

The histone deacetylase inhibitor trichostatin A induces cell cycle arrest and apoptosis in colorectal cancer cells via p53-dependent and -independent pathways

JIN MENG^{1,4}, HAI-HUA ZHANG², CHUN-XIANG ZHOU³, CHEN LI¹, FENG ZHANG¹ and QI-BING MEI¹

¹Department of Pharmacology, School of Pharmacy, ²School of Clinical Medicine and Stomatology,

³Department of Pharmacy, Xijing Hospital, The Fourth Military Medical University, Xi'an 710032;

⁴Department of Pharmacy, The 309th Hospital of PLA, Beijing 100091, P.R. China

Received January 3, 2012; Accepted March 13, 2012

DOI: 10.3892/or.2012.1793

Abstract. Many chemotherapeutic agents induce apoptosis via a p53-dependent pathway. However, up to 50% of human cancers have p53 mutation and loss of p53 function. Histone deacetylase inhibitors (HDACIs) are emerging as a potentially important new class of anticancer agents. Here, we report that, Trichostatin A (TSA), a pan-HDAC inhibitor, could induce G2/M cell cycle arrest and apoptosis in both colorectal cancer cell lines with wild-type p53 (HT116 cells) and mutant p53 (HT29 cells), although HCT116 cells had more apoptotic cells than HT29 cells. TSA induces apoptosis in both cell lines via the mitochondrial pathway as indicated by decrease of the mitochondrial membrane potential (MMP) and activation of caspase-3. Additionally, TSA induces expression of the pro-apoptotic protein Bax and decreases the expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL in both cell lines. Bax knockdown by siRNA significantly impaired TSA-induced apoptosis in both cell lines. These data suggest that TSA induces G2/M cell cycle arrest and Bax-dependent apoptosis in colorectal cancer cells (HCT116 cells and HT29 cells) by both p53-dependent and -independent mechanisms. However, cells with normal p53 function are more sensitive to TSA-induced apoptosis.

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related mortality with 1,000,000 cases of all over the world in 2009 (1). Despite the fact that early-stage colorectal cancer is amenable and may be cured by surgical resection, recurrence rates remain high. Hence, there is an urgent need to develop novel treatment strategies.

Histone deacetylase inhibitors (HDACIs) have been extensively studied in basic biological research to gain an understanding of chromatin structure and transcriptional control and have recently been introduced as a potentially important new class of anticancer agents (2-4). Trichostatin A (TSA), a pan-HDAC inhibitor, has been shown to induce cell cycle arrest, promote cell differentiation and apoptosis, and inhibit metastasis (5) in various types of tumors (6). It is well known that p53 may play an important role in the anti-cancer effects induced by TSA. p53 stimulates the promoter activities of p21^{Waf1/Cip1} (p21) and Bax genes to enhance their expression as a transcriptional factor (7). The downstream events include cell cycle arrest and activation of apoptosis, as evidenced by changes in cell cycle kinetics and induction of caspase activity (8-10). However, the mechanisms by which TSA induces cell growth arrest and cell death remain to be fully defined.

Many chemotherapeutic agents induce apoptosis via p53-dependent pathway. However, there are up to 50% of human cancers that have p53 mutation and loss of p53 function (10). It is not clear whether TSA-induced anticancer effects are dependent on normal p53 function in CRC cells. In the present study, we treated CRC cells which have normal p53 function (HCT116 cells) and mutant p53 (HT29 cells), with HDAC inhibitor TSA and investigate its anticancer effects. We have demonstrated that TSA is effective in inducing G2/M cell cycle arrest and apoptosis in both CRC cell lines by both p53-dependent and -independent mechanisms. Cells with normal p53 function are more sensitive to TSA-induced apoptosis, which is via Bax-dependent mitochondrial pathway. This study may provide evidence for the use of HDAC inhibitor in clinical treatment of colorectal cancers with wild-type or mutant p53.

