# Butyrate inhibits the proliferation and induces the apoptosis of colorectal cancer HCT116 cells via the deactivation of mTOR/S6K1 signaling mediated partly by SIRT1 downregulation

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**Abstract.** Butyrate, a histone deacetylase inhibitor, is a typical short chain fatty acid produced by gut microbiota, the dysmetabolism of which has been consistently associated with colorectal diseases. However, its role in tumorigenesis and progression of colorectal cancer cells remains under-investigated. The present study examined the antitumor function of butyrate in the colorectal cancer cell line HCT116 and investigated the underlying molecular mechanism. MTT assay was used to measure cell proliferation and ELISA assay was used to determine cell apoptosis by measuring histone release and caspase-3 activation. The results demonstrated that butyrate treatment significantly inhibited proliferation and induced apoptosis in HCT116 cells with an increased B-cell lymphoma-2 (Bcl-2)-associated X protein/Bcl-2 ratio. Western blotting demonstrated that the phosphorylation of mammalian target of rapamycin (mTOR) at Ser2448, ribosomal protein S6 kinase β-1 (S6K1) at Thr389, S6 at Ser235/236 and expression of silent mating type information regulation 2 homolog (SIRT)1 were decreased following butyrate treatment, while the acetylation of S6K1 was indicated to be increased. Silencing of SIRT1 by small interfering RNA technology demonstrated a similar inhibition on growth, induction of apoptosis, elevation of S6K1 acetylation and deactivation of mTOR/S6K1 signaling. Butyrate treatment also enhanced the inhibition of SIRT1 silencing on cell proliferation and activity of mTOR/S6K1. The activation of mTOR/S6K1 signaling and upregulation of cell proliferation mediated by overexpression of SIRT1 were blocked by butyrate. These data suggested that butyrate inhibited proliferation and induced apoptosis in HCT116 cells by deactivating mTOR/S6K1 signaling, possibly through its inhibition of SIRT1.

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## Introduction

Colorectal cancer (CRC) is a widespread malignant tumor, representing the third most common cancer in men and women globally (1). It is unfortunate that only a small fraction of patients with metastatic CRC can undergo curative resection and experience disease-free survival (2). Surgical treatment and radiotherapy are currently used, but both have certain limitations. It has been identified that radiotherapy and surgery can trigger undesirable invasion or metastasis in certain cases (3-7). Therefore, chemotherapy is frequently applied in cancer therapy. The majority of anti-cancer drugs are investigated and used based on their inhibitory effect on the growth and induction of apoptosis in tumor cells. Understanding the mechanisms underlying cellular responses is important to improve chemotherapeutic efficacy.

Butyrate, a short chain fatty acid, is produced by fermenting dietary fiber using gut microbiota (8). Butyrate serves an important role in inhibiting cell growth and inducing the apoptosis of tumors (9,10) and it functions by regulating the activity of histone deacetylase (HDAC), silent mating type information regulation 2 homolog (SIRT)1, and caspase-3 (11). Butyrate has been identified as a chemotherapeutic strategy based on its effects on cell growth, proliferation and apoptosis in tumor cells (12). However, the role of butyrate in the tumorigenesis and progression of CRC, and the underlying anti-cancer mechanism of butyrate remains to be elucidated.

SIRT1, as a member of the HDAC family, is known to be positively correlated with tumor growth (13). Knockdown of SIRT1 has been reported to increase the chemosensitivity of hepatic cancer cells to anti-tumor agent cisplatin and to inhibit cell metastasis (14). It has been identified that the expression

of SIRT1 is involved in transcription factor nanog expression in CRC (15). SIRT1 has been employed as the target of anti-tumor drugs to inhibit cell growth in cancers (16). It has also been demonstrated that butyrate can inhibit SIRT1 expression to induce apoptosis in hepatic cancer cells (17). Whether butyrate, as a HDAC inhibitor, can modulate cell apoptosis and proliferation via SIRT1 regulation in CRC remains to be investigated.

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that affects cell metabolism and growth (18), and regulates protein synthesis and controls cell growth by mediating the phosphorylation of p70S6 ribosomal kinase (S6K) and eukaryotic translation initiation factor 4E binding protein, thus activating S6 via phosphorylation (19). Phosphorylation of S6K at T389 has been reported to be enhanced by SIRT1-mediated deacetylation (20). However, the exact association between activity of mTOR/S6K signaling and SIRT1 in CRC requires investigation.

The present study confirmed the effect of butyrate on cell proliferation and apoptosis in CRC cells *in vitro*, and explored the underlying molecular mechanism in order to reveal the potential therapeutic target for CRC cells.

