ROMIDEPSIN AFFECTS THE GROWTH, PROLIFERATION, AND APOPTOSIS OF COLON CANCER CELLS THROUGH ACTIVATING AKT/ERK/NF-KB PATHWAY

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ABSTRACT

Objective*: This study investigates the effect of romidepsin on the growth, proliferation and apoptosis of colon cancer cells by activating the AKT/ERK/NF-κB pathway.*

Methods: *Human colon cancer cell lines were cultured in vitro to establish a mouse subcutaneous xenograft tumour model. When the tumours reached approximately 10 mm, the mice were randomly divided into a saline group and a romidepsin group (10 mg/kg), and the tumour size of the two groups were measured. Human colon cancer cell lines were treated with 1, 5 and 10 mg/L romidepsin. The proliferation of colon cancer cells was detected by CCK-8, the apoptosis by flow cytometry, and the phosphorylation levels of protein kinase B (AKT), externally regulated protein kinase (ERK) and nuclear transcription factor-κB (NF-κB) proteins by a western blot.*

Results: *T26 cells were inoculated subcutaneously into the backs of mice. The tumour size was measured after its formation. It was noted that, compared with the saline group, the growth of colon cancer in mice was significantly inhibited in the romidepsin group (10 mg/L). In addition, compared with the control group, the proliferation rate was not significantly restrained in the romidepsin group (1 mg/L) (P>0.05) but greatly inhibited in the romidepsin group (5 and 10 mg/L), which was time- and dose-dependent (P<0.05). After being treated with 1 mg/L, 5 mg/L and 10 mg/L romidepsin, the apoptosis rate of colon cancer cells gradually increased, and with the increase of drug concentration, the percentage of apoptosis elevated from the normal 5.42%±0.15% to 41.83 %±2.79% (P<0.05). The expression levels of p-AKT, p-ERK and p-NF-κB in colon cancer cells treated with 10 mg/L romidepsin were significantly lower than those in the control group.*

Conclusions: *Romidepsin can inhibit the growth and proliferation of colon cancer cells but can promote their apoptosis. The related mechanism may be achieved by regulating the AKT/ERK/NF-κB signalling pathway.*

Keywords: *Romidepsin, AKT/ERK/NF-κB pathway, colon cancer, growth, proliferation, apoptosis.*

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Introduction

Colon cancer typically occurs in the junction of the duodenum and sigmoid colon. It ranks fourth among cancer incidences and mortality rates worldwide and third in gastrointestinal tumours, and it is increasing each year[(1)]. Colon cancer can develop in a circular shape along the intestinal wall, travel along the intestinal lumen longitudinally, and can invade the deep wall of the intestine and spread to the lymphatics, abdominal cavity or along an incision[(2)]. In the middle and late stages, patients can present with bloating, indigestion, anaemia, weight loss, abdominal pain and mucus stool. According to the tumour sites, clinical manifestations vary. It has been believed that the onset of colon cancer is mainly related to high-fat and low-fibre diets, and the incidence of colon cancer is higher in individuals with familial multiple intestinal polyposis syndrome[(3)]. In clinics, traditional medicines such as oxaliplatin and fluorouracil have relatively severe toxic side effects and easily lead to drug resistance. Histone deacetylase (HDAC) as a drug target has become a hot spot in clinical anti-tumour research. Romidepsin is an HDAC inhibitor[(4)], and related data show that it can inhibit tumour cell proliferation, tumour

angiogenesis, and metastasis, thus having an antitumor effect[5]. Therefore, this study analyses the relevant mechanisms that affect the growth, proliferation and apoptosis of colon cancer cells.

Materials and methods

Experimental reagents and instruments

RPMI 1640 medium and foetal bovine serum were purchased from Gibco; a protein quantification kit was purchased from Thermo Fisher Scientific Co., Ltd.; the Tris HCI buffer was purchased from Tianjin Umbrella Company; the PBS buffer was purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.; and the protein lysate and AKT, p-AKT, p-ERK and NF-κB antibodies were purchased from CST (Shanghai) Biological Reagents Co., Ltd.

The ultra-clean bench was purchased from Esco Technologies, Inc.; the CO2 incubator was purchased from Thermo Fisher Scientific Co., Ltd.; the automatic microplate reader was purchased from the BioTek Instruments, Inc.; the cryogenic refrigerator was purchased from Haier Group Corporation; the flow cytometry was purchased from BD Biosciences; the refrigerated centrifuge was purchased from Eppendorf; and the thermostatic water bath was purchased from Shanghai Zhixin Experimental Instrument Co., Ltd.