Correspondence to: Dr Qi-Bing Mei or Dr Feng Zhang, Department of Pharmacology, School of Pharmacy, The Fourth Military Medical University, 17 Changlexi Road, Xi'an 710032, P.R. China
E-mail: meiqb@yahoo.cn
E-mail: zhangf37@yahoo.cn

Abbreviations: TSA, trichostatin A; CRC, colorectal cancer; MMP, mitochondrial transmembrane potential; HDACIs, histone deacetylase inhibitors

Key words: trichostatin A, colorectal cancer cells, apoptosis, cell cycle arrest, p53

Materials and methods

Cell culture and treatments. Colorectal cancer cell lines HCT116 and HT29 cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum, 10 mg/ml antibiotics (penicillin and streptomycin) and 2 mmol/l L-glutamine at 37°C under 5% CO₂ and saturated moisture. Trichostatin A (TSA) (Sigma, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO, Sigma), final concentrations of 0.1, 1 and 5 μ M were used to treat the cells and proper amount of DMSO was used as vehicle control.

Cell cycle analysis. Cells were treated with varying concentrations of TSA for 24 h. At the time of harvesting, cells were digested with 0.25% trypsin (Gibco) and re-suspended in phosphate-buffered saline (PBS), fixed in 70% ethanol at 4°C overnight. When analyzing, cells were washed with PBS and treated with 20 μ g/ml ribonuclease A (RNase A, Sigma) at 37°C for 30 min. Cells were then stained with 50 μ g/ml propidium iodide (PI, Sigma) for 30 min and DNA content was analyzed by flow cytometry with FACScan (Becton-Dickinson, Mountain View, CA, USA) using the CELLQuest program (Becton-Dickinson). Cell cycle distribution was analyzed by WinMDI software.

Annexin V-FITC and PI staining. Cells were treated as above and were digested with trypsin, stained with 5 μ l Annexin V-FITC (BD Pharmingen) and 5 μ l propidium iodide (PI, BD Pharmingen) staining solution in the dark at room temperature (RT) for 15 min. The cell samples were analyzed by flow cytometry on a FACScan station with CellQuest software using the FL1 and FL2 range for Annexin V FITC and PI, respectively.

Flow cytometry assessment of the changes in mitochondrial transmembrane potential (MMP). MMP was measured by flow cytometry with MitoTracker Red (Invitrogen) probe which is a mitochondrion-selective stain concentrated by active mitochondria. The probe accumulates in normal mitochondria and the reduction of MMP leads to the release of the stain. The probe was dissolved in DMSO and diluted in PBS before use. Cells were treated with MitoTracker for 45 min before trypsinization. Cells were then washed twice with PBS and analyzed by flow cytometry on a FACScan station with CellQuest software using the FL2 for MitoTracker Red staining.

Bax siRNA transfection. Bax siRNA and scramble siRNA were purchased from Santa Cruz Biotechnology Inc. siPORT NeoFX Transfection Agent (Ambion) was used for the siRNA transfection. Twenty-four hours after transfection, cells were treated with TSA for another 24 h. Knock-down of Bax expression was confirmed by western blotting.

Western blotting. Cells were lysed in lysis buffer containing 150 mM NaCl, 1% NP40, 0.5% deoxycholic acid, 0.1% SDS, 50 mM Tris (pH 8.0), and 1:25 protease inhibitor cocktail. Protein concentrations of the lysates were determined by the Bradford protein assay system (Bio-Rad Laboratories,