## Materials and methods

*Cell culture*. The human CRC cell line HCT116 was purchased from the Cell Bank of the Chinese Academy of Sciences Institute (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Lonza Group, Ltd., Basel, Switzerland), 5 mM L-glutamine, 5 mM non-essential amino acids, 100 U/ml penicillin and streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) in a humidified 5% $CO_2$ incubator at 37˚C.

*Proliferation assay*. Cell proliferation was evaluated using MTT (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). A total of 2,000 cells were seeded into each well of a 96-well plate in 100 $\mu$l medium and incubated with or without 0.5, 1, 2, 3, 4 and 5 mM butyrate for 24, 48 and 72 h at 37˚C in a 5% $CO_2$ incubator. Subsequently, cells were incubated with 20 $\mu$l 5 mg/ml MTT at 37˚C for 4 h, and then lysed for 10 min with the addition of 200 $\mu$l DMSO (OriGen Biomedical, Inc., Austin, TX, USA). Absorbance was measured at 490 nm using a Rainbow microplate reader (Tecan Group, Ltd., Mannedorf, Switzerland).

*Apoptosis assay*. HCT116 cells ($4x10^3$) were seeded into each well of a 96-well plate and were cultured to 80% confluence at 37˚C, and then treated with 1 mM butyrate for 0, 24, 48 and 72 h. Cells were collected and cell apoptosis was detected using a Cell Death Detection ELISA$^{PLUS}$ kit (cat. no. 11774425001; Roche Applied Science, Mannheim, Germany) according to the manufacturer's protocols. Results are presented as the fold induction of DNA fragmentation relative to untreated control. To verify the effect of butyrate on apoptosis, activation of caspase-3 was also assessed in butyrate-treated or untreated cells using human active caspase-3 ELISA (cat. no. AF835; R&D Systems, Inc., Minneapolis, MN, USA). Cells ($5x10^5$/well) were cultured in 6-well plates to 80% confluence at 37˚C. Then the cells were treated with 1 mM butyrate for 0, 24, 48 and 72 h. The cells were lysed and detected in accordance with the manufacturer's protocol.

*Western blot analysis*. Cells were cultured to 80% confluence at 37˚C. The cells ($5x10^6$/well) were washed twice with PBS and proteins were extracted using the M-PER Mammalian Protein Extraction Reagent (Pierce; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Following centrifugation at 12,000 x g for 10 min at 4˚C, the supernatant was collected and quantified using a bicinchoninic acid quantification kit (Beyotime Institute of Biotechnology, Haimen, China). The proteins (50 $\mu$g) were separated by SDS-PAGE on a 12% gel (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and transferred onto polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat dried milk in Tris-buffered saline with 0.1% Tween-20 for 1 h at room temperature, and incubated with specific primary antibodies overnight at 4˚C. Mouse monoclonal anti-B-cell lymphoma-2 (Bcl-2; 1:500; cat. no. sc7382; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), rabbit polyclonal anti-Bcl-2-associated X protein (Bax; 1:500, cat. no. sc493; Santa Cruz Biotechnology, Inc.), mouse monoclonal anti-GAPDH antibody (1:3,000; cat. no. sc-365062; Santa Cruz Biotechnology, Inc.), rabbit polyclonal anti-mTOR (1:1,000; cat. no. 2972; Cell Signaling Technology, Inc., Danvers, MA, USA), rabbit monoclonal anti-phosphorylated (p)-mTOR (Ser2448; 1:1,000; cat. no. 5536; Cell Signaling Technology, Inc.), rabbit polyclonal anti-S6K1 (1:1,000; cat. no. 9202; Cell Signaling Technology, Inc.), rabbit monoclonal anti-p-S6K1 (Thr389; 1:1,000; cat. no. 9205; Cell Signaling Technology, Inc.), rabbit polyclonal anti-acetylated-lysine (1:1,000; cat. no. 9441; Cell Signaling Technology, Inc.), rabbit monoclonal anti-S6 (1:1,000; cat. no. 2217; Cell Signaling Technology, Inc.), rabbit monoclonal anti-p-S6 (Ser235/236; 1:2,000; cat. no. 4858; Cell Signaling Technology, Inc.) and mouse monoclonal anti-SIRT1 (1:1,000; cat. no. 8469; Cell Signaling Technology, Inc.) antibodies were used, followed by the horseradish peroxidase-conjugated secondary antibodies goat anti-mouse (1:2,000; cat. no. sc-2005; Santa Cruz Biotechnology, Inc.) and anti-rabbit immunoglobulin G (1:2,000; cat. no. sc-2004; Santa Cruz Biotechnology, Inc.) for 2 h at room temperature. Development was performed using an enhanced chemiluminescence detecting reagent (GE Healthcare Life Sciences, Little Chalfont, UK). The protein blots were quantified by densitometry using Quantity One software version 4.6.2 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and the amounts were expressed relative to the internal reference GAPDH.