Cell culture

Frozen colon cancer cells were removed from the refrigerator and thawed. The thawed solution in a cryopreserved tube was dropped into a sterile centrifuge tube, followed by adding 3-5 ml of PBS. The solution was centrifuged, and the supernatant was discarded. The solution was then mixed with the RMPI 1640 medium containing 10% FBS for cell counting. An appropriate amount of culture medium and a certain volume and concentration of penicillin double-antibody solution were added. The cells were cultured in a 5% CO_2 incubator at 37 °C and passaged when the cells covered 80%~90% of the culture flask.

Methods

The effect of romidepsin on the growth of cell carcinoma cells was observed. The cells in the logarithmic growth phase were collected, and the medium was replaced with a new one. The cells were washed twice with PBS and then digested with trypsin. The cells were also washed repeatedly with PBS after centrifugation to remove the serum and 10 ml PBS was added for resuspension, mixed evenly. The supernatant was discarded after centrifugation. Using a 1 ml syringe, 100 μl of the cell suspension was administered subcutaneously to the back of the mice. It was expected that tumours would form in 10-15 days. When the tumours grew to about 10 mm, the mice were randomly divided into a saline group and a romidepsin group (10 mg/kg) and sacrificed at 40 days. Tumour sizes were measured at day 10, 20, 30 and 40 after drug administration.

The CCK-8 method was used to examine the effect of romidepsin on colon cancer cell proliferation. Colon cancer cells in the logarithmic growth phase were digested with trypsin, which was made into a cell suspension with a serum-containing culture solution. The suspension was inoculated within a 96-well plate, with about 2×105 cells per well, and 100 μl of cell suspension was added to each well and cultured in a CO_2 incubator for 24 h. The culture mediums containing 1, 5 and 10 mg/L romidepsin were prepared. A control group containing 1% DMSO and no romidepsin was formed. Then, 10 μl of each medium with various concentrations of romidepsin was added to the culture plate, incubated for 24, 48 and 72 h, and 10 μl of CCK solution was dropped into each well and incubated for 3 h. The absorbance at 450 nm was determined using a microplate reader. The inhibition rate of cell proliferation = (1-OD value of experimental group/OD value of control group).

Flow cytometry was used to detect the effect of romidepsin on the apoptosis of colon cancer cells. Colon cancer cells in the logarithmic growth phase were added to the culture medium, and 100 μl of single-cell suspension was added to a well plate, with 2.5×104 cells/ml per well, and cultured in an incubator with 5% CO_2 at 37 °C. After the cells adhered, colon cancer cell lines were treated with 1, 5 and 10 mg/L of romidepsin (the control group was not given romidepsin) and cultured again in a 5% CO_2 incubator at 37 °C for 48 h. The cells were digested with 0.25% trypsin. The remaining cells in each well of the 6-well plate were washed with PBS buffer, centrifuged at 2,000 rpm/separation for 5 minutes, and the supernatant was discarded. The apoptosis rate of each group was detected. The levels of protein kinase B (AKT), extracellular regulated protein kinases (ERK) and nuclear factor-κB (nuclear factor-κB, NF-κB) protein phosphorylation were detected with the western blot. The colon cancer cells in the logarithmic growth phase were digested with trypsin and placed in a constant temperature incubator containing 5% CO_2 overnight. Next, 1, 5 and 10 mg/L ro-

midepsin culture solutions were added to the culture bottle for 48 h for detection. After being washed with PBS and centrifugated, the cells were added to 100 μL of cell lysate. The supernatant was collected for testing. Then, 10 μL of protein extract was aspirated and the amount of protein was detected using the BCA kit. SDS-PAGE electrophoresis was performed, and the membrane was transferred and sealed.

The diluted primary antibody was incubated overnight at 4 °C, after which its PVDF membrane was removed, and it was stored in a refrigerator at -20 °C. The PVDF membrane was then washed and put into the secondary antibody dilution, incubated for at least 1 h, and washed 3 times with TBST solution. The membrane was treated with a colour-developing solution for 4 min and scanned with an Odyssey infrared scanner. Protein bands were scanned, developed and quantified.

Statistical analysis

The measurement data in this study were expressed by ($\bar{x}\pm s$). The comparison between the two groups was analysed using the t-test. The repeated measurement analysis of variance was used to compare the data between multiple groups. $P<0.05$ was considered a statistical difference. All data were analysed using the SPSS21.0 software.

