available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Histone deacetylase inhibitors and transforming growth factor-β induce 15-hydroxyprostaglandin dehydrogenase expression in human lung adenocarcinoma cells

Min Tong, Yunfei Ding, Hsin-Hsiung Tai *

Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082, United States

ARTICLE INFO

Article history:
Received 24 March 2006
Accepted 7 June 2006

Keywords:
15-PGDH
COX-2
HDAC Inhibitors
TGF-β
Wnt
Tumor suppressor

ABSTRACT

Histone deacetylase (HDAC) inhibitors have been actively exploited as potential anticancer agents. To identify gene targets of HDAC inhibitors, we found that HDAC inhibitors such as sodium butyrate, scriptaid, apicidin and oxamflatin induced the expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), a potential cyclooxygenase-2 (COX-2) antagonist and tumor suppressor, in a time and concentration dependent manner in A549 and H1435 lung adenocarcinoma cells. Detailed analyses indicated that HDAC inhibitors activated the 15-PGDH promoter-luciferase reporter construct in transfected A549 cells. A representative HDAC inhibitor, scriptaid, and its negative structural analog control, nullscript, were further evaluated at the chromatin level. Scriptaid but not nullscript induced a significant accumulation of acetylated histones H3 and H4 which were associated with the 15-PGDH promoter as determined by chromatin immunoprecipitation assay. Transforming growth factor-β1 (TGF-β1) also induced the expression of 15-PGDH in a time and concentration dependent manner in A549 and H1435 cells. Induction of 15-PGDH expression by TGF-β1 was synergistically stimulated by the addition of Wnt3A which was inactive by itself. However, combination of TGF-β and an HDAC inhibitor, scriptaid, only resulted in an additive effect. Together, our results indicate that 15-PGDH is one of the target genes that HDAC inhibitors and TGF-β may induce to exhibit tumor suppressive effects.

1. Introduction

15-Hydroxyprostaglandin dehydrogenase (15-PGDH) is a NAD^+-dependent enzyme that catalyzes the oxidation of prostaglandins and lipoxins at 15(S)-hydroxyl group to 15-keto-metabolites which exhibited greatly reduced biological activities [1]. This enzyme is widely expressed in mammalian tissues and primarily functions to control tissue and circulating levels of prostaglandins and lipoxins. Lung is particularly enriched in this enzyme for rapid removal of circulating vasoactive prostaglandins in order to cytoprotect the cardiovascular system [2]. In addition to vasoactivity of prostaglandins, prostaglandin E_2 (PGE_2) has been shown to stimulate cell proliferation, angiogenesis, cell migration, invasion, and resistance of apoptosis [3,4]; prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been demonstrated to induce cell rounding and the formation of actin stress fibers [5]; thromboxane A_2 (TXA_2) has been found to mediate endothelial migration and angiogenesis [6]. These cellular effects of prostaglandins are very much related to cell transformation and carcinogenesis. Increased levels of these prostaglandins have been reported in multiple types of tumors and have been attributed to an enhanced expression of

* *Corresponding author*. Tel.: +1 859 257 1837; fax: +1 859 257 7585.
E-mail address: htai1@uky.edu (H.-H. Tai).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.06.004

a prostaglandin synthetic enzyme, cyclooxygenase-2 (COX-2), in tumors [7]. However, tissue and circulating levels of prostaglandins are regulated not only by synthetic enzymes, but also by catabolic enzymes. It has been shown that the expression of 15-PGDH is down-regulated in lung, colon and other tumors [8–10] as opposed to COX-2 being up-regulated in most tumors [7]. The consequence of this reciprocal expression of the synthetic enzyme and the catabolic enzyme is the amplified tissue and cellular levels of prostaglandins which are conducive to carcinogenesis. Therefore, a rational approach to cancer therapy would be to inhibit the expression and/or activity of COX-2 or to stimulate the expression of 15-PGDH by chemopreventive agents. The former approach has been intensively studied and well documented [11].

