Gastric cancer cell lines induced by trichostatin A

Xiao-Ming Zou, Yun-Long Li, Hao Wang, Wu Cui, Xiao-Lin Li, Song-Bin Fu, Hong-Chi Jiang

AIM: To explore the effect of trichostatin A (TSA) on apoptosis and acetylated histone H3 levels in gastric cancer cell lines BGC-823 and SGC-7901.

METHODS: The effect of TSA on growth inhibition and apoptosis was examined by MTT, fluorescence microscopy and PI single-labeled flow cytometry. The acetylated histone H3 level was detected by Western blot.

RESULTS: TSA induced apoptosis in gastric cancer cell lines BGC-823 and SGC-7901 was in a dose and time-dependent manner. Apoptotic cells varied significantly between TSA treated groups (37.5 ng/mL 72 h for BGC-823 cell line and 75 ng/mL 72 h for SGC-7901 cell line) and control group (0.85 ± 0.14 vs 1.14 ± 0.07, P = 0.02; 0.94 ± 0.07 vs 1.15 ± 0.06, P = 0.02). Morphologic changes of apoptosis, including nuclear chromatin condensation and fluorescence strength, were observed under fluorescence microscopy. TSA treatment in BGC-823 and SGC-7901 cell lines obviously induced cell apoptosis, which was demonstrated by the increased percentage of sub-G1 phase cells, the reduction of G1-phase cells and the increase of apoptosis rates in flow cytometric analysis. The result of Western blot showed that the expression of acetylated histone H3 increased in BGC-823 and SGC-7901 TSA treatment groups as compared with the control group.

CONCLUSION: TSA can induce cell apoptosis in BGC-823 and SGC-7901 cell lines. The expression of acetylated histone H3 might be correlated with apoptosis.

© 2008 The WJG Press. All rights reserved.

Key words: BGC-823; SGC-7901; Trichostatin A; Apoptosis; Acetylated histone H3; Gastric cancer

Peer reviewer: Dr. Jordi Camps, Centre de Recerca Biomèdica, Hospital Universitari de Sant Joan, C. Sant Joan s/n, Reus 43201, Spain

Zou XM, Li YL, Wang H, Cui W, Li XL, Fu SB, Jiang HC. Gastric cancer cell lines induced by trichostatin A. World J Gastroenterol 2008; 14(30): 4810-4815 Available from: URL: http://www.wjgnet.com/1007-9327/14/4810.asp DOI: http://dx.doi.org/10.3748/wjg.14.4810

INTRODUCTION

Gastric cancer is the second most common cancer worldwide[10]. It is often not detected until an advanced stage; consequently, the 5-year survival rates are low (10%-20%). Owing to local invasion and metastasis, radiation therapy or chemotherapy does not significantly increase the length or improve the quality of life of patients with advanced gastric cancer. Therefore, there is growing interest in the development of novel neoadjuvant and adjuvant treatment modalities.

It is widely accepted that histone acetylation is essential to establish a transcriptional competent state of chromatin[12-14]. The reversible (de)acetylation of the N-terminal histone tails by specific histone acetylases and deacetylases (HDAC) is involved in the regulation of gene expression. Dysfunction of histone acetylases and HDACs are associated with different types of cancer[8-10]. Various HDAC inhibitors (HDACIs) have been described to induce cell cycle arrest, differentiation, and apoptosis in cell lines[9,12]. Many of these have potent antitumor activities in vivo[13]. One of the most effective and best studied HDAC inhibitors is trichostatin A (TSA). The crystallographic analysis of TSA and a histone deacetylase homologue indicates that TSA interacts reversibly with the HDAC catalytic site preventing binding of the substrate[13]. Considering that HDAC inhibitors are able to induce apoptosis in different cell types[14-16], we intend to know their potential
to induce apoptosis in gastric cancer cell lines BGC-823 and SGC-7901. We also studied the effect of TSA-induced acetylated histone H3 level on these cell lines.