*RNA interference and overexpression of SIRT1*. Small interfering (si)-RNA against SIRT1 and the negative control were designed and chemically synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). The target sequence of siRNA against SIRT1 was 5'-GAAGTGCCTCAGATATTA A-3'. The siRNA sequences of the negative control (siCtrl) was: Sense, 5'-UUCUCCGAACGUGUCACGUTT-3' and antisense, 5'-ACGUGACACGUUCGGAGAATT-3'. A total of $2x10^4$ HCT116 cells were seeded into each well of a 12-well

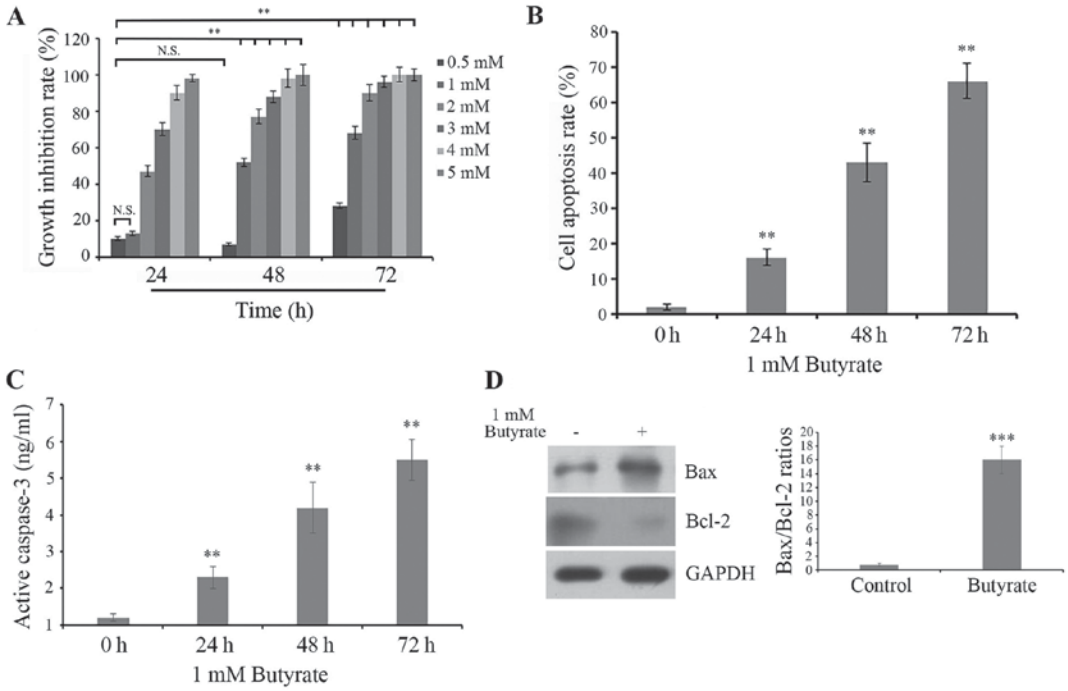


Figure 1. Butyrate treatment inhibits proliferation and promotes apoptosis in HCT116 cells. (A) Cells were treated with 0.5, 1, 2, 3, 4 and 5 mM butyrate for 24, 48 and 72 h, respectively. Cell growth was evaluated by MTT assay and the cell growth inhibition rate was calculated. N.S., no significance; $^{**}$P<0.01 vs. 0.5 mM butyrate for 24. (B) Cells were treated with 1 mM butyrate for 0, 24, 48 and 72 h. DNA fragmentation was determined by histone release. Histone release was measured by ELISA assay, indicating cell apoptosis. The fold induction of DNA fragmentation in HCT116 cells treated with butyrate was presented relative to untreated control cells. (C) Caspase-3 activation was determined by ELISA assay. $^{**}$P<0.01 vs. 0 h. (D) Alterations in Bax and Bcl-2 protein expression in HCT116 cells with or without 1 mM butyrate for 48 h were assessed by western blot analysis with anti-Bax or anti-Bcl-2 antibodies, respectively. Bax/Bcl-2 ratios were calculated by protein expression/Bcl-2 protein expression. GAPDH was detected as the internal reference. $^{***}$P<0.001 vs. control. Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein.

plate and were cultured to 80% confluence in incubator with 5% $CO_2$ at 37˚C in DMEM medium supplemented with 10% FBS. Cell transfections were performed using 100 nmol siRNA and 5 $\mu$l Lipofectamine® 2000™ (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The pcDNA3.1-His-SIRT1 plasmid was obtained from Dr Tony Kouzarides (University of Cambridge, Cambridge, UK) (21). Cells were further cultured at 37˚C for 48 h following transfection, and cells were subsequently lysed and analyzed for the protein expression of SIRT1 by western blotting as aforementioned. In addition, cells were treated with 1 mM butyrate for 48 h and then the cell proliferation and protein levels of the mTOR/S6K1 signaling pathway were detected.

*Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).* Total RNA was extracted using an RNA isolation kit (A&A Biotechnology, Gdynia, Poland), according to the manufacturer's protocol. cDNA was obtained by RT using the RevertAid™ First Strand cDNA synthesis kit (Fermentas; Thermo Fisher Scientific, Inc., Pittsburgh, PA, USA) according to the manufacturer's protocol. The RT reaction was performed at 25˚C for 10 min, 42˚C for 60 min and 70˚C for 10 min. cDNA was amplified via qPCR using a TaqMan® Gene Expression Assay (Applied Biosystems; Thermo Fisher Scientific, Inc.) with fluorogenic carboxyfluorescein-labeled probes using specific primers for target proteins. The specific primers for PCR were: Forward, 5'-TCGGCAGGTCCCTTTGTCATCC-3' and reverse, 5'-TGCAGGTCAACTGGTGTCGT-3' for SIRT1; and forward, 5'-GATCCCTCCAAAATCAAGTG-3' and reverse, 5'-GAGTCCTTCCACGATACCAA-3' for GAPDH. Real-time fluorescence detection was performed with the ABI PRISM 7700 Sequence Detector (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions for RT-qPCR were as follows: 40 amplification cycles of 94˚C for 10 sec, 53˚C for 30 sec and 72˚C for 40 sec, followed by a final extension at 72˚C for 10 min. mRNA expression of the target proteins was calculated using the formula $2^{-\Delta\Delta Cq}$ (22) and was normalized to the level of GAPDH. The relative mRNA level was presented as a percentage of the control.

*Statistical analysis.* Data were obtained from at least three experiments. Values were expressed as the mean ± standard error of the mean. Statistical analysis was performed using SPSS version 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance was used to assess differences between groups. The Duncan method was employed for pairwise comparison followed by Bonferroni correction. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Butyrate treatment inhibits proliferation and induces apoptosis in HCT116 cells.* HCT116 cells were treated with butyrate at 0.5, 1, 2, 3, 4 and 5 mM for 24, 48 and 72 h, respectively. Cell viability was evaluated by MTT assay and the cellular growth inhibition rate was calculated. As presented in Fig. 1A, butyrate treatment inhibited the proliferation of

HCT116 cells in a dose- and time-dependent manner; 0.5 mM butyrate treatment for 24 and 48 h and 1 mM butyrate treatment for 24 h did not demonstrate significant inhibition of proliferation in HCT116 cells, while 1 mM butyrate treatment for 48 h and butyrate treatment at all concentrations for 72 h demonstrated significant inhibition of proliferation activity (Fig. 1A). In addition, cells were treated with 1 mM butyrate for 24, 48 and 72 h, and DNA fragmentation and caspase-3 activity were evaluated by ELISA assay. The results demonstrated that butyrate treatment induced the apoptosis of HCT116 cells in a time-dependent manner (Fig. 1B and C). To further confirm the role of butyrate in the apoptosis of HCT116 cells, the pro-apoptosis protein Bax and anti-apoptosis protein Bcl-2 were evaluated. Western blot analysis indicated that butyrate-treated HCT116 cells demonstrated higher expression of Bax and lower expression of Bcl-2 in comparison with control cells, with increased Bax/Bcl-2 ratios (Fig. 1D). These results indicated that butyrate treatment inhibited cell proliferation by inducing apoptosis signaling via the modulation of Bax and Bcl-2 expression.

*mTOR/S6K1 signaling is deactivated by butyrate treatment.* To investigate the underlying mechanism of butyrate inhibiting proliferation and inducing apoptosis in HCT116 cells, the cells were treated with 1 mM butyrate for 48 h. Then the activity of mTOR/S6K1 signaling were investigated using western blot analysis. The results demonstrated that the phosphorylation levels of mTOR at Ser2448, S6K1 at Thr389 and S6 at Ser235/236 were significantly downregulated, while the acetylation level of S6K1 was significantly upregulated and the expression of SIRT1 was decreased following butyrate treatment (Fig. 2). Based on these data, it was speculated that butyrate treatment inhibited cell proliferation, possibly by deactivating mTOR/S6K1 signaling, which may be mediated by the downregulation of SIRT1 and the enhancement of S6K1 acetylation.

