



RAPID COMMUNICATION

Effect of histone deacetylase inhibitor on proliferation of biliary tract cancer cell lines

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Abstract

AIM: To explore the effect of histone deacetylase inhibitor, trichostatin A (TSA) on the growth of biliary tract cancer cell lines (gallbladder carcinoma cell line and cholangiocarcinoma cell line) *in vivo* and *in vitro*, and to investigate the perspective of histone deacetylase inhibitor in its clinical application.

METHODS: The survival rates of gallbladder carcinoma cell line (Mz-ChA-I cell line) and cholangiocarcinoma cell lines (QBC939, KMBC and OZ cell lines) treated with various doses of TSA were detected by methylthiazol tetrazolium (MTT) assay. A nude mouse model of transplanted gallbladder carcinoma (Mz-ChA-I cell line) was successfully established, and changes in the growth of transplanted tumor after treated with TSA were measured.

RESULTS: TSA could inhibit the proliferation of gallbladder carcinoma cell line (Mz-ChA-I cell line) and cholangiocarcinoma cell lines (QBC939, KMBC and OZ cell lines) in a dose-dependent manner. After the nude mouse model of transplanted gallbladder carcinoma (Mz-ChA-I cell line) was successfully established, the growth of cancer was inhibited in the model after treated with TSA.

CONCLUSION: TSA can inhibit the growth of cholangiocarcinoma and gallbladder carcinoma cell lines *in vitro* and *in vivo*.

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Key words: Biliary tract cancer; Gallbladder carcinoma; Cholangiocarcinoma; Proliferation; Trichostatin A

INTRODUCTION

Biliary tract cancer, consisting of gallbladder carcinoma and cholangiocarcinoma, presents many challenges to physicians. It is a relatively rare cancer often causing a diagnostic dilemma, as its presentation may be similar to that of non-malignant conditions^[1,2]. It was reported that the misdiagnosis rate of biliary duct cancer is 19.1% and the median survival time of biliary tract cancer patients is about 7 mo^[3]. At present, treatment of biliary tract cancer is also difficult^[4] and complex due to a morbid patient population and limited data on the optimal therapeutic approach. Surgery remains the mainstay of treatment, although the extent of resection required is still controversial. Despite recent advances in imaging modalities, most of the patients are at the advanced stage of the disease at presentation, thus making radical surgery not feasible, which seriously affects its prognosis. The role of adjuvant therapy is also controversial. Different chemotherapeutic regimens have been investigated in small uncontrolled studies, with generally disappointed results^[5]. In patients with unresectable biliary duct cancer, combination of chemotherapy and radiotherapy can result in a prolonged survival time of some patients. In a palliative setting, biliary stenting and other supportive measures can alleviate symptoms and improve survival. Ultimately, treatment decisions should be individualized and participation in clinical trials is encouraged. Further progress in the management of biliary tract cancer is anticipated using biological therapies and continued research is essential to discover the optimal treatment for this challenging disease^[2]. It is, thus, important to research its biological characteristics and biotherapy.

Cancer is a disease resulting from both genetic and epigenetic changes. There are many important epigenetic changes in its early carcinogenesis. Accumulating evidence indicates, however, that disparities in gene expression resulting from variable modifications in DNA methylation and chromatin structure in response to the environ-

ment also play a role in differential susceptibility to the disease^[6,7]. Histone acetylation/deacetylation constitutes the most relevant chromatin remodelling mechanism underlying the DNA access to nuclear machinery and mutagenic agents^[8]. It has been recently shown that histone acetylation/deacetylation is closely related with tumorigenesis^[9,10]. Acetylation of histone plays a very important but incompletely understood role in genetic regulation^[11]. Histone deacetylase inhibitors could markedly inhibit the growth of tumor cell lines^[12,13]. It has been shown that expression of p53BP1, a non-histone protein, is associated with HDAC4 and plays a role in histone acetylation of fully-grown oocytes^[14]. Other data indicate that the known non-histone targets may play a role in the pathogenesis of cholangiocarcinoma^[15].

Experiments *in vitro* have shown that TSA can be used in the treatment of ovarian cancer, pancreatic endocrine carcinoma, prostate cancer, *etc*^[16-18]. The results of this study focusing on the effect of histone deacetylase inhibitor, trichostatin A (TSA) on the proliferation of gallbladder carcinoma cell line (Mz-ChA-I cell line) and cholangiocarcinoma cell lines (QBC939, KMBC and OZ cell lines) *in vivo* and *in vitro*, have demonstrated the value of biotherapy for biliary tract cancer with TSA.