MATERIALS AND METHODS

Reagents and antibodies
Stock solutions of TSA (Sigma-Aldrich, USA) in ethanol were stored at -20°C. Dimethylthiazole diphenyl tetrazolium bromide (MTT), propidium iodide (PI, Beijing Zhongshan Golden Bridge Biotechnology, China) and Hoechst 33342 (Sigma-Aldrich) were used. Antibody against GAPDH was purchased from Santa Cruz Biotechnology (Santa Cruz Biotechnology, USA). Anti-acetyl-histone H3 (rabbit polyclonal IgG) and Goat Anti-Rabbit IgG, HRP-conjugate were purchased from Upstate Biotechnology (Upstate Biotechnology, USA).

Cell culture and treatment
Human gastric epithelial cell lines BGC-823 and SGC-7901 were obtained from Institute of Tumor Research of Heilongjiang. BGC-823 cells and SGC-7901 cells were cultured and maintained in RPMI 1640, supplemented with fetal bovine serum 10% (v/v), penicillin 100 IU/mL, and streptomycin 100 μg/mL in a humidified atmosphere of 5% CO2 in air at 37°C.

MTT assay
MTT assay was used to obtain the number of living cells in the sample. Cells were seeded on 96-well plates at a predetermined optimal cell density to ensure exponential growth in the duration of the assay. After 24 h preincubation, growth medium was replaced with experimental medium containing the appropriate drug or control. Six duplicate wells were set up for each sample, and cells untreated with drug served as control. Treatment was conducted at 12, 24, 48 and 72 h with final TSA concentrations of 37.5, 75, 150, 300 and 600 ng/mL, respectively. After incubation, 10 μL MTT (6 g/L, Sigma) was added to each well and the incubation was continued for 4 h at 37°C. After removal of the medium, MTT stabilization solution (dimethylsulphoxide: ethanol = 1:1) was added to each well, and shaken for 10 min until all crystals were dissolved. Then, optical density was detected in a microplate reader at 550 nm wavelength using an ELISA reader. The negative control well without cells was used as zero point of absorbance. Each assay was performed in triplicate.

Detection of chromatin condensation
Chromatin condensation was detected by nuclear staining with Hoechst 33342. BGC-823 and SGC-7901 cells were collected by centrifugation (500 × g for 5 min at 4°C) and washed twice with PBS. Cells were fixed in 10% formaldehyde and stored at 4°C. For analysis, cells were washed in PBS, then Hoechst 33342 (5 mg/L) was directly added to the medium by gently shaking at 4°C for 5 min. Stained nuclei were visualized under a Zeiss Axiophot fluorescence microscope at 400 × magnification with an excitation wavelength of 355-366 nm and an emission wavelength of 465-480 nm. Four independent replicates were used. In this way, apoptotic BGC-823 and SGC-7901 cells were stained brightly blue because of their chromatin condensation, while normal BGC-823 and SGC-7901 cells were evenly stained slightly blue.

Cell cycle and apoptosis assays
BGC-823 and SGC-7901 cells were treated as indicated. Floating and adherent cells were collected by centrifugation (500 × g for 5 min at 4°C) and washed twice with PBS. Cells were fixed in 90% ethanol and stored at -20°C. For analysis, cells were washed in PBS and stained by suspension in PI (50 mg/L) containing RNase A (2 mg/L) for 30 min at 4°C. Stained cells were analyzed on a FACSscan (Becton- Dickinson, Heidelberg, Germany).

Western blotting
Cells treated as indicated were harvested in 5 mL of medium, pelleted by centrifugation (1000 × g for 5 min at 4°C), then washed twice with ice-cold PBS and lysed in ice-cold HEPES buffer [HEPES (pH 7.5) 50 mmol/L, NaCl 10 mmol/L, MgCl2 5 mmol/L, EDTA 1 mmol/L, glycerol 110% (v/v), Triton X-100 1% (v/v), a cocktail of protease inhibitors, and 1 mg/L TSA on ice for 30 min. The lysates were clarified by centrifugation (15000 × g for 10 min at 4°C) and the supernatants then either analyzed immediately or stored at -80°C. Equivalent amounts of protein (50 μg) from total cell lysates were resolved by SDS-PAGE using precast 12% Bis-Tris gradient gels and transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were blocked overnight at 4°C in blocking buffer [nonfat dried milk 5% (v/v), NaCl 150 mmol/L, Tris(pH 8.0) 10 mmol/L and 0.05% Tween 20 (v/v)]. Proteins were detected by incubation with primary antibodies at appropriate dilutions in blocking buffer overnight at 4°C. Unbound antibody was removed by washing with Tris-buffered saline (pH 7.2) containing 0.5% Tween 20 (TBS-T). The membrane was then incubated at room temperature with horseradish peroxidase-conjugated secondary antibody. After extensive washing with TBS-T, bands were visualized by enhanced chemiluminescence followed by exposure to autoradiography.