*SIRT1 silencing enhances the inhibition of butyrate treatment on mTOR/S6K1 signaling and cell growth.* To verify the above hypothesis, SIRT1 was knocked down using siRNA technology. The mRNA and protein expression of SIRT1 were evaluated by RT-qPCR analysis and western blotting. The results demonstrated that SIRT1 expression in SIRT1-silenced cells was significantly decreased compared with non-silenced control cells (Fig. 3A and B). Subsequently, SIRT1-silenced and non-silenced HCT116 cells were treated with or without 1 mM butyrate for 48 h. Activity of mTOR/S6K1 signaling in cells was detected by western blot analysis. The results demonstrated that SIRT1 silencing increased the acetylation of S6K1 and decreased the phosphorylation of mTOR at Ser2448 and S6K1 at Thr389. In addition, butyrate treatment further reduced the phosphorylations of mTOR at Ser2448 and S6K1 at Thr389 in SIRT1-silenced HCT116 cells (Fig. 3C). Additionally, cell proliferation assay demonstrated that SIRT1 silencing further reduced cell proliferation of HCT116 cells treated with butyrate (Fig. 3D). The cellular apoptosis assay revealed that SIRT1 silencing promoted the inducing effect of butyrate on apoptosis in HCT116 cells (Fig. 3E). In addition, it was identified that butyrate could still inhibit proliferation and induce apoptosis. These data suggested that SIRT1 may

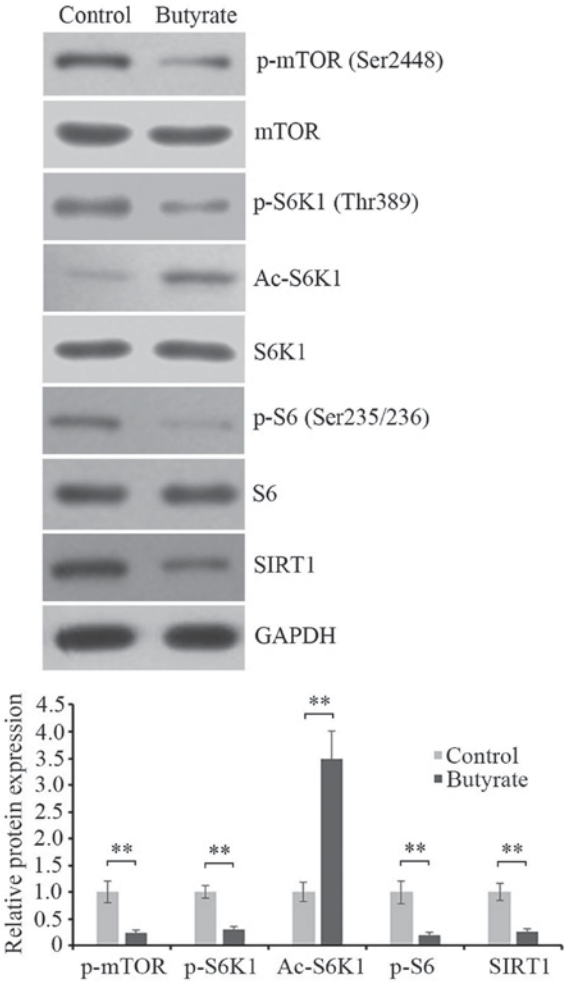


Figure 2. mTOR/S6K1 signaling is inhibited and the acetylation of S6K1 is increased by butyrate treatment. Protein expression levels of the phosphorylation of mTOR at Ser2448 (p-mTOR), mTOR, phosphorylation of S6K1 at Thr389 (p-S6K1), acetylation of S6K1 (Ac-S6K1), S6K1, phosphorylation of S6 at Ser235/236 (p-S6), S6 and SIRT1 in HCT116 cells with or without 1 mM butyrate for 48 h were assessed by western blot analysis using their corresponding antibodies. GAPDH was detected as the internal reference. The blots were quantified by densitometry, and data were presented as the ratios of p-mTOR/mTOR, p-S6K1/S6K1, Ac-S6K1/S6K1, p-S6/S6 and SIRT1/GAPDH. The results were expressed as a ratio relative to the value obtained in untreated control cells. $^{**}P<0.01$, as indicated. mTOR, mammalian target of rapamycin; S6K1, S6 kinase β-1; p-, phosphorylated; Ac-, acetylated; SIRT, silent mating type information regulation 2 homolog.

be involved in the regulation of butyrate in mTOR/S6K1 signaling, and thus affect cell proliferation and apoptosis, but it may not be the only target of butyrate; it is possible that the acetylation of S6K1 is associated with the inhibition of butyrate on mTOR/S6K1 signaling.