MATERIALS AND METHODS

Cell lines

QBC939 cell line (a human cholangiocarcinoma cell line), KMBC cell line (a human cholangiocarcinoma cell line), OZ cell line (a human cholangiocarcinoma cell line) and Mz-ChA-I cell line (originally isolated from human gallbladder adenocarcinoma) were used in our study. QBC939 cell line was kindly provided by Professor Shu-Guang Wang, The Third Military Medical University, Chongqing, China. KMBC, OZ and Mz-ChA-I cell lines were kindly provided by Dr. Ke-Qin Hu, University of California, Irvine Medical Center, California, USA. Cell culture media and supplements were purchased from GIBCO Invitrogen Corporation (Carlsbad, CA, USA).

Animals

Sixteen male BALB/c nude mice at the age of 4 wk, weighing 10.0 ± 2.1 g, were purchased from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). The mice were caged individually under specific pathogen-free conditions and fed with standard maintenance diet and water throughout the experimental period. All animals received care treatment in compliance with the Guidelines of Ministry of Public Health of China.

Cell culture

All biliary tract cancer cell lines (QBC939, KMBC, OZ and Mz-ChA-I cell lines) were grown as a monolayer in RPMI-1640 medium (Sigma, USA) supplemented with 10% fetal bovine serum, penicillin (100 000 U/L), cultured in T-75 cm² culture flasks, maintained at 37°C in a humidified atmosphere containing 50 mL/L CO₂. At the beginning of experiment, cells at the exponential growth phase were removed from the flask with a solution containing

0.25% trypsin and 0.02% EDTA and seeded in 96-well plates containing RPMI-1640 medium supplemented with 10% fetal bovine serum.

Methylthiazol tetrazolium (MTT) assay

QBC939, KMBC, OZ and Mz-ChA-I cells at logarithmic growth phase were digested with 0.25% trypsin and then suspended. Cells were calculated under microscope with the cell suspension adjusted to 2×10^5 /mL. The wells in the plate were divided into 6 experiment groups and 1 blank control group. Cells were added into the plate (200 μ L per well, 0.4×10^5), and medium into the blank control wells. After 18 h, the medium for the experiment groups was replaced with a medium containing 0.10, 0.25, 0.50, 1.0, 1.5, 2.0 μ mol/L TSA, respectively. The culture was continued, then 20 μ L of 5 mg/mL MTT was added into each well after 4, 12, 24, 36 and 48 h, respectively. After another 4 h, the medium was discarded and 150 μ L DMSO was added into each well. The *A* value for each well was measured at the wave length of 490 nm. The survival rate was then calculated and the survival curve was plotted following the formula: survival rate (%) = the *A* value of experiment groups - the *A* value of blank control group / the *A* value of negative group - the *A* value of blank control group $\times 100\%$.

Treatment of Mz-ChA-I cell lines and establishment of gallbladder carcinoma transplanted tumor model

Mz-ChA-I cells at the logarithmic phase were divided into two groups: one group was treated with 0.75 μ mol/L TSA, the other group was cultured in medium. Cells were digested and counted after 24 h. The 16 male BALB/c nude mice at the age of 4 wk were randomly divided into group A (control group) and group B (TSA group) randomly, 8 in each group. The mice in group A were subcutaneously inoculated with untreated Mz-ChA-I cell suspension *via* the back, while those in group B were subcutaneously inoculated with Mz-ChA-I cell suspension treated with TSA for 24 h *via* the back. The wound was slightly pressed with a cotton swab for hemostasis and closed. The volume of cell suspension was 0.2 mL, with a concentration of 1×10^5 /mL. The volume of tumors was calculated in the sixth week according to the following formula: the volume = the biggest diameter \times transverse diameter²/2.

Statistical analysis

Statistical analysis was performed using the rank sum test (*H* test) for multi-sample comparison and *t*-test for two-sample comparison. The data were expressed as mean \pm SE. *P* < 0.05 was considered statistically significant.

