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Original Article

Effect of histone deacetylase on prostate carcinoma

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Abstract: Commonly occurred in aged males, the incidence of prostate carcinoma is increasing by years. Histone deacetylase (HDACs) as one key enzyme in regulating gene transcription has been found to be related with cancer occurrence. Trichostatin A (TSA) is one HDAC inhibitor for suppressing tumor growth. This study thus treated prostate carcinoma cell line PC3 with TSA, to analyze the effect of HDAC on the occurrence and progression of HDAC. PC3 cells were treated with gradient concentrations of TSA. MTT assay was employed to detect the proliferation of PC3 cells, while flow cytometry was used to detect the cell apoptosis and cell cycle. Apoptotic proteins including caspase-3, caspase-9 and bcl-2 were further quantified by Western blotting. MTT assays showed a dose- and time-dependent manner of TSA in inhibiting PC3 cell proliferation. Most of PC3 cells were arrested at G1 phase after treating with TSA. The apoptotic ratio of cells was also elevated by higher concentrations of drugs. Apoptotic proteins including caspase-3, caspase-9 and bcl-2 were all up-regulated by TSA. HDAC inhibitor can effectively suppress the proliferation of prostate carcinoma cells, which can be arrested at G1 phase. The elevated apoptotic ratio was caused by up-regulation of apoptosis-related proteins caspase-3, caspase-9 and bcl-2, in both dose- and time-dependent manners.

Keywords: Histone deacetylase, HDAC inhibitor, prostate carcinoma

Introduction

Commonly occurred in aged males, prostate carcinoma is the leading malignant tumor in Western countries [1]. The occurrence of cancer is now widely believed to be related with abnormal epigenetic regulation. Histones, including H2A, H2B, H3 and H4, participate in almost all DNA expression processes including replication, transcription, and repair via ubiquitination, phosphorylation, methylation and acetylation. The acetylation and de-acetylation are two hall marks for facilitating and inhibiting of transcription, as one antagonistic pair of factors [2]. The acetylation of histones is dynamically regulated by acetyl transferase and histone deacetylase (HDACs), both of which play crucial roles in the pathogenesis of tumors. HDACs have been found to exist in various tumor cells, in which they can inhibit the expression of cell cycle inhibitors [3]. Trichostatin A (TSA) is one specific HDAC inhibitor for inducing tumor differentiation and thus suppressing tumor growth. Previous study found that low dosage of TSA will not interfere with normal cell functions or cause cytotoxicity for normal cells [4]. This study thus selected prostate carcinoma cell line PC3, on which different concentrations of TSA will be applied for analyzing its effect on proliferation, apoptosis and cell cycle regulation, in an attempt to investigate the role of HDAC on progression of prostate cancer.

Materials and methods

Cell culture

Prostate carcinoma cell line PC3 (Shanghai Institute of Cell Biology, China) were cultivated in RPMI1640 medium (Sigma, US) in humidified chamber at 37°C with 5% CO_2. Cells were passed every 3~5 days, and were digested in 0.25% trypsin. Cell suspensions were then seeded into 96-well plate for further use.

MTT assay

Cells at log-phase were adjusted to 1.0 × 105 per mL and were seeded into 96-well plate.

