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Curcumin Inhibits Cell Viability by Inducing Apoptosis and Autophagy in Human Colon Cancer Cells

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Abstract: ***Objective:*** To explore the inhibitory effect of curcumin on colon cancer in vitro. ***Methods:*** Human colon cancer cell lines HT29 and SW620 were cultured in vitro, which were divided into test group and control group. Cells in the test groups were treated with curcumin at different concentrations, while the control groups were treated with dimethylsulfoxide (DMSO). Subsequently, methyl thiazolyl tetrazolium (MTT) assay, were carried out to estimate cellular proliferative activity. Flow cytometry using annexin-V/propidium iodide (PI) staining was performed to detect cell apoptosis. Formation of autophagosomes in the cytoplasm in colon cancer cells were detected by TEM (transmission electron microscopy analysis), and Western blot to measure the expressions of proteins related to apoptosis and autophagy. ***Results:*** MTT assay showed that curcumin inhibited the proliferative activity of both HT29 and SW620 cells, significantly different from the control group ($P<0.001$). The half-maximal inhibitory concentration (IC50) of curcumin at 24 hours was 20μM for both HT29 and SW620 cells. Annexin-V FITC/PI double labeling showed that, After treatment with curcumin of 20μM for 24 hours, a significant increase was observed in apoptosis rate in both HT219 cells ((10.00 ± 0.60)% vs. (4.00 ± 0.70)%, $P<0.01$; (10.50 ± 0.40)% vs. (4.30 ± 0.26)%, $P<0.01$) compared with the control cells. TEM analysis showed that curcumin increased the accumulation of autophagosomes in the cytoplasm in HT29 and SW620 cells. Western blot showed an increase in the expressions of apoptosis-related proteins including cleaved caspase-3 and cleaved poly ADP-ribose polymerase(PARP) but decrease of P62 protein in both HT29 and SW620 cells after 24-hour treatment with 20μM curcumin for 24 hours compared with the control groups. ***Conclusion:*** Curcumin can inhibit the proliferation, induce apoptosis and autophagy in human colon cancer cells.

Keywords: Colon cancer, Curcumin, Apoptosis, Autophagy

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1 Introduction

Colon cancer is one of the most common causes of cancer-related deaths worldwide. The current primary treatment for colon cancer is surgical excision, plus or minus adjuvant chemotherapy[1,2]. Despite advances in early surgical treatment, the overall prognosis is poor. 5-fluorouracil (5-FU) is considered the gold standard for colon cancer treatment. However, 5-FU resistance has become the primary cause of failure in colon cancer treatment[2].

Curcumin is a phenolic pigment extracted from the rhizome of the herbaceous turmeric. It is the main active ingredient of turmeric and has anti-inflammatory, antioxidant and antitumor effects[3]. Our previous study found that curcumin can induce autophagy in malignant melanoma diseased cells and inhibit the proliferation and invasion of malignant cells[4,5]. The aim of this study was to investigate the effects of curcumin on proliferation, apoptosis and autophagy of colon cancer cells and to provide a theoretical basis for subsequent

experimental studies, thus providing the experimental basis for its broader application in clinical practice.

2 Materials and methods

2.1 Materials

Cell lines: Human colon cancer HT29 and SW620 cell lines were purchased from the Chinese Academy of Sciences cell bank. Curcumin (No. A0086, CAS Accession No. 458-37-7, purity ≥ 98%) was purchased from Chengdu Mansite Biotechnology Co., Ltd. It was dissolved in dimethyl sulfoxide (DMSO) (Sigma, USA) to a concentration of 250 mM and stored at 4 °C until use. RPMI 1640 medium, trypsin was purchased from Gibco, USA, fetal bovine serum (FBS) was purchased from Zhejiang Tianhang Biotechnology Co., Ltd, thiazolyl blue tetrazolium bromide (MTT) and Matrigel was purchased from American BD company, Annexin V-FITC kits were purchased from Nanjing Kanji Biotechnology Co., Ltd.

2.2 Method

2.2.1 Cell culture

Colon cancer HT29 and SW620 cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum in a conventional 37 °C, 5% CO2 incubator and logarithmic growth phase cells were used for further experiment.

2.2.2 MTT method to detect the effect of different drugs on cell proliferation

Colon cancer HT29 and SW620 cells (1 x 104/ml) were seeded in 96-well plates at 200 μl per well and each group was given 3 replicate wells to a final concentration of 10, 20, 30, 40, 50, 60, 70 μM while the control group was added with an equal amount of DMSO. The cells were collected at 24, 48, 72 and 96 h, and the absorbance values of each well were measured by a microplate reader at a wavelength of 490 nm (A).