RESULTS

Effect of TSA on survival of QBC939, KMBC, OZ and Mz-ChA-I cell lines *in vitro*

QBC939, KMBC, OZ and Mz-ChA-I cell lines were shrunk and died when TSA was added at various concentrations. MTT assay showed that TSA could shorten the survival time of QBC939, KMBC, OZ and Mz-ChA-I cell lines in a dose-dependent manner. The inhibition of cell survival increased with the increased dose of TSA (Table 1, Figure 1).

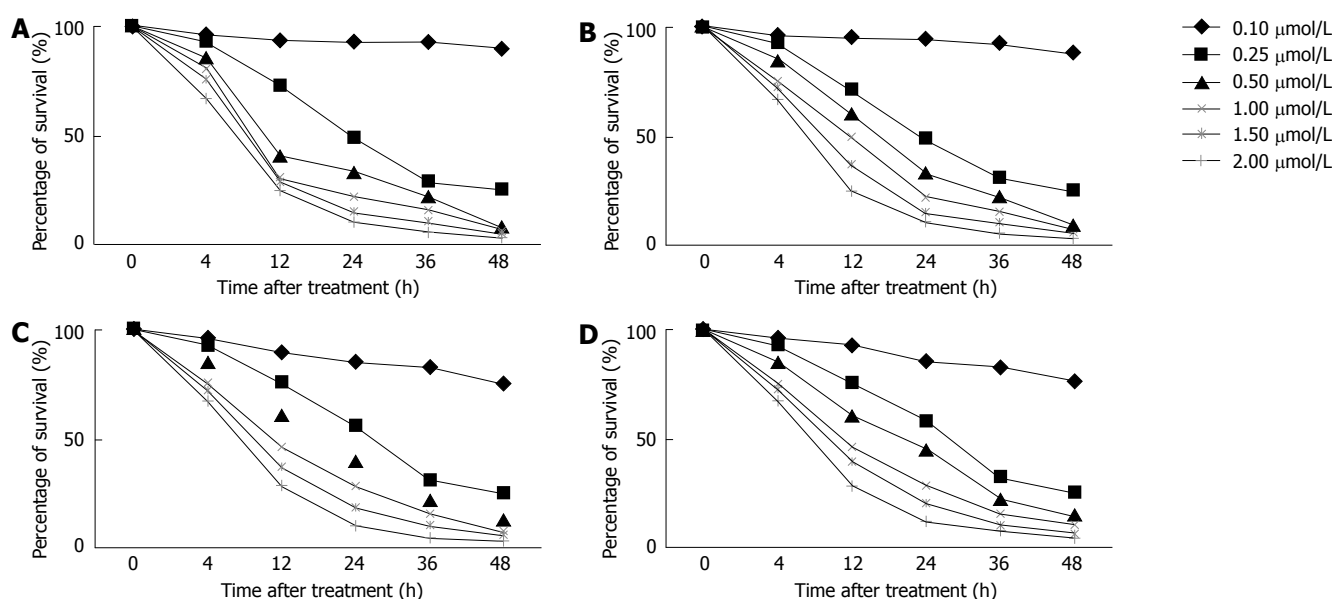


Figure 1 Effect of TSA on the survival of QBC₉₃₉ (A), KMBC (B), OZ (C), and Mz-ChA-I (D).

Table 1 Effect of TSA on the survival of biliary tract cancer cell lines

Concentration of TSA	Percentage of survival of cell lines (median and quartile)			
	QBC ₉₃₉	KMBC	OZ	Mz-ChA-I
0.10 μmol/L	92.87 (93.09)	93.27 (94.87)	83.07 (87.72)	83.26 (88.75)
0.25 μmol/L	33.38 (60.61)	34.88 (59.60)	36.63 (65.60)	38.58 (66.51)
0.50 μmol/L	24.90 (36.45)	24.86 (46.64)	26.90 (50.64)	27.90 (52.64)
1.00 μmol/L	16.99 (26.30)	17.09 (35.80)	18.46 (37.23)	18.46 (37.23)
1.50 μmol/L	11.29 (21.63)	11.32 (25.36)	12.29 (27.36)	12.79 (29.86)
2.00 μmol/L	6.52 (17.35)	6.28 (16.36)	6.28 (19.25)	9.03 (20.25)
Value of <i>H</i>	15.66	16.11	14.23	14.32
<i>P</i>	0.0082	0.0076	0.046	0.043

The survival percentage of cell lines treated with various doses of TSA was detected after 4, 12, 24, 36 and 48 h, respectively, by MTT assay. The MTT value was expressed as median and quartile.