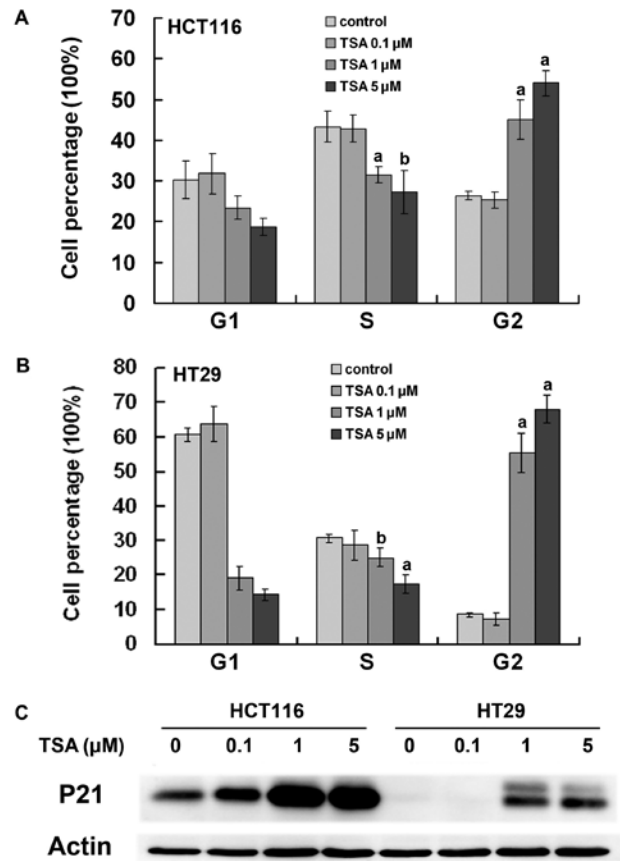


Figure 1. TSA induces G2/M arrest in CRC HCT116 and HT29 cells in a dose-dependent way. Cells were exposed to different concentrations of Trichostatin A (TSA) for 24 h. Cell cycle was measured by flow cytometry after PI staining. The percentage of cells in different cell cycle phases was analyzed using WinMDI software. Data are presented as means plus SD from 3 independent experiments and shown in the bar graph. (A) HCT116 cells. ^aP<0.01 vs. corresponding control. ^bP<0.05 vs. corresponding control. (B) HT29 cells. ^aP<0.01 vs. corresponding control. ^bP<0.05 vs. corresponding control. (C) p21 expression was detected by western blotting. Actin blot was included to show equal protein loading for all the samples.

Hercules, CA). Equal amounts of protein (30 μ g protein each lane) were separated by SDS-PAGE, transferred to nitrocellulose membranes (Hybond C, Amersham, UK). Immunoblots were blocked with 5% skim milk in TBS/Tween-20 (0.05%, v/v) for 1 h at RT. The membrane was incubated with primary antibody overnight at 4°C. Antibody against polyadenylribosyl polymerase (PARP) were from BD Pharmingen, antibodies against p21, Bcl-2, Bcl-xL and MCL-1 were purchased from Santa Cruz Biotechnology Inc., antibodies against Bax and active caspase-3 were purchased from Abcam, and actin antibody was purchased from Sigma. The membrane was incubated with corresponding secondary antibody conjugated with horseradish peroxidase (Sigma) (1:5000) at RT for 1 h. The blots were developed using an enhanced chemiluminescence western blotting detection system (Amersham Biosciences, UK).

Statistical analysis. The data represent at least three independent experiments. Statistical comparisons were made using Student's t-test. P<0.05 was considered to represent a statistically significant difference.