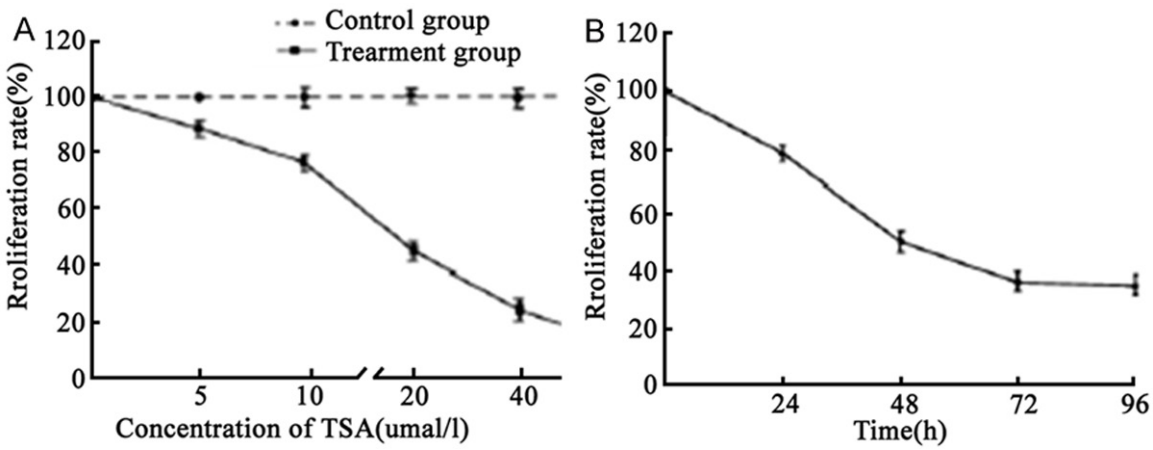

Figure 1. Proliferation rate of PC3 cells under different concentration (left) or time periods (right) of TSA treatment.

Table 1. Cell cycle and apoptotic ratios of PC3 cells

Group	Cell cycle ratio (%)			Apoptotic ratio (%)
	G1	S	G2	
Control	57.19±2.26	25.41±1.85	10.07±1.14	3.97±1.56
TSA				
5 μM	60.12±1.02*	24.68±1.82*	10.58±1.23*	20.12±1.01*
10 μM	63.44±1.21$^{*,\#}$	23.56±1.64$^{*,\#}$	11.26±1.43$^{*,\#}$	22.17±1.25$^{*,\#}$
20 μM	65.05±1.34$^{*,\#,\&}$	21.23±1.12$^{*,\#,\&}$	12.08±1.44$^{*,\#,\&}$	23.89±1.43$^{*,\#,\&}$
40 μM	67.89±1.43$^{*,\#,\&,@}$	19.89±1.03$^{*,\#,\&,@}$	14.15±1.76$^{*,\#,\&,@}$	26.12±1.55$^{*,\#,\&,@}$

Note: *P<0.05 compared to control group; $^{\#}$P<0.05 compared to 5 μM TSA group; $^{\&}$P<0.05 compared to 10 μM group; $^{@}$P<0.05 compared to 20 μM group.

Table 2. Apoptotic proteins expression levels

Protein (group)	2 hours TSA	4 hours TSA
Caspase-3		
0	16.23±0.97	10.50±0.42*
10 μM	13.74±0.92$^{\#}$	9.67±0.57$^{*,\#}$
20 μM	9.67±1.2$^{\#,\&}$	6.81±0.66$^{*,\#,\&}$
40 μM	6.25±1.34$^{\#,\&,@}$	4.28±0.78$^{*,\#,\&,@}$
Caspase-9		
0	14.12±0.82	11.47±0.51*
10 μM	12.21±0.81$^{\#}$	8.62±0.48$^{*,\#}$
20 μM	9.23±1.18$^{\#,\&}$	7.57±0.32$^{*,\#,\&}$
40 μM	5.64±1.22$^{\#,\&,@}$	3.51±0.21$^{*,\#,\&,@}$
Bcl-2		
0	15.22±0.81	11.12±0.45*
10 μM	11.53±0.73$^{\#}$	9.52±0.44$^{*,\#}$
20 μM	8.75±0.45$^{\#,\&}$	6.27±0.41$^{*,\#,\&}$
40 μM	6.08±0.33$^{\#,\&,@}$	3.79±0.17$^{*,\#,\&,@}$

Note: *P<0.05 compared to 2-hour TSA group; $^{\#}$P<0.05 compared to no TSA group; $^{\&}$P<0.05 compared to 10 μM group; $^{@}$P<0.05 compared to 20 μM group.

Gradient concentrations of TSA (0, 5, 10, 20 and 40 μM) were added in each group. At different time points (24, 48, 72 and 96 hours after drug treatment), 5 mg/mL MTT (Huamei, China) were added for continuous incubation. Cells were then centrifuged at 1000 g for 5 min, with supernatants being discarded. 0.1 mL DMSO was mixed with cells for reading absorbance (A) value on a microplate reader. The cell proliferation rate (%) was calculated as: $(A_{Experiment}-A_{Blank})/(A_{Control}-A_{Blank}) \times 100\%$.