A variety of chemopreventive agents have been tested for their anticancer effects. Histone deacetylase (HDAC) inhibitors have emerged recently as promising chemopreventive agents because they can cause cell growth arrest, differentiation, and/or apoptosis in many types of cancer cells and can prevent the formation of tumors in animal models [12,13]. HDAC inhibitors have been shown to induce the expression of a small number of tumor suppressive genes, such as $p21^{WAF1}$ [14], transforming growth factor-β (TGF-β) receptors [15,16] and growth-differentiation factor 11, a member of the TGF-β superfamily [17]. TGF-β and Wnt ligands are known to regulate cell fate determination [17]. These two extracellular factors induce their effects through two distinct signaling cascades. The TGF-β signaling is initiated by type I and type II receptors-activated phosphorylation and association of Smad proteins which translocate into the nucleus where they interact with DNA binding partners including histone deacetylase to regulate gene expression [18]. The Wnt signaling is activated by ligand initiated and receptor-mediated inhibition of glycogen synthase kinase-3 that allows unphosphorylated β-catenin to translocate into the nucleus where it associates with the lymphoid enhancer binding factor/T-cell factor (LEF/TCF) transcription factors and activates Wnt target genes [18]. Several studies have shown that cooperation between TGF-β and Wnt signaling pathways plays a significant role in regulating differentiation and cell fate determination [19,20].

Stimulation of the expression of 15-PGDH in mammalian cells by various agents has been described in several reports. Phorbol 12-myristate 13-acetate (PMA) was shown to induce the expression of 15-PGDH in HL-60 cells [21] and HEL cells [22]. 1,25-Dihydroxyvitamin D_3 was found to stimulate the induction of the enzyme in human neonatal monocytes [23] and in human prostate cancer cells [24]. Androgens were shown to induce the expression of the enzyme in human prostate cancer cells [25] and the induction of the enzyme was synergistically stimulated by IL-6 and forskolin [26]. Dexamethasone and other glucocorticoids were demonstrated to induce the enzyme in human lung cancer cells [27] and HEL cells [28]. Very recently, TGF-β1 was reported to stimulate the expression of the enzyme in colon cancer cells [28]. Among these stimulants, TGF-β1 [18], glucocorticoids [28] and 1,25-dihydroxyvitamin D_3 [29] have been shown to have anti-proliferative and prodifferentiation effects. Androgens were also reported to enhance TGF-β-induced apoptosis in human prostate cancer cells [30].

In this report, we show that HDAC inhibitors and TGF-β1 induces the expression of 15-PGDH, a potential tumor suppressive gene [8–10], in A549 human lung adenocarcinoma cells. Induction by TGF-β1 is synergistically stimulated by the addition of Wnt3A. These findings support the notion that HDAC inhibitors and TGF-β1 may exhibit their tumor suppressive effects by stimulating, at least in part, the expression of 15-PGDH.

2. Materials and methods

2.1. Materials

Human kidney cell line AD293 and human non-small cell lung carcinoma (NSCLC) cell lines A549 and H1435 were obtained from the American Type Culture Collection. NSCLC cell lines H157 and H460 were kindly provided by Dr. John Yannelli and Dr. Kyungbo Kim, respectively of the University of Kentucky. Sodium butyrate (NaBT), sodium dodecyl sulfate (SDS), dithiothreitol (DTT), leupeptin, soybean trypsin inhibitor, phenylmethylsulfonyl fluoride (PMSF), and RPMI 1640 were obtained from Sigma Chemical Co. TGF-β1 and Wnt3A were supplied by the R & D System. Apicidin, oxamflatin, scriptaid, MS-275 and trichostatin A (TSA) were purchased from Alexis Biochemicals. Nullscript was supplied by BIOMOL. SB 431542 was obtained from Tocris Bioscience. Polyvinylidene fluoride (PVDF) membrane was obtained from the Millipore Corp. Electro-chemiluminescence (ECL^+) plus Western Blotting Detection System RPN 2132 was purchased from Amersham Biosciences. A proximal 15-PGDH promoter-luciferase reporter construct (388-bp) was kindly provided by Dr. B. Gellersen of the University of Hamburg, Germany [31]. This construct was modified by subcloning the promoter sequence into the pGL2-enhancer vector for lower background activity. Rabbit antiserum against human placental 15-PGDH was generated as described previously [32]. Rabbit antiserum against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was generated as reported previously from our laboratory [33]. Hyperacetylated H3 and H4 antibodies and chromatin immunoprecipitation assay kit were obtained from Upstate Biotechnology. Horseradish peroxidase (HRP)-labeled goat anti mouse IgG was supplied by Transduction Laboratories. HRP-labeled goat anti rabbit IgG was from Zymed. PGE_2 was supplied by Cayman Chemical Co. 15(S)-[15-^{3}H] PGE_2 was prepared according to a previously published procedure [34]. Other reagents were obtained from the best commercial sources.