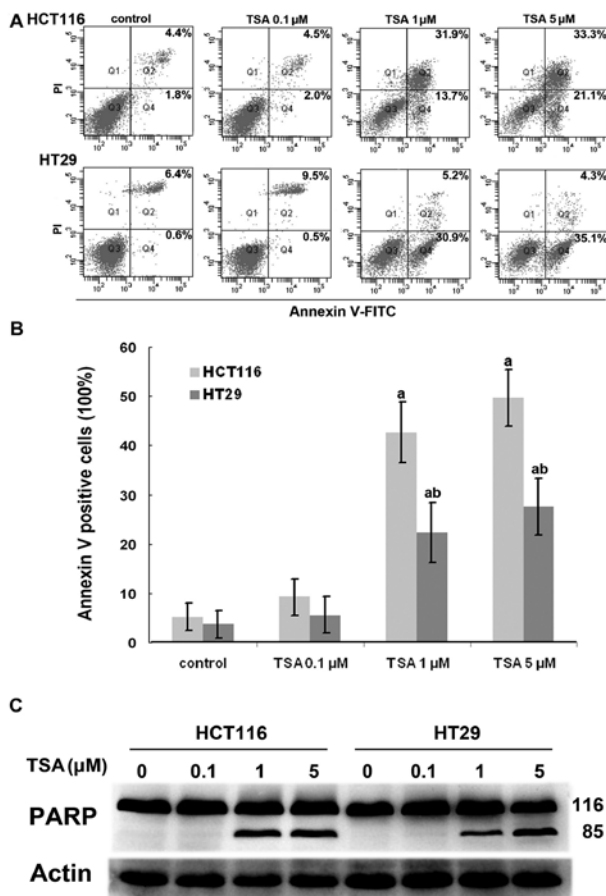


Figure 2. TSA induces apoptosis in CRC cell lines. (A) Apoptosis was analyzed by Annexin V-FITC and PI staining with flow cytometry. Representative plots of one set of triplicate experiments. Early apoptotic cells (Annexin V⁺ and PI⁻) are displayed in the lower right quadrant and late apoptotic cells (Annexin V⁺ and PI⁺) are shown in the upper right quadrant. (B) The percentages of apoptotic cells are indicated by Annexin V⁺ cells shown as means plus SD from 3 independent experiments. Data are shown as means plus SD from 3 independent experiments with representative plots of one set of the experiments. ^aP<0.01 vs. corresponding control. ^bP<0.05 vs. corresponding HCT116 cells. (C) PARP cleavage was detected by western blotting. Actin blot was included to show equal protein loading for all the samples.

Results

TSA induces G2/M arrest in CRC HCT116 and HT29 cells. Histone deacetylase (HDAC) inhibitors have been reported to cause growth arrest at the G1 and/or G2/M phases in a wide variety of tumour cells (11). We measured cell cycle distribution 24 h after varying concentrations of TSA (0.1, 1 and 5 μM) treatment by PI staining in both HCT116 cells with wild-type p53 and HT29 cells with mutant p53. As shown in Fig. 1A and B, TSA induced dose-dependent G2/M phase arrest in both cell lines, with decrease of S phase cells, indicating the inhibition of DNA replication. Since p21 is the principle cyclin dependent kinase inhibitor (CDKI) which is involved in cell cycle arrest upon DNA damage (12), we analyzed p21 expression by western blotting after TSA treatments. As shown in Fig. 1C, p21 expression was upregulated after TSA treatment in a dose-dependent manner in HCT116 cells, while in HT29 cells, p21 level was much lower, which