2.2.3 Annexin V-FITC/PI double labeling method to detect the apoptosis rate

Colon cells HT29 and SW620 cells of appropriate density were seeded in 6 cm culture dish before treating with 20 μM curcumin for 24 h, and the control group was added with equal amount of DMSO. The treated HT29 and SW620 cells were collected and washed twice with PBS to adjust the cell concentration to 1 x 10^6/ml before staining with Annexin V and PI. It was incubated for 15 min at room temperature and protected from light before detecting by flow cytometry.

2.2.4 Observation of intracellular autophagosome formation under a transmission electron microscope

Colon cancer HT29 and SW620 cells were seeded in 6 cm culture dish before treating with 20 μM curcumin for 24 h and the control group was added with equal amount of DMSO. The original culture solution was discarded, washed twice with PBS and the cells were collected by trypsinization. It was then washed with PBS and placed in an EP tube before centrifuging at 2000 rpm for 5 min. The supernatant was then discarded. After double-fixing the cells with 2.5% glutaraldehyde and 1% citric acid were used, they were dehydrated with ethanol and acetone. Cells were embedded in epoxy resin and double-stained with uranyl acetate and lead citrate before observing under a transmission electron microscope (JEM 1011 CX, MA, USA) and photographed.

2.2.5 Western blot analysis

HT29 and SW620 cells were treated with 20 μM curcumin or DMSO for 24 h, respectively. The cells were then collected, and protein lysate was mixed and lysed on ice for 30 min. The protein concentration of the total protein extracted from the supernatant after centrifugation was determined by Coomassie Brilliant Blue method. Then, 25 μg of protein sample was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, protein was transferred using electrophoresis onto a nitrocellulose membrane before blocking with 5% skim milk for 2 h. The corresponding primary antibody was added to incubate at 4 °C overnight. The membrane was washed on the second day before adding human horseradish peroxidase-labelled secondary antibody (1:5000) for 1 h at room temperature. After washing the membrane was washed, ECL chromogenic kit was used for 1 min. It was then subjected for imaging with the Bio-Rad gel imaging system using β-actin as an internal reference.

2.2.6 Statistical processing

Data were expressed as X ± S, and statistical analysis was performed using SPSS 23.0 software. The t-test was used for comparison between the two groups. There was a statistically significant difference between the groups and the control group (*P < 0.05, **P < 0.01

and ***P < 0.001).

3 Results

3.1 Inhibition of cell proliferation by curcumin

The effect of curcumin on the activity of colon cancer cells was detected by MTT assay. Curcumin inhibits the activity of HT29 and SW620 cells (Fig. 1A, B). The inhibitory effect was dose-effect in the range of 10 – 40 μM, and it was time-dependent in 0 – 48 h. The IC50 of 24 h was 20 μM which was statistically significant compared with the DMSO control group (P < 0.05).

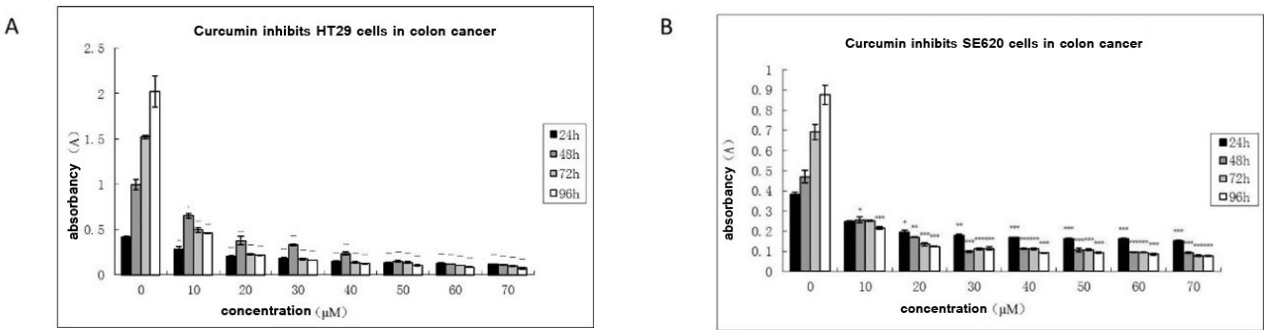

Figure 1. MTT assay for cell viability

The inhibitory effects of different concentrations of curcumin on the proliferation of HT29 cells (A) and SW620 cells (B) at different times were statistically significant compared with the DMSO control group (*P < 0.05, **P < 0.01, ***P < 0.001).