Results

Effect of romidepsin on colon cancer cell growth

CT26 cells were inoculated subcutaneously into the backs of mice. Tumour sizes were measured after formation. It was noted that, compared with the saline group, the growth of colon cancer in mice was significantly inhibited in the romidepsin group (10 mg/L), as shown in Figure 1.

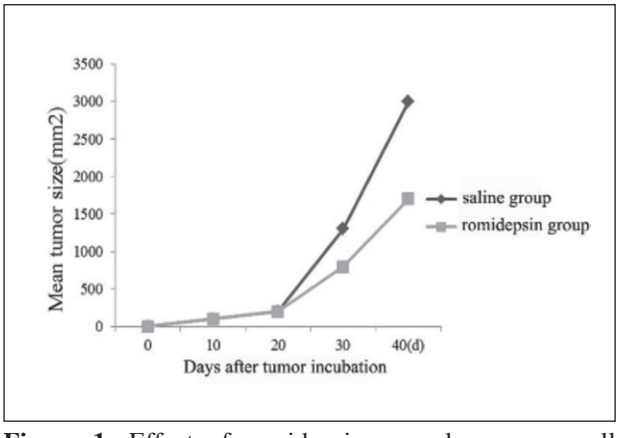

Figure 1: Effect of romidepsin on colon cancer cell growth.

Effect of romidepsin on colon cancer cell proliferation

As shown in Table 1 and Figure 2, the colon cancer cells were treated with 1, 5 and 10 mg/L of romidepsin for 24, 48 and 72 h. It was suggested that compared with the control group, the proliferation rate of colon cancer cells was not greatly inhibited in the mice treated with 1 mg/L of romidepsin ($P>0.05$), but significantly controlled in the mice treated with 5 and 10 mg/L of romidepsin, which was time- and dose-dependent ($P<0.05$).

Time (h)	Control group	Romidepsin concentration (mg/L)		
		1	5	10
24h	2.65±1.03	2.79±1.20	9.74±2.56*	13.22±2.09*
48h	3.18±1.47	3.63±1.68	23.37±3.43*	32.65±2.53*
72h	6.50±2.95	6.89±2.60	31.50±3.39*	48.46±3.06*

Table 1: Effect of romidepsin on colon cancer cell proliferation ($\bar{x}\pm s$).
*Note: *indicates $P<0.05$ compared with the control group and the 1 mg/L romidepsin group.*

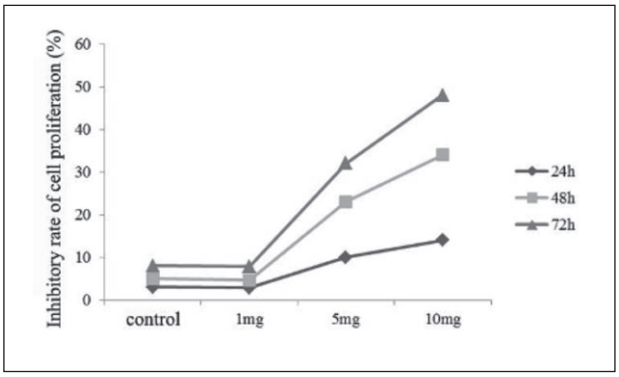

Figure 2: Effect of romidepsin with various concentrations on colon cancer cell proliferation.

Effect of romidepsin on colon cancer cell apoptosis

After being treated with 1 mg/L, 5 mg/L and 10 mg/L romidepsin, the apoptosis rate of the colon cancer cells gradually increased, and with the increase of drug concentration, the percentage of apoptosis elevated from the normal 5.42%±0.15% to 41.83%±2.79% ($P<0.05$), as shown in Table 2.

Group	Concentration (mg/L)	Apoptosis rate (%)
Control group	-	5.42±0.15
Romidepsin	1	5.89±0.30
Romidepsin	5	33.29±2.58*
Romidepsin	10	41.83±2.79*

Table 2: Effect of romidepsin on colon cancer cell apoptosis ($\bar{x}\pm s$).
*Note: *indicates $P<0.05$ compared with the control group and the 1 mg/L romidepsin group.*

Effect of romidepsin on phosphorylation of AKT/ERK/NF-κB protein in colon cancer cells

The findings of the western blot showed that the expression levels of p-AKT, p-ERK and p-NF-κB in colon cancer cells treated with 10 mg/L romidepsin significantly decreased compared with the control group, as shown in Figure 3.