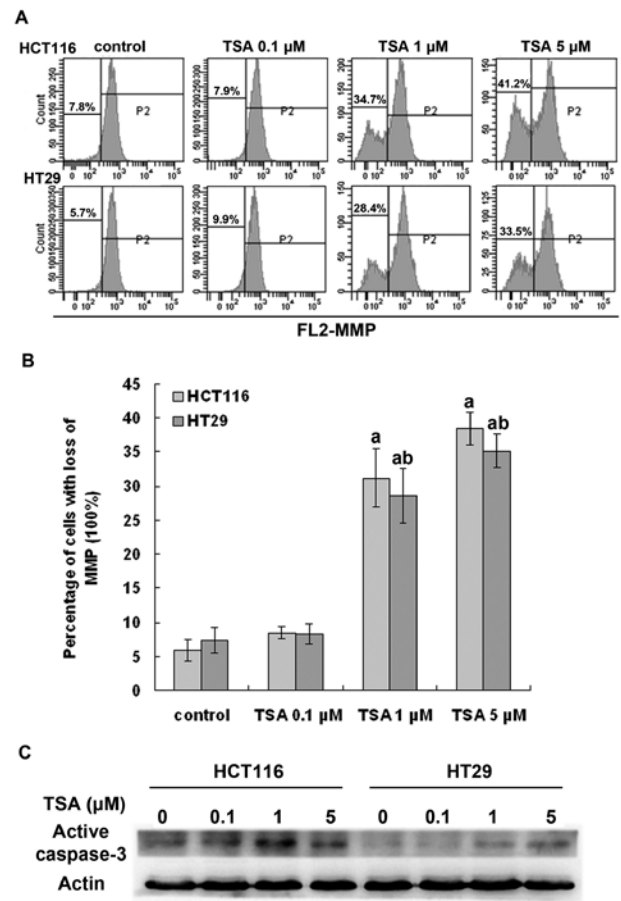


Figure 3. TSA induces apoptosis in CRC cells via mitochondrial pathway by causing dissipation of MMP and caspase-3 activation. (A) MMP changes were measured by flow cytometry. Representative plots of one set of triplicate experiments. (B) Data are shown as means plus SD from 3 independent experiments. ^aP<0.01 vs. corresponding control. ^bP<0.05 vs. corresponding HCT116 cells. (C) Protein from each group of cells was extracted and analyzed by western blotting using anti-active caspase-3 antibody. Actin blot was included to show equal protein loading for all the samples.

could be detected only when treated with TSA 1 and 5 μM (Fig. 1B).

TSA induces apoptosis in CRC cell lines with normal or mutant p53 function. We measured TSA-induced apoptosis with Annexin V-PI staining in both HCT116 cells and HT29 cells. Both types of cells were exposed to varying concentrations of TSA (0.1, 1 and 5 μM) for 24 h. As shown in Fig. 2A and B, TSA caused dose-dependent apoptosis in both cell lines, suggesting that TSA could induce apoptosis in CRC cells through both p53-dependent and -independent way. However, in HCT116 cells which have normal p53 function, cells were more sensitive to TSA-induced apoptosis than HT29 cells with mutant p53, indicating that normal p53 function could promote TSA-induced apoptosis in CRC cells. Apoptosis was further confirmed by PARP cleavage detected with western blotting (Fig. 2C). The cleaved PARP 85 kDa band was constantly detected in TSA treated CRC cells in a similar pattern with Annexin V-positive cells.

TSA induces apoptosis in CRC cells via mitochondrial pathway by causing dissipation of MMP and caspase-3

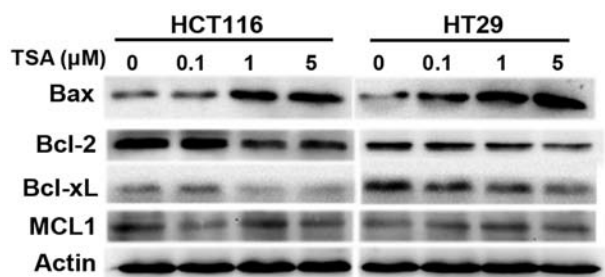


Figure 4. TSA induces changes of apoptosis related proteins. Protein from each group of cells was extracted and analyzed by western blotting using antibodies against Bax, Bcl-2, Bcl-xL and MCL1. Actin blot was included to show equal protein loading for all the samples.

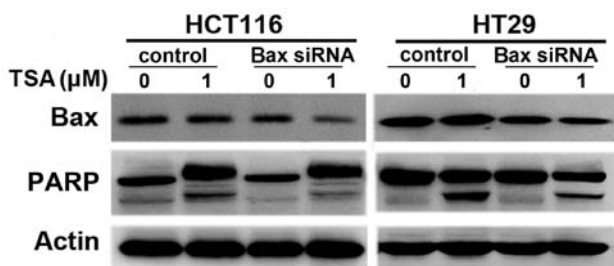


Figure 5. TSA induces apoptosis in CRC cells in a Bax-dependent pathway. Cells were transfected with Bax siRNA or scramble siRNA as control and then treated with TSA 1 μ M. Apoptosis was detected by western blotting using anti-PARP antibody which could recognize the cleaved band. Actin blot was included to show equal protein loading for all the samples.

activation. We then asked whether TSA induced apoptosis in CRC cells via different pathways. Mitochondrial membrane injury and caspase-3 activation are key steps in the progression of apoptosis. We first measured MMP dissipation, which plays a pivotal role in the initiation of apoptosis. The results showed that TSA caused increased loss of MMP in a dose-dependent way in both cell lines (Fig. 3A and B), there was not much difference between the two cell lines. We next detected caspase-3 activation by western blotting with antibody which could recognize the cleaved active form of caspase-3. Our results showed that TSA induced caspase-3 activation in a similar manner with MMP dissipation (Fig. 3C). These results demonstrated that TSA induced apoptosis in both CRC cell lines via mitochondrial pathway and dependent on caspase-3 activation.