Effect of TSA on survival of Mz-ChA-I cell line

All the male BALB/c nude mice survived. The mice in group A were subcutaneously inoculated with untreated Mz-ChA-I cell suspension *via* the back. The mice in group B were subcutaneously inoculated with Mz-ChA-I cell suspension *via* the back after treated with TSA for 24 h. After the Mz-ChA-I cell line was transplanted into the nude mice for 6 wk, the tumor volume of group A was $930.25 \pm 261.64 \text{ mm}^3$, the transplanted tumor volume of group B was smaller than that of group A, and there was a significant difference between them ($t = 2.50$, $P = 0.036$).

DISCUSSION

Regulation of genes is a new topic^[19]. Genetic information is regulated and expressed precisely. Gene expression can be greatly regulated by histone remodeling. This is a kind of basic post formation theory of gene regulation^[20]. Histone deacetylase (HDAC) and histone acetyl transferase (HAT) are two counteracting enzymes. Their activities can control the acetylation of protein lysine residues, notably those contained in the N-terminal extensions of the core

histones. Acetylation of histones affects gene expression through its influence on chromatin conformation^[21,22]. A lot of data have shown that HDAC is one of the promising targets of cancer treatment as many HDAC inhibitors of solid and liquid tumors have entered clinical trials^[23]. HDAC inhibitor is a kind of chemical compound regulating gene expression at the transcription level by changing chromosome structure through inhibiting HDAC. The HDAC inhibitors-inhibited HDAC enzymes shift the balance between the deacetylation activity of HDAC enzymes and the acetylation activity of histone acetyltransferases, resulting in hyperacetylation of core histones. Exposure of cancer cells to HDAC inhibitors is associated with a multitude of molecular and biological effects, ranging from transcriptional control, chromatin plasticity, protein DNA interaction to cellular differentiation, growth arrest and apoptosis. HDAC inhibitors are an exciting new addition to the arsenal of cancer therapeutics^[24,25].

This study focused on the effect of histone deacetylase inhibitor, trichostatin A (TSA) on the survival of QBC939, KMBC, OZ and Mz-ChA-I cell lines and determined if TSA can be used in the treatment of biliary tract cancer. Our results show that TSA could shorten the survival time of QBC939, KMBC, OZ and Mz-ChA-I cell lines *in vitro* in a dose-dependent manner. We have successfully established a nude mouse model of transplanted gallbladder carcinoma. The growth of biliary tract cancer was inhibited in the mice after treatment with TSA. Our results show that TSA could shorten the survival time of gallbladder carcinoma and cholangiocarcinoma cell lines *in vivo* and *in vitro*, indicating that TSA is a potential drug for the treatment of biliary tract cancer. It was reported that HDACIs MS-275, NVP-LBH589 and NVP-LAQ824 can effectively inhibit the growth of human biliary tract cancer cells^[26,27]. However, early diagnosis and treatment of biliary tract cancer are still difficult^[29-34]. In order to improve its diagnosis and treatment, further study on its epidemiology, clinicopathology and molecular biology is needed.

COMMENTS

Background

Biliary tract cancer, consisting of gallbladder carcinoma and cholangiocarcinoma, presents many challenges to physicians. At present, treatment of biliary tract cancer is difficult. This study focused on the effect of trichostatin A (TSA) on the proliferation of biliary tract cancer cells.

Research frontiers

Cancer is a disease resulting from both genetic and epigenetic changes. There are many important epigenetic changes in its early carcinogenesis. It has been recently shown that histone acetylation/deacetylation is closely related with tumorigenesis. Acetylation of histone plays a very important but incompletely understood role in genetic regulation. Histone deacetylase inhibitors could markedly inhibit the growth of tumor cells.

Innovations and breakthroughs

TSA can inhibit the growth of cholangiocarcinoma and gallbladder carcinoma cell lines *in vitro* and *in vivo*.

Applications

TSA can be used as a potential drug for the treatment of biliary tract cancer.

Peer review

This manuscript describes the effect of histone deacetylase inhibitor on the proliferation of four biliary tract cancer cell lines. The study was well designed, and had novel findings. The conclusion is of clinical value.

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