*Butyrate treatment attenuates the induction of SIRT1 overexpression in the proliferation of HCT116 cells.* To verify the role of SIRT1 in the modulation of butyrate on mTOR/S6K1 signaling and cell proliferation, SIRT1 was overexpressed in HCT116 cells and the effect of butyrate on mTOR/S6K1 signaling in SIRT1-overexpressed cells was detected. Western blotting demonstrated that SIRT1 overexpression increased the phosphorylations of mTOR at Ser2448 and S6K1 at Thr389, while butyrate treatment attenuated the promotive effect (Fig. 4A). In addition, the cell proliferation assay

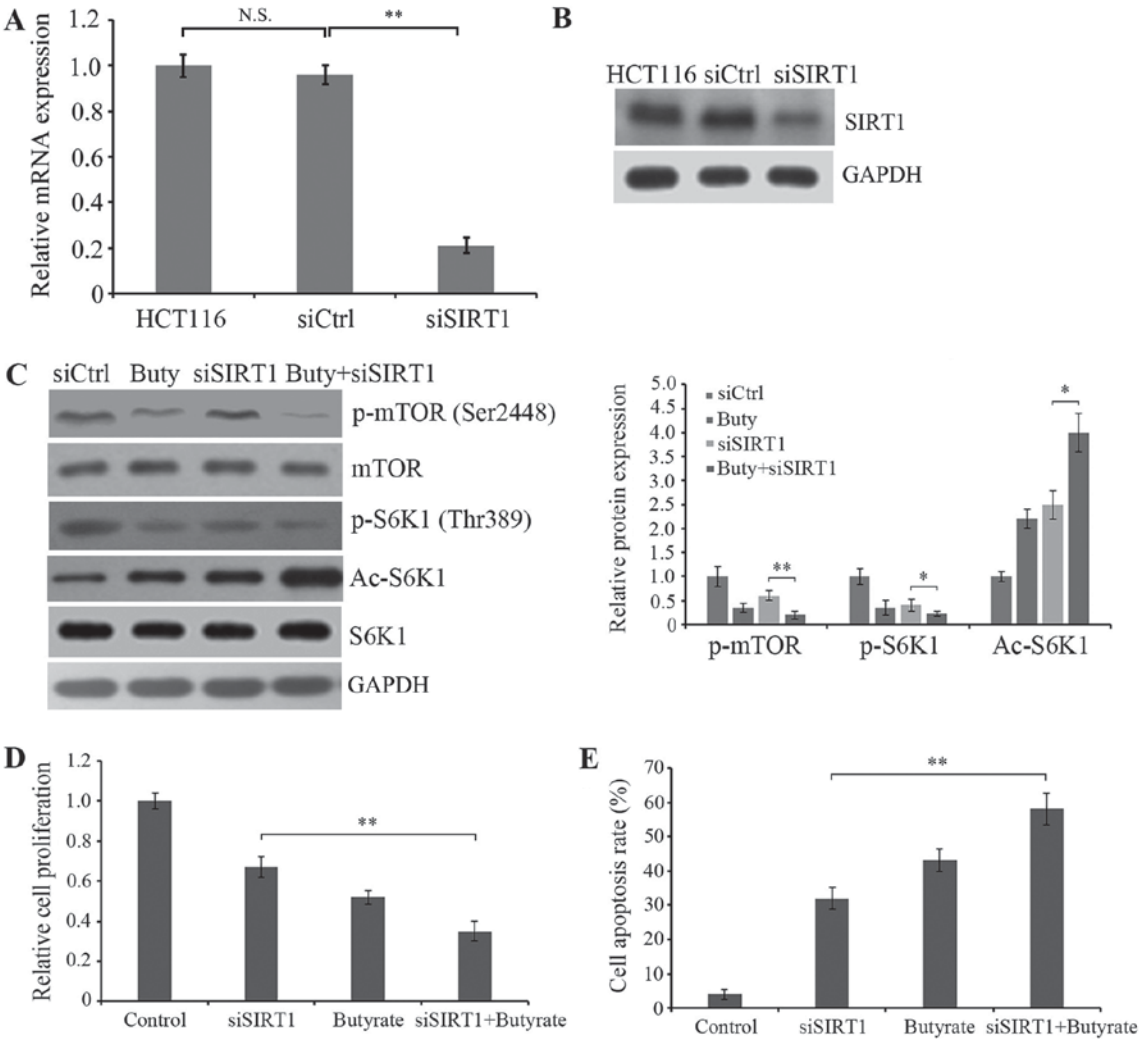


Figure 3. Butyrate treatment enhances the inhibition of cell proliferation and the activity of mTOR/S6K1 induced by SIRT1 silencing. Cells were transfected with siCtrl or siSIRT1 for 48 h and the (A) mRNA and (B) protein expressions of SIRT1 cells were analyzed by reverse transcription-quantitative polymerase chain reaction and western blot analyses, respectively. GAPDH was detected as the internal standard. N.S., no significance; $^{**}$P<0.01 vs. siCtrl. (C) SIRT1-silenced and non-silenced HCT116 cells were treated with or without 1 mM butyrate for 48 h. The protein expression of p-mTOR, mTOR, p-S6K1, Ac-S6K1 and S6K1 in cells was assessed by western blot analysis using the corresponding antibodies. GAPDH was detected as an internal standard. The blots were quantified by densitometry, and data were presented as the ratios of p-mTOR/mTOR, p-S6K1/S6K1 and Ac-S6K1/S6K1. The results were expressed as a ratio relative to the value obtained in control cells. (D) SIRT1-silenced and non-silenced HCT116 cells were incubated with fresh medium with or without 1 mM butyrate for 48 h. Cell proliferation was detected by an MTT assay. (E) Cellular apoptosis was evaluated by detecting the fold induction of DNA fragmentation using ELISA assay. $^{*}$P<0.05 and $^{**}$P<0.01, as indicated. SIRT, silent mating type information regulation 2 homolog; mTOR, mammalian target of rapamycin; S6K1, S6 kinase β-1; siCtrl, control small interfering RNA; siSIRT1, SIRT1 small interfering RNA; p-, phosphorylated; Ac-, acetylated; Buty, butyrate.