Flow cytometry

PC3 cells at log-phase were adjusted to 1.0×10^5 per mL and were seeded into 96-well plate. TSA were added as those in MTT assay. Cells were collected by centrifugation at 1000 g for 5 min, washed by PBS and were fixed in absolute ethanol overnight. PI dye was then added to stain cells, which were detected by flow cytometry for apoptotic cell ratio.

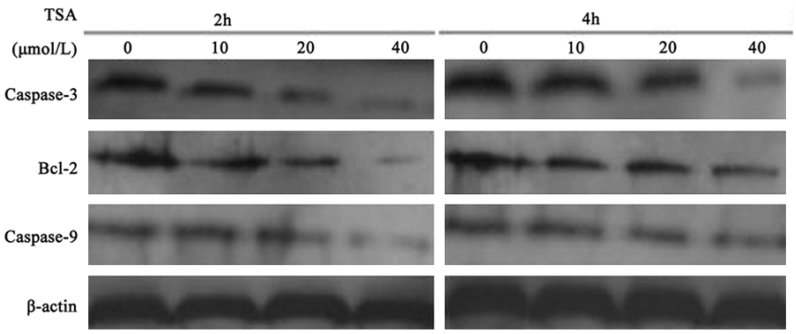

Figure 2. Apoptotic proteins expression.

Western blotting

Log-phased cells were adjusted to 1.0 × 10^5 per mL and were seeded into 96-well plate. TSA were added as those in MTT assay. Cells were collected by centrifugation at 1000 g for 5 min, and were lysed on ice. Proteins were extracted by centrifugation and were denatured. 60 μg proteins were separated by SDS-PAGE and were transferred to PVDF membrane. The membrane was firstly blocked in 5% defatted milk powder at room temperature for 1 hour, followed by primary antibody incubation overnight. Secondary antibody with horseradish peroxidase conjugated was then applied for 1-hour incubation at room temperature, followed by development and exposure.

Statistical analysis

SPSS 17.0 software was used to process all collected data, which were presented as mean ± standard deviation (SD). Measurement data were compared by student t-test, while enumeration data were analyzed by chi-square test. A statistical significance was defined when $P<0.05$.

Results

PC3 cell proliferation

Under different concentrations of TSA, PC3 cells' proliferation rate was gradually suppressed (100%, 88.4%, 76.3%, 44.7% and 24.4% against the control group for 0, 5, 10, 20 and 40 μM drugs, respectively; **Figure 1A**). A time-dependent manner was also revealed, as 24, 48, 72 and 96 hours of 40 μM TSA treatment depressed the proliferation rate to 78.4%, 44.7%, 35.6% and 33.4%, respectively (**Figure 1B**).

Cell cycle and apoptosis

Using flow cytometry assay, we found the arrest of PC3 cells at G1 phase after TSA treatment (57.19%±2.26%, 60.12%±1.02%, 63.44%±1.21%, 65.05%±1.34% and 67.89%±1.43% for 0, 5, 10, 20 and 40 μM drugs, respectively; **Table 1**). With elevated TSA concentration, the cell cycle arrest ratio was significantly increased ($P<0.05$), suggesting the inhibition of TSA on cellular DNA replication and G1 stage arrest.

The total apoptotic ratio of PC3 cells was 3.97%±1.56%, 20.12%±1.01%, 20.12%±1.01%, 22.17%±1.25% and 26.12%±1.55% for 0, 5, 10, 20 and 40 μM drugs, respectively ($P<0.05$, Table The elevated concentration of TSA also potentiated cell apoptosis.

Apoptotic proteins expressions

We further utilized Western blotting to detect the expression level of apoptotic proteins including caspase-3, caspase-9 and bcl-2 in PC3 cells. Results showed that after TSA treatment, expression levels of those proteins were all decreased in both dose- and time-dependent manners ($P<0.05$, **Table 2** and **Figure 2**).

Discussion

The acetylation of histones is one critical mechanism underlying the pathogenesis of malignant tumors. Inside the body there are both histone acetyltransferase (HATs) and HDACs, whose dynamic balance determines the activity of histone acetyl in an antagonistic manner [5]. In the process, the abnormal binding of HDAC with gene promoter may inhibit the transcription of tumor suppressor genes, thus facilitating the occurrence of cancer.