3.2 Effect of curcumin on apoptosis

The effect of curcumin on apoptosis of colon cancer cells was detected by flow cytometry. The results showed that curcumin induces apoptosis in HT29 cells (Fig. 2A) and SW620 (Fig. 2C). After treatment with 20 μM curcumin for 24 h, the apoptotic rate of HT29 cells was (10.00 ± 0.60)%, which was significantly different from that of the control group (4.00 ± 0.70)% (P < 0.001, Fig. 2B) while SW620 cell apoptotic rate was (10.50 ± 0.40)%, which was significantly different from the control group (4.30 ± 0.26)% (P < 0.001, Fig. 2D).

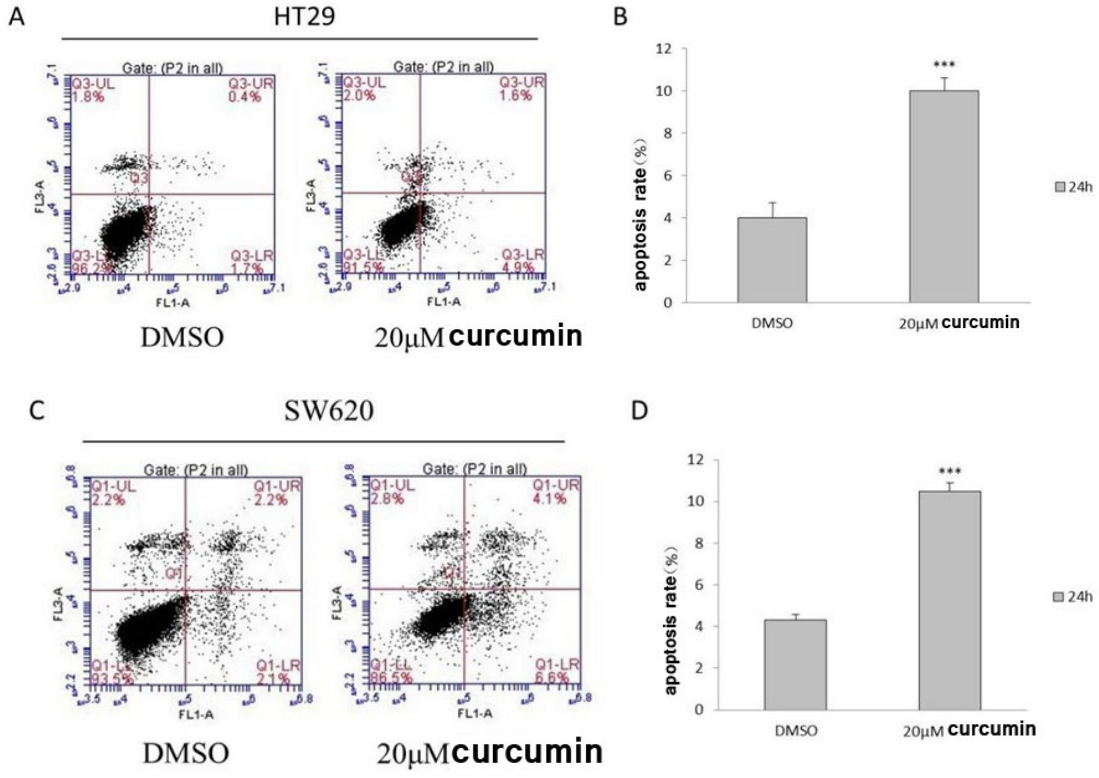

Figure 2. Curcumin induces apoptosis in HT29 and SW620 cells

Flow cytometry showed the apoptosis of HT29 cells (A), and SW620 cells (C) was induced by 20 μM curcumin for 24 h, which was significantly different from DMSO group, ***P < 0.001 (B, D).

3.3 Effect of curcumin on autophagy

Transmission electron microscopy was used to detect the effect of curcumin on the production of autophagosomes in colon cancer cells. The results showed the effect after 24 h of incubation with 20 μM curcumin and the same amount of DMSO in the control group. The structure of the organelles in the control group was almost normal; however, a large number of phagocytic vacuoles and autophagosomes (shown in black arrows) were observed in the curcumin-treated group (Fig. 3B, D).