RESULTS

TSA inhibited the proliferation of BGC-823 and SGC-7901 cells
TSA inhibited cellular proliferation and survival in BGC-823 and SGC-7901 cell lines. It resulted in a significant decrease in the cell population of BGC-823 and SGC-7901 compared with control, following treatment with TSA. Inhibition of TSA was dependent on the dose and incubation time (Tables 1 and 2).

TSA induced apoptosis of BGC-823 and SGC-7901 cells
To investigate the effects of TSA induced cytotoxicity,
morphologic changes of apoptosis were observed under fluorescence microscope. At 72 h, cells treated with or without TSA (37.5 ng/mL in BGC-823 and 75 ng/mL in SGC-7901) were stained by Hoechst 33342, a classical way of identifying apoptotic cells, to observe nuclei morphology. The result indicated that nuclei of most BGC-823 and SGC-7901 cells treated with TSA were stained highly condensed, bright nucleus; while the cells in control group were stained average slightly blue (Figure 1).

TSA treatment (37.5 ng/mL per 72 h) sensitively induced apoptosis of BGC-823 cells, which was demonstrated by the raised percentage of sub-G1 phase cells, the increase of apoptosis rates (20.12%) in flow cytometry (Figure 2). Cell cycle effects were examined by FACS analysis. The reduction of G1-phase cells (65.40%-49.83%) and the increment of S-phase cells (32.75%-49.56%) were observed in BGC-823 cells (Figure 2A, B). TSA induced apoptosis in SGC-7901 cells. Upon treatment with TSA (75 ng/mL) for 72 h in SGC-7901 cells, the reduction of G1-phase cells (72.12%-65.51%), S-phase cells (21.52%-12.88%) and the increment of G2-phase cells (6.35%-21.61%), and apoptosis rates (29.54%) were observed by FACS analysis (Figure 2C, D).

TSA regulated the level of acetylated histone H3 in BGC-823 and SGC-7901 cell lines

Western blot analysis was used to detect the level of acetylated histone H3 of BGC-823 cells and SGC-7901 cells treated with TSA (37.5 ng/mL per 48 h in BGC-823 cells and 75 ng/mL per 48 h in SGC-7901 cells). It was
shown that there was an increase of acetylated histone H3 level in TSA treated cells (Figure 3).

DISCUSSION

Histones are small-sized and basic-charged proteins essential for chromatin folding. Posttranslational modifications such as acetylation, methylation, and phosphorylation have been suggested to be involved in the regulation of gene expression, cell division, nucleosome assembly, and DNA repair processes via alterations in the nucleosome architecture[17]. To
Control groups  TSA-treated groups

This suggests that induction of histone hyperacetylation by HDAC inhibitors is responsible for the antiproliferative activity through selective induction of genes that play important roles in the cell cycle and cell morphology\[20]. It has been shown that reversible acetylation of lysine on histone H3 plays an important role in regulating gene transcription\[20]. Our findings indicate that acetylated histone H3 expression levels increased in BGC-823 and SGC-7901 cells following TSA treatment. Increased acetylated histone H3 expression levels in BGC-823 and SGC-7901 cells may be an important event in mediating the apoptosis of BGC-823 and SGC-7901 cells induced by TSA. These findings generate the necessity to investigate the mechanism of TSA in the treatment of BGC-823 and SGC-7901 cells. Therefore, we suppose that HDAC inhibitors cause acetylated histones to accumulate in tumor tissues, and this accumulation can be used as a trigger of the biologic activity of the HDAC inhibitors.