Studies have revealed the absence of RARβ in various solid tumors. In prostate carcinoma cells, the negative expression of RARβ2 and acetylation of histones H3 and H4 exist. The RARβ2 expression can be induced by HDACs inhibitors [6]. Pathological examination on prostate cancer tissues found elevated mRNA

and protein levels of HDACs, suggesting their participation in both occurrence and progression of prostate cancer [7]. Prostate cancer is one hormone-dependent tumor, with potent nuclear expression of HDAC1, 2 and 3 compared to normal epithelial cells, which only had slight expression of those proteins [8]. The expression level of nuclear HDAC1 is positively correlated with the malignancy of tumors, as more advanced tumors had higher expression levels [9, 10]. The higher expression level of HDAC1 and 2 thus suggested advanced Gleason stage with higher proliferation potency and shorter survival window. The specific inhibition of HDAC1 but not HDAC2 can increase the expression of E-cadherin for suppressing tumor infiltration, making HDAC2 as one independent factor for tumor prognosis [11]. As one effective HDACs inhibitor, TSA can inhibit the deacetylation of histones at Ki values of 3.4 nM TSA and RA have been reported to induce the differentiation of primary cells into metamyelocytes and neutrophils in AML patients with positive AML1-ETO [13].

In this study, we treated prostate carcinoma cells PC3 with different concentrations of TSA. MTT assay showed decreased cell proliferation rate (100%, 88.4%, 76.3%, 44.7% and 24.4% against the control group for 0, 5, 10, 20 and 40 μM drugs, respectively), and time-dependent cell growth inhibition (78.4%, 44.7%, 35.6% and 33.4% against the control group for 0, 5, 10, 20 and 40 μM drugs, respectively). These results suggested a negative correlation between PC3 cells' proliferation rate and TSA concentration or treatment time. Past study has revealed the inhibition on HDACi by TSA, for further inducing alternations of various tumor cells, including growth arrest, differentiation induction, and apoptosis. Such time- and dosage- dependency of TSA on PC3 cell growth in previous reports [14] were consistent with our results.

Flow cytometry results showed cell cycle arrest at G1 phase after applying TSA. Further elevated concentrations of TSA caused more cells to be arrested, suggesting the role of TSA in impeding DNA replication. Furthermore, the apoptotic ratio was also elevated by higher concentration of TSA. These data collectively suggest the transition of cells with HDAC1 deficiency from mitosis to programmed cell death directed by caspase-3. Therefore, the knockout of HDAC1 gene may inhibit tumor growth [15, 16]. Previous studies using HDAC1 inhibitor also found the arrest of tumor cells at G1 or G2/M phase, thus suppressing both proliferation and differentiation of tumor cells, and the formation of solid tumors [17, 18].

This study utilized Western blotting to detect the expression level of apoptotic proteins including caspase-3, caspase-9 and bcl-2. Results showed more potent expression of those proteins after TSA application in both time- and dosage-dependent manners. Caspase protein family participates in regulating signal pathway for body cell's apoptosis for initiating the cellular response of apoptosis signal. Multiple proteins are involved in the signaling pathway of apoptosis. As the terminal factor of caspase cascade reaction, caspase-3 is the key enzyme for inducing apoptosis after being stimulated by multiple factors [19]. Moreover, mitochondria also plays a role in cell apoptosis by altering the reductive-oxidative electrical potential, for increasing the related of cytochrome c into cytoplasm and initiating caspase 3 or caspase 9 [20]. Our results collectively suggested the enhancement of TSA on expression of apoptotic proteins in PC3 cells for inducing programmed cell death of tumor cells.

In summary, HDACs inhibitor TSA can effectively the proliferation rate of prostate carcinoma cells by arresting cells at G1 phase and increasing apoptotic ratio, which is caused by elevate apoptotic proteins including caspase-3, caspase-9 and bcl-2. Those inhibitor effects were of both drug dosage- and time-dependency, although detailed pro-apoptotic signaling pathways require further studies.

Disclosure of conflict of interest

None.

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