TSA induces changes of apoptosis-related proteins. It is known that the ratio of pro-apoptotic protein (Bax) and anti-apoptotic proteins (Bcl-2 and Bcl-xL) of Bcl-2 family determines whether cancer cells respond to an apoptotic signal by mediating the disruption of the mitochondrial membrane and the release of caspase activators (13). By western blotting, we showed that (Fig. 4) Bax was upregulated after TSA treatment in both HCT116 and HT29 cells in a dose-dependent manner. On the contrary, the anti-apoptotic proteins Bcl-2 and Bcl-xL were downregulated in both cell lines, while Mcl-1 level did not change by TSA. The expression level of all three proteins were different in the cell lines, which might explain the difference of apoptosis induced by TSA in those cells.

TSA induces apoptosis in CRC cells in a Bax-dependent pathway. Bax is an important pro-apoptotic protein in triggering mitochondria-mediated apoptosis by permeabilizing mitochondrial outer membranes. Since TSA induces apoptosis in both cell lines via mitochondrial pathway, we examined whether Bax is essential in both p53-dependent and -independent apoptosis. As shown in Fig. 5, Bax siRNA impaired TSA-induced apoptosis in both HCT116 and HT29 cells, as indicated by the weakened cleaved bands of PARP. These results suggest that TSA induces apoptosis in CRC cells in a Bax-dependent way.

Discussion

Acetylation and deacetylation of histones, are known to be involved in gene expression regulation, catalyzed by histone acetyl transferases (HATs) and histone deacetylases (HDACs), respectively (14). HDAC inhibitors (HDACIs) are introduced as potential clinical treatments for cancer that inhibit deacetylase activity, thereby increasing acetylation of many proteins, including histones (15-17). TSA, a pan-HDAC inhibitor, has shown anti-neoplasia effects in many kinds of tumor cells. It is known that conventional chemotherapeutic agents induce apoptosis of cancer cells mostly through p53-dependent pathway. However, p53 mutations and inactivation have been found in more than half of all human carcinoma cells (10). We therefore observed whether TSA was effective in inducing cell cycle arrest and apoptosis in CRC cell lines with wild-type p53 (HCT116 cells) and mutant p53 (HT29 cells).

p21, a member of the cyclin-dependent kinase (CDK) inhibitor protein family, acts by binding cyclin-CDK complexes, inhibiting their kinase activity and effectively blocking DNA synthesis and cell cycle progression (18-20). The growth arrest at G1 phase by HDAC inhibitors is thought to be highly dependent on the upregulation of p21 (11). It is well established that p21 induced G1/S phase arrest through its interactions with cyclin E/CDK2 (21). p21 is also involved in G2/M arrest via inhibiting the CDK-activating kinase (CAK) and consequently inactivation of CDK1 (8-10). Some studies have reported that p21 induction in several p53 null cells also could induce G2/M arrest (22). In the current study, TSA was shown to induce cell cycle arrest at G2/M phase in both cell lines, with S phase cells reduced, indicating the inhibition of DNA replication in S phase. By western blotting, we showed that TSA increased p21 expression in HCT116 cells with wild-type p53, suggesting that HDACIs may activate the transactivation of p53. While the expression of p21 in p53 mutant HT29 cells was only slightly induced. The mechanisms by which TSA induces G2/M arrest in HT29 cells are not known. TSA has been shown to induce G2/M arrest in p53 (-/-) MG63 osteosarcoma cells through the induction of Gadd45 (23). The above results suggest that TSA induce cell cycle arrest in CRC cells through p53-dependent and -independent pathway.

