high. The latest statistics show that only 376 thousand new cases of CRC in China in 2015 reached 191 thousand people, accounting for fifth of all cancers (11). At present in the treatment of colon cancer has made important progress, progress and prognosis of patients with colon cancer mainly depends on the new target and explores the treatment mechanism of the occurrence and development of colon cancer has become the focus of the present research. Recent studies have shown that miRNA-375 was a role of the miRNAs family might be involved in the development and progression of multiple tumors (12-15). In our present study, the results were shown that miRNA-375 over-expression had effects to suppress cell proliferation and enhance cell apoptosis via staining the cell cycle in G1 phase in HT-29 and SW 620 which were two kinds of colon cancer cell lines. To investigation the mechanism of miRNA-375 in suppression colon cancer, we determined the target of miRNA-375 was CTGF by targetscan software and previous study (16).

CTGF plays an important role in wound repair and fibrosis, meanwhile, CTGF also plays an important role in the development, progression and survival of tumor cells (17-19). EGFR is a member of the erbB receptor family on the cell surface, mainly related to cell proliferation, growth, migration, invasion and survival, and plays an important role in the formation and metastasis of colon cancer (20). Many drugs and substances can inhibit the proliferation of colon cancer stem cells through EGFR signaling pathway (21, 22). Depending on previous studies, the results were shown EGFR signaling induced cancer development by regulation Erk and PI3K/Akt pathway (23-25). The activation of PI3K/AKT signaling pathway can lead to a series of biological activities such as tumor growth, reproduction, anti apoptosis, metastasis and metastasis. Overexpression and aberrant activation of PI3K and AKT are involved in the development and progression of human cancers such as ovarian cancer,

pancreatic cancer, breast cancer and gastric cancer (26-28). ERK signaling pathway plays an important role of cancer occurrence and development (29, 30). Depending on our present study, we found that the CEGF which is the target of miRNA-375, EGFR, PI3K, AKT and Erk protein expressions were significantly suppressed with miRNA-375 over-expression in HT-29 and SW 620 cells experiments. That might the results that the cell proliferation suppression, cell apoptosis rate increasing and cell cycle staining in G1 phase.

In conclusion, microRNA-375 regulates proliferation and apoptosis of colon cancer by suppressing CTGF-EGFR/PI3K/AKT/Erk signaling pathway in vitro cell experiments.

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