In summary, in this study we showed that TSA can induce apoptosis in gastric cancer cell lines BGC-823 and SGC-7901. We demonstrated that the expression of acetylated histone H3 might be correlated with apoptosis. Further work will be necessary to explore additional mechanisms that lead to induction of apoptosis.

**COMMENTS**

**Background**
Histones are small-sized and basic-charged proteins essential for chromatin folding. The acetylation state of histones is reversibly regulated by histone acetyltransferase (HAT) and deacetylase (HDAC). An imbalance of this reaction leads to an aberrant behavior of the cells in morphology, cell cycle, differentiation, and carcinogenesis. HDAC is especially known to play an important role in carcinogenesis.

**Research frontiers**
There is increasing evidence that HDAC inhibitors are effective therapeutic agents in the treatment of a variety of cancers refractory to conventional anticancer agents. Several structurally diverse HDAC inhibitors, such as trichostatin A (TSA), amicrobial metabolite, or butyrates, have been identified and their in vitro activity in transformed cells makes them promising agents for cancer therapy. Although extensive studies have been done, roles of HDAC inhibitor in gastric cancer are still unclear.

**Innovations and breakthroughs**
This study investigates the effects of the HDAC inhibitor TSA on gastric cancer cell lines BGC-823 and SGC-7901. The results demonstrate that low concentrations of TSA can significantly reduce the growth of BGC-823 and SGC-7901 cells and the effects of TSA on BGC-823 and SGC-7901 cells are dose and time-dependent. These findings indicate that acetylated histone H3 expression levels increased in BGC-823 and SGC-7901 cells following TSA treatment.

**Applications**
It can be seen from this paper that the HDAC inhibitor, TSA, can induce apoptosis of BGC-823 and SGC-7901 cells. It suggests that HDAC is a promising target for the development of anticancer drugs for gastric cancer.

**Peer review**
This paper examined the role of TSA in gastric cancer cell lines BGC-823...
REFERENCES

1. Dicken BJ, Bigam DL, Cass C, Mackey JR, Joy AA, Hamilton SM. Gastric adenocarcinoma: review and considerations for future directions. *Ann Surg* 2005; 241: 27-39

2. Kadonaga JT. Eukaryotic transcription: an interlaced network of transcription factors and chromatin-modifying machines. *Cell* 1998; 92: 307-313

3. Zhang W, Bone JR, Edmondson DG, Turner BM, Roth SY. Essential and redundant functions of histone acetylation revealed by mutation of target lysines and loss of the Gcn5p acetyltransferase. *EMBO J* 1998; 17: 3155-3167

4. Strahl BD, Allis CD. The language of covalent histone modifications. *Nature* 2000; 403: 41-45

5. Pruiitt K, Zinn RL, Ohm JE, McGarvey KM, Kang SH, Watkins DN, Herman JG, Baylin SB. Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation. *PloS Genet* 2006; 2: e40

6. Shukla SD, Aroor AR. Epigenetic effects of ethanol on liver and gastrointestinal injury. *World J Gastroenterol* 2006; 12: 5265-5271

7. De Schepper S, Bruviere H, Verhulst T, Steller U, Andries L, Wouters W, Janicot M, Arts J, Van Heusden J. Inhibition of histone deacetylases by chlamydoin induces apoptosis and proteasome-mediated degradation of survivin. *J Pharmacol Exp Ther* 2003; 304: 881-888

8. Chen YX, Fang JY, Zhu HY, Lu R, Cheng ZH, Qiu DK. Histone acetylation regulates p21WAF1 expression in human colon cancer cell lines. *World J Gastroenterol* 2004; 10: 2643-2646

9. Subramanian C, Opipari AW Jr, Bian X, Castle VP, Kwok RP. Ku70 acetylation mediates neuroblastoma cell death induced by histone deacetylase inhibitors. *Proc Natl Acad Sci USA* 2005; 102: 4842-4847