indicated that butyrate treatment also significantly suppressed the enhancement of proliferation induced by SIRT1 overexpression and the upregulation of SIRT1 attenuated the effect of butyrate on cell proliferation (Fig. 4B). Therefore, these data demonstrated that butyrate treatment inhibited cell proliferation by deactivating mTOR/S6K1 signaling, which was possibly mediated by SIRT1 reduction.

## Discussion

Butyrate, a HDAC inhibitor, has been considered as a promising antitumor agent for a variety of different types of human cancers (11,23,24). Chemotherapy commonly kills tumor cells by activating apoptosis. It has been reported that butyrate is able to reduce cell proliferation and stimulate cell apoptosis in multiple types of cancers (25-27). However, the exact role of butyrate in CRC and the underlying mechanism of butyrate-induced apoptosis remains unclear. The present study investigated the effect of butyrate on cell proliferation and apoptosis, and explored the possible molecular mechanism in human CRC HCT116 cells. It was identified that butyrate significantly inhibited cell proliferation and induced apoptosis in HCT116 cells. mTOR/S6K1 signaling was revealed to be regulated by butyrate treatment and SIRT1 expression was demonstrated to be involved in the regulation.

In addition, it was also revealed that butyrate treatment induced apoptosis in CRC HCT116 cells and significantly increased the expression of the pro-apoptosis protein Bax and decreased the expression the anti-apoptosis protein Bcl-2. In the Bcl-2 family, the ratio of pro-apoptotic to anti-apoptotic proteins is a key factor in determining the occurrence and level of apoptosis. The proportion of pro-apoptotic proteins in