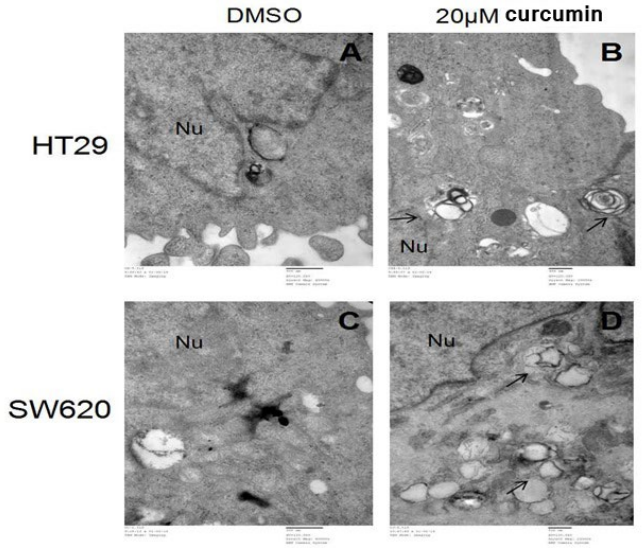

Figure 3. Curcumin induces autophagy in HT29 and SW620 cells

20 μM curcumin induced the production of HT29 cells (B) and SW620 cells (D) autophagosomes (shown in black arrows). Nu showed the nucleus, and the vascular control structure of the DMSO control group was almost normal.

3.4 Effect of curcumin on the expression of caspase 3, PARP and P62 proteins in colon cancer cells

The effect of curcumin on apoptosis-related protein caspase 3 and PARP in colon cancer cells was detected by western blot. The results showed that the expression of cleaved caspase 3 and cleaved PARP protein in HT29 cells (Fig. 4A) and SW620 cells (Fig. 4B) increased significantly compared with the control group after 24 h of treatment with 20 μM curcumin, while the expression of P62 protein was decreased.

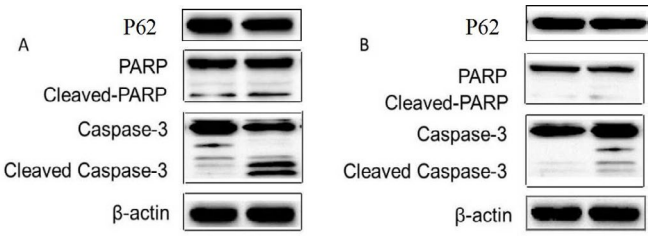

Figure 4. Western blot detection of curcumin on caspase 3 and PARP in colon cancer cells

The expression levels of cleaved caspase 3 and cleaved PARP after treating with 25 μM curcumin for 24 h in HT29 cells (A) and SW620 cells (B) were significantly higher than those in DMSO control group, while P62 protein expression was decreased with β-actin as internal reference.

4 Discussion

Due to the advantages of minor side effects of natural or plant-derived drugs and good patient tolerance, many researchers are committed to the research of adjuvant therapy for malignant tumors. Studies have shown that curcumin can induce different patterns of cell death, such as interference with mitosis, apoptosis and autophagy. Das T *et al*[6] found that curcumin can cause cell growth arrest and cell death by affecting multiple cellular signaling pathways and this process is independent of p53 activation or inhibition. Grazyna Mosieniak *et al*[7] found that curcumin can inhibit the growth of colon cancer cells by inducing cell senescence. This study confirmed that curcumin can inhibit the proliferation of HT29 and SW620 colon cancer cells and prevent its invasive ability.

Defects in the process of programmed cell death (PCD) are the primary factor in tumorigenesis. Inhibition of apoptosis is usually associated with increased expression of antiapoptotic proteins and decreased expression of proapoptotic proteins[8]. Defects in the apoptotic signaling pathway clinically confer multidrug resistance to cancer cells for chemotherapy and radiation therapy, as both therapies kill target cells primarily by inducing apoptosis[9]. Apoptosis is involved in cancer cell death induced by chemotherapy drugs, suggesting that apoptosis can be used as a primary targeting mechanism for new therapeutic drugs[10]. The study of autophagy in the development and progression of colon cancer is limited and controversial. Studies have reported that targeting of Atg7 by 3-methyladenine (3-MA) or small interfering RNA inhibits autophagy and enhances 5-FU-induced apoptosis and cytotoxicity

in human colon cancer[11]. Another study suggested that the human monoclonal antibody panitumumab is used to treat metastatic colorectal cancer by inducing autophagy to exert its cytotoxic effect[12]. Recent studies have shown that both autophagy and apoptosis are involved in chemotherapy drug-induced cancer cell death, suggesting that autophagy and apoptosis may be the primarily targeting mechanisms for new therapeutic drugs[13]. P62 is an autophagy degradation substrate whose level is negatively correlated with autophagy activity[14]. This study found that curcumin can promote the expression of apoptosis-related proteins cleaved caspase 3 and cleaved PARP in human colon cancer HT29 and SW620 cells, inhibit the expression of P62 protein as well as induce apoptosis and autophagy. This may be one of several mechanisms by which curcumin inhibits colon cancer.

In conclusion, curcumin is an effective substance that inhibits the activity of colon cancer cells and induces apoptosis and autophagy. Its mechanism of inhibition of colon cancer cells needs to be further investigated in order to provide a theoretical basis for curcumin as an adjuvant treatment for colon cancer.

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