2.2. Cell culture

A549 cells and other human lung adenocarcinoma cells were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (FBS) and 1 mg/100 ml gentamicin in a humidified atmosphere containing 5% CO_2 at 37 °C. Cells were plated in 12-well plates (2 ml/well) at about 10^5 cells/well in duplicate and grown for 24 h before the cells were starved for 24 h in a medium containing 0.1% FBS. The cells were treated with the stimulant for the indicated length of time as indicated in the figure legends. Cells were then washed once with PBS

buffer and sonicated in 50 mM Tris–HCl, pH 8.0 containing 0.1 mM DTT before assaying for enzyme activity or cells were lysed in cell lysis buffer (20 mM Tris–HCl, pH 8.0 containing 137 mM NaCl, 1 mM $CaCl_2$, 10% glycerol, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 100 mM 4-(2-aminoethy)benzensulfonyl fluoride, and mixed protease inhibitors) and then spun down to remove debris. The supernatant was subjected to SDS/PAGE and Western blot analysis.

2.3. Promoter transfection and luciferase assay

The proximal (388 bp) 15-PGDH promoter-luciferase reporter construct was modified as described above. A549 or AD293 cells were split to 12-well plates (1 ml) at about 2×10^5 cells/well and cultured overnight in RPMI-1640 medium supplemented with 10% fetal calf serum (FBS) and 1 mg/100 ml gentamicin. The cells were transfected with the 15-PGDH promoter-luciferase reporter construct or the pGL2-enhancer vector at the concentration of 1 μg/well using Lipofectamine 2000 according to the manufacturer's instructions. A luciferase reporter vector was also transfected as a control. Briefly, 1 μg DNA and 1 μl Lipofectamine 2000 reagent was added separately into 50 μl OpTi-MEMI medium for 5 min at room temperature, then they were combined and mixed gently by pipetting up and down for several times. The DNA complex was then left at room temperature for about 20 min. Serum- and antibiotics-free RPMI 1640 medium (0.6 ml) was added to the DNA complex. Following transfection the cells were incubated in the same medium for 5 h before incubation in the RPMI-1640 medium containing 10% FBS for 18 h. Various HDAC inhibitors were respectively added and incubated for additional 48 h. The cells were then washed, lysed completely in lysis buffer (0.1 M K_2HPO_4/KH_2PO_4, pH 7.8, 0.5% Triton X-100 and 1 mM DTT) and spun briefly in a microcentrifuge tube to pellet the cell debris. The luciferase values were determined by adding the lysate supernatant to a Titertek fluoroplate and using 20 mM Tricine (pH 7.8), 2.67 mM magnesium sulfate, 0.1 mM EDTA, 1.07 mM magnesium carbonate, 33.3 mM DTT, 270 μM coenzyme A, 470 μM beetle luciferin, and 530 μM ATP as a substrate solution in a Titertek Luminoscan.

2.4. Chromatin immunoprecipitation (ChIP) assay

A549 cells were cultured with scriptaid (1 μM) for 4, 12 or 24 h, or with nullscript (1 μM) and scriptaid (1 μM) for 4 h. ChIP assay using acetylated histone H3 or H4 was carried out according to the acetylated histone H3 ChIP kit manufacturer's instructions. Briefly, histone/DNA complexes were fixed with 10% formaldehyde for 10 min, and the treated cells scraped from plates were lysed in lysis buffer. The DNA was sheared by 2×10 s of a probe sonicator set at 20% power. An aliquot of the chromatin preparation was set aside and designated as the input fraction. The histone/DNA complexes were precleared and incubated with 2 μg of antiacetylated histone H3 or H4 antibody. The overnight immunoprecipitate was harvested with salmon sperm DNA/protein A-agarose beads. The protein was digested with proteinase K and the DNA was recovered by phenol/chloroform extraction and ethanol precipitation followed by analysis by the PCR. The PCR reaction was: 94 °C for 3 min followed by 30 cycles of 94 °C for 20 s, 59 °C for 30 s, 72 °C for 30 s, and final extension at 72 °C for 10 min. Primers for the 15-PGDH promoter were sense: 5′-GGTAGGCTACCAGCGGCTCT-3′ and antisense: 5′-GTTCCCATCTCGTAATCAGTGG-3′. The PCR product was 280-bp in length. Five microliters of the reaction mixture was separated by electrophoresis on a 3% agarose gel and the PCR product was visualized under UV using ethidium bromide staining.

2.5. 15-PGDH assay

15-PGDH was routinely assayed by measuring the transfer of tritium from 15(*S*)-[15-^{3}H]-PGE_2 to glutamate by coupling 15-PGDH with glutamate dehydrogenase as described previously [