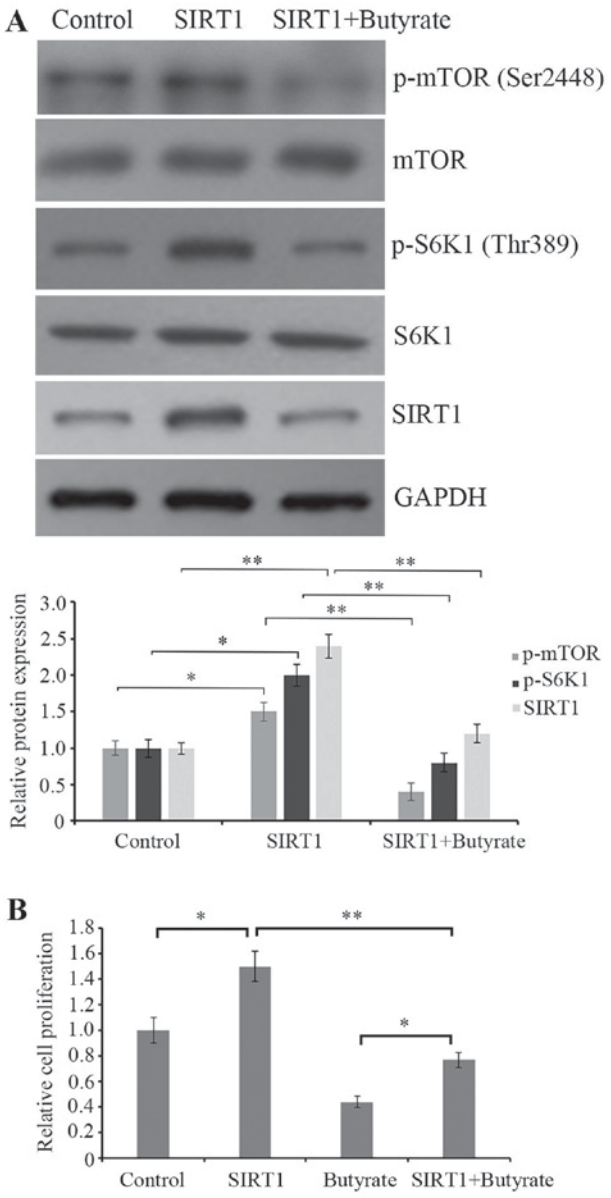


Figure 4. Butyrate treatment attenuates SIRT1 overexpression mediated activation of mTOR/S6K1 and proliferation elevation. (A) Cells were transfected with or without SIRT1-expressing plasmid for 48 h, and then the protein expression of p-mTOR, mTOR, p-S6K1, S6K1 and SIRT1 in cells was assessed by western blot analysis using the corresponding antibodies. GAPDH was detected as an internal standard. The western blots were quantified by densitometry and data were presented as the ratio of p-mTOR/mTOR, p-S6K1/S6K1 and SIRT1/GAPDH. The results were expressed as a ratio relative to the value obtained in control cells. (B) Cell proliferation was detected by an MTT assay. $^{*}P<0.05$ and $^{**}P<0.01$, as indicated. SIRT, silent mating type information regulation 2 homolog; mTOR, mammalian target of rapamycin; S6K1, S6 kinase β-1; p-, phosphorylated.

cells also determines the cellular response to death signals and cell destiny (28,29). SIRT1 is commonly highly expressed in cancer cells and serves an important role in promoting cell proliferation and blocking apoptosis (13,30). It has been demonstrated that downregulation of SIRT1 expression mediated by chemotherapeutic drugs can inhibit tumor growth and induce cell death (24,31). SIRT1 downregulation by butyrate reduced Bcl-2 expression and elevated caspase-3 expression in hepatic cancer cells (17). In the present study, SIRT1 expression was decreased by butyrate treatment. Therefore, it is possible that butyrate treatment regulated apoptosis-associated proteins by decreasing SIRT1 expression.

It has also been reported that SIRT1, as a deacetylase, can inhibit the acetylation of S6K1 and thereby promote the phosphorylation of Thr389 (a critical phosphorylation site for S6K1 activity) (20). S6K1, one of the substrates of mTOR, is responsible for modulating multiple cell biological functions, including protein synthesis, cell survival and growth (19). In the present study, it was demonstrated that SIRT1 silencing markedly increased the acetylation of S6K1, decreased its phosphorylation at Thr389 and slightly decreased the phosphorylation of mTOR at Ser2448 (the activity site for mTOR). However, SIRT1 overexpression induced the opposite effect to SIRT1 silencing. Butyrate treatment further enhanced the promotive effect of SIRT1 silencing on S6K1 acetylation and the inhibitory effect on S6K1 activity, whereas butyrate treatment reversed the promotion of SIRT1 overexpression on mTOR/S6K1 signaling and cell proliferation. However, it was identified that butyrate treatment further inhibited the proliferation and induced cell apoptosis in SIRT1-silenced HCT116 cells, suggesting that SIRT1 may not be the only target of butyrate to regulate cell proliferation and apoptosis via the mTOR/S6K1 signaling pathway, and that there may be other signaling pathways involved in this regulation. It has also been reported that the Lys484, Lys485 and Lys493 sites of S6K1 are acetylated (20). Whether these Lys sites of S6K1 are involved in the modulation of SIRT1 on S6K1 activity and the mechanism underlying the regulation of mTOR activity have yet to be elucidated.

In conclusion, the results of the present study demonstrated that butyrate treatment inhibited cell proliferation and induced apoptosis by inhibiting the activation of the mTOR/S6K1 signaling pathway, partly via SIRT1 inhibition. Therefore, the present study provides a promising target for butyrate to suppress the proliferation and induce apoptosis in CRC HCT116 cells.

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## Availability of data and materials

The analyzed datasets generated during the study are available from the corresponding author on reasonable request.

## Authors' contributions

XL was the major contributor to the conception and design of the research, and critically revised the manuscript for important intellectual content. MC and ZZ performed the

experiments and acquired the data. SH performed statistical analysis, and analyzed and interpreted the data. XL and MC obtained the funding. MC and ZZ drafted the manuscript and gave final approval of the version to be published.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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