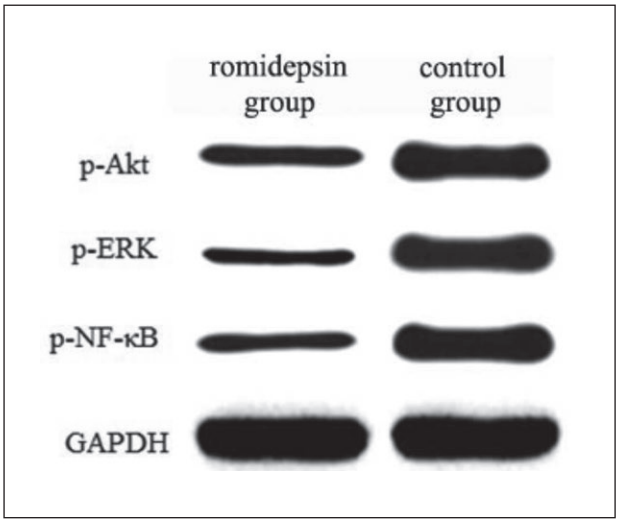

Figure 3: Effect of romidepsin on phosphorylation of AKT, ERK and NF-κB protein in colon cancer cells.

Discussion

Colon cancer, which typically occurs in the colon, cecum, ascending colon or descending colon, has become a serious social medical problem, with incidences increasing each year. Its onset is the result of the synergy of multiple genes, closely related to environmental, genetic and other factors[6]. At present, colon cancer is still mainly treated by surgery and postoperative radiotherapy and chemotherapy. Because patients have no obvious symptoms in the early stage, in general, tumours have already exceeded the submucosa when patients go to the hospital. Postoperative tumour recurrence and metastasis are still the main cause of death for patients[7-8].

Histone deacetylases inhibitor (HDACi), a new type of tumour-targeting therapeutic drug with high efficiency and low toxicity, can advance tumour cell apoptosis with minimal effect on normal cells and have other anti-tumour effects such as differentiation, thus providing significant therapeutic prospects[9-10]. Related data show that the inhibition of HDACi on tumour cell growth is mainly associated with increasing the level of histone acetylation in tumour cells and promoting the transcription of the tumour suppressor gene P21[11]. Romidepsin is a class I HDACi inhibitor and its specific structure, namely a disulphide bond, is key to exerting its activity. It is often used clinically to treat cutaneous T-cell lymphoma, and its effective rate is reported to be about 30%[12]. Some scholars have found that romidepsin can accumulate acetylated histones to interfere with the cancer cell proliferation cycle, and it can also inhibit the level of p2l/p27 and change cell cycles through elevating the cell cycle-dependent protein kinase[13]. It can also induce tumour cell apoptosis by disturbing the apoptotic protein balance and promote high levels of reactive oxygen species in tumour cells by limiting the expression of antioxidant proteins[14]. Some scholars[15] have confirmed that romidepsin can significantly inhibit the proliferation of human colon cancer cell HCT-116 without obvious nephrotoxicity and haematological damage.

The results of this study revealed that the romidepsin group (10 mg/L) could significantly inhibit the growth of colon cancer in mice: the colon cancer cell proliferation of the romidepsin group (5 and 10 mg/L) was significantly inhibited, which was time- and dose-dependent ($P<0.05$). After being treated with 1 mg/L, 5 mg/L and 10 mg/L romidepsin, the apoptosis rate of the colon cancer cells gradually increased, and with the increase of drug concentration, the percentage of apoptosis elevated from the normal 5.42%±0.15% to 41.83%±2.79% ($P<0.05$), which suggests that romidepsin can significantly inhibit the growth and proliferation of colon cancer cells, and advance the apoptosis of colon cancer cells.

Abnormal activation of any signal transduction pathway in the cell may be closely related to the development of tumour cells. Some data have shown that AKT/ERK/NF-κB signalling pathways are imbalanced and abnormally activated in a variety of malignant tumours[16]. The results of this study have also revealed that the expression levels of p-AKT, p-ERK and p-NF-κB in colon cancer cells treated with 10 mg/L romidepsin are significantly lower than those in the control group. This suggests that these signalling pathway changes may be associated with the effects of romidepsin on colon cancer cell growth, proliferation and apoptosis.

In summary, romidepsin can inhibit the growth and proliferation of colon cancer cells and promote their apoptosis, which may be achieved by regulating the AKT/ERK/NF-κB signalling pathway.

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