Some HDAC inhibitors, including TSA, have been reported to induce apoptosis in cancer cells through activation of p53 by phosphorylation or acetylation (24,25), suggesting a p53-dependent apoptotic pathway by TSA. Here, we showed that TSA was effective in both p53 wild-type HCT116 cells and p53 mutant HT29 cells, indicating that TSA could induce

apoptosis in CRC cells via both p53-dependent and -independent mechanisms. However, apoptotic rates in HCT116 cells treated with TSA was higher than that in HT29 cells, demonstrating that the susceptibility to TSA-induced apoptosis in CRC cells might be regulated by p53 status.

Bax plays an important role in apoptosis, which is downstream of the p53 pathway and a direct target of p53. Exogenously expressed p53 increases Bax expression in several cancer cell types, which correlates with the induction of apoptosis (26). Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization, allowing cytochrome c to be released from mitochondria, which associates with the 47 kDa procaspase-9/Apaf 1 and activates caspase cascade, leading to apoptosis (27). Bax and Bcl-2 are homologous proteins that have opposite effects on cell fate (28). In the present study, the increase in apoptosis was associated with upregulation of pro-apoptotic protein Bax, and downregulation of anti-apoptotic proteins Bcl-2 and Bcl-xL. Since mutant p53 gene is unable to transactivate downstream target genes, such as Bax (10), the expression of Bax might be induced by other transcription factors (29). We also showed that TSA dose-dependently decreased MMP in both cell lines, suggesting the involvement of mitochondrial pathway. Caspase-3, the key executor of apoptosis, has been described to be essential for drug-induced apoptosis. We have detected the activation of caspase-3 after TSA treatment in both p53 wild-type and mutant CRC cell lines. These results demonstrate that TSA induces apoptosis in both CRC cell lines via mitochondrial pathway and dependent on caspase-3 activation, regardless of p53 status.

Bax is an initiator in mitochondrial apoptotic pathway. In the current study, Bax knock-down by siRNA significantly impaired TSA-induced apoptosis in both HCT116 and HT29 CRC cells. We concluded that Bax is an essential mediator for both p53-dependent and -independent apoptosis induced by TSA in CRC cells. TSA might induce cell death in CRC cells by increasing the acetylation of Ku70, a Bax-binding protein, which causes Bax release and become free to translocate from the cytoplasm to mitochondria to stimulate apoptosis (30).

In summary, based on these findings, we concluded that TSA induces G2/M cell cycle arrest and Bax-dependent apoptosis in colorectal cancer cells (HT29 cells and HT29 cells) by both p53-dependent and -independent mechanisms. This study may provide useful information for the use of HDAC inhibitors to treat clinical patients with colorectal cancer.

Acknowledgements

This study was supported by the National 973 project of China (2010CB535002).