10. Fenici I, Sonnack V, Failing K, Bergmann M, Steger K. In vivo effects of histone-deacetylase inhibitor trichostatin-A on murine spermatogenesis. *J Androl* 2004; 25: 811-819

11. Toth KF, Knoch TA, Wachsmuth M, Frank-Stoehr M, Stohr M, Bacher CP, Muller G, Rippe K. Trichostatin A-induced histone acetylation causes decondensation of interphase chromatin. *Cell Sci* 2004; 117: 4277-4287

12. Romanski A, Bacic B, Bug G, Pfeifer H, Gul H, Remiszewski S, Hoezler D, Atadja P, Ruthardt M, Ottmann OG. Use of a novel histone deacetylase inhibitor to induce apoptosis in cell lines of acute lymphoblastic leukaemia. *Haematologica* 2004; 89: 419-426

13. Marks PA, Richon VM, Rifkind RA. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. *J Natl Cancer Inst* 2000; 92: 1210-1216

14. Batova A, Shao LE, Dicianni MB, Yu AL, Tanaka T, Rephaeli A, Nudelman A, Yu J. The histone deacetylase inhibitor AN-9 has selective toxicity to acute leukemia and drug-resistant primary leukemia and cancer cell lines. *Blood* 2002; 100: 3319-3324

15. Glick RD, Swendeman SL, Coffey DC, Rifkind RA, Marks PA, Richon VM, La Quaglia MP. Hybrid polar histone deacetylase inhibitor induces apoptosis and CD95/CD95 ligand expression in human neuroblastoma. *Cancer Res* 1999; 59: 4302-4309

16. Kim DH, Kim M, Kwon HJ. Histone deacetylase in carcinogenesis and its inhibitors as anti-cancer agents. *J Biochem Mol Biol* 2003; 36: 110-119

17. Grunstein M. Histone acetylation in chromatin structure and transcription. *Nature* 1997; 389: 349-352

18. de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J* 2003; 370: 737-749

19. Saito A, Yamashita T, Mariko Y, Nosaka Y, Tsuchiya K, Ando T, Suzuki T, Tsuroo T, Nakanishi O. A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors. *Proc Natl Acad Sci USA* 1999; 96: 4592-4597

20. Butler LM, Agus DB, Scher HI, Higgins B, Rose A, Cordon-Cardo C, Thaler HT, Rifkind RA, Marks PA, Richon VM. Suiberyolaniumide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in vitro and in vivo. *Cancer Res* 2000; 60: 5165-5170

21. Chen WK, Chen Y, Gu JX, Cui GH. [Effect of trichostatin A on histone acetylation level and apoptosis in HL-60 cells]. *Zhongguo Shiyan Xueyixue ZaZhi* 2004; 12: 324-328

22. Duan H, Beckman CA, Boxer LM. Histone deacetylase inhibitors down-regulate bcl-2 expression and induce apoptosis in t(14;18) lymphomas. *Mol Cell Biol* 2005; 25: 1608-1619

23. Hong ZY, Yi LS, Miao XY, Lu YP, Zhou JF, Liu WL. [Mechanism of apoptosis induced by trichostatin a in leukemia Molt-4 cells analyzed by microarray]. *Ai Zheng* 2006; 25: 946-953

24. Wang J, Saunthararajah Y, Redner RL, Liu JM. Inhibitors of histone deacetylase relieve ETO-mediated repression and induce differentiation of AML1-ETO leukemia cells. *Cancer Res* 1999; 59: 2766-2769

25. Han JW, Ahn SH, Park SH, Wang SY, Bae GU, Seo DW, Kwon HK, Hong S, Lee HY, Lee YW, Lee HW. Apicidin, a histone deacetylase inhibitor, inhibits proliferation of tumor cells via induction of p21WAF1/Cip1 and gelsolin. *Cancer Res* 2000; 60: 6068-6074

26. Davie JR. Covalent modifications of histones: expression from chromatin templates. *Curr Opin Genet Dev* 1998; 8: 173-178

S-Editor Zhong XY  L-Editor Ma JY  E-Editor Yin DH