References

- Jernall A, Siegel R, Ward E, *et al*: Cancer statistics, 2009. *CA Cancer J Clin* 59: 225-249, 2009.
- Johnstone RW: Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat Rev Drug Discov* 1: 287-299, 2002.
- Yu X, Guo ZS, Marcu MG, *et al*: Modulation of p53, Erbb1, Erbb2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228. *J Natl Cancer Inst* 94: 504-513, 2002.
- Zhu WG and Otterson GA: The interaction of histone deacetylase inhibitors and DNA methyltransferase inhibitors in the treatment of human cancer cells. *Curr Med Chem AntiCancer Agents* 3: 187-199, 2003.
- Meinke PT and Liberators P: Histone deacetylase: a target for antiproliferative and antiprotazoal agents. *Curr Med Chem* 8: 211-235, 2001.
- Yoshida M, Kijima M, Akita M and Beppu T: Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J Biol Chem* 265: 17174-17179, 1990.
- Laguer G, Doetzlhofer A, Schuettengruber B, *et al*: The tumor suppressor p53 and histone deacetylase 1 are antagonistic regulators of the cyclin-dependent kinase inhibitor p21/WAF1/CIP1 gene. *Mol Cell Biol* 23: 2669-2679, 2003.
- Levine AJ: p53, the cellular gatekeeper for growth and division. *Cell* 88: 323-331, 1997.
- Prives C and Hall PA: The p53 pathway. *J Pathol* 187: 112-126, 1999.
- Vogelstein B, Lane D and Levine AJ: Surfing the p53 network. *Nature* 408: 307-310, 2000.
- Hirose T, Sowa Y, Takahashi S, *et al*: p53-independent induction of Gadd45 by histone deacetylase inhibitor: coordinate regulation by transcription factors Oct-1 and NF-Y. *Oncogene* 22: 7762-7773, 2003.
- Rich T, Allen RL and Wyllie AH: Defying death after DNA damage. *Nature* 407: 777-783, 2000.
- Pommier Y, Sordet O, Antony S, Hayward RL and Kohn KW: Apoptosis defects and chemotherapy resistance: molecular interaction maps and networks. *Oncogene* 23: 2934-2949, 2004.
- Emiliani S, Fischle W, Van Lint C, Al-Abed Y and Verdin E: Characterization of a human RPD3 ortholog, HDAC3. *Proc Natl Acad Sci USA* 95: 2795-2800, 1998.
- Krämer OH, Gottlicher M and Heinzel T: Histone deacetylase as a therapeutic target. *Trends Endocrinol Metab* 12: 294-300, 2001.
- Marks PA, Richon VM, Breslow R and Rifkind RA: Histone deacetylase inhibitors as new cancer drugs. *Curr Opin Oncol* 13: 477-483, 2001.
- Melnick A and Licht JD: Histone deacetylases as therapeutic targets in hematologic malignancies. *Curr Opin Hematol* 9: 322-332, 2002.
- Janicke RU, Sohn D, Essmann F and Schulze-Osthoff K: The multiple battles fought by anti-apoptotic p21. *Cell Cycle* 6: 407-413, 2007.
- Le HV, Minn AJ and Massague J: Cyclin-dependent kinase inhibitors uncouple cell cycle progression from mitochondrial apoptotic functions in DNA-damaged cancer cells. *J Biol Chem* 280: 32018-32025, 2005.
- Seoane J, Le HV and Massague J: Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. *Nature* 419: 729-734, 2002.
- Bartek J and Lukas J: Pathways governing G1/S transition and their response to DNA damage. *FEBS Lett* 490: 117-122, 2001.
- Choi YH, Lee WH, Park KY and Zhang L: p53-independent induction of p21 (WAF1/CIP1), reduction of cyclin B1 and G2/M arrest by the isoflavone genistein in human prostate carcinoma cells. *Jpn J Cancer Res* 91: 164-173, 2000.
- Hirose T, Sowa Y, Takahashi S, *et al*: p53-independent induction of Gadd45 by histone deacetylase inhibitor: coordinate regulation by transcription factors Oct-1 and NF-Y. *Oncogene* 22: 7762-7773, 2003.
- Hsu YF, Sheu JR, Hsiao G, *et al*: p53 in trichostatin A induced C6 glioma cell death. *Biochim Biophys Acta* 1810: 504-513, 2011.
- Oh ET, Park MT, Choi BH, *et al*: Novel histone deacetylase inhibitor CG200745 induces clonogenic cell death by modulating acetylation of p53 in cancer cells. *Invest New Drugs* 30: 435-442, 2010.
- Miyashita T and Reed JC: Tumor suppressor p53 is a direct transcriptional activator of the human Bax gene. *Cell* 80: 293-299, 1995.
- Li P, Nijhawan D, Budihardjo I, *et al*: Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91: 479-489, 1997.
- Sugita K, Koizumi K and Yoshida H: Morphological reversion of sis-transformed NIH3T3 cells by trichostatin A. *Cancer Res* 52: 168-172, 1992.
- Levrero M, De Laurenzi V, Costanzo A, *et al*: The p53/p63/p73 family of transcription factors: overlapping and distinct functions. *J Cell Sci* 113: 1661-1670, 2000.
- Cohen HY, Lavu S, Bitterman KJ, *et al*: Acetylation of the C terminus of Ku70 by CBP and PCAF controls Bax-mediated apoptosis. *Mol Cell* 13: 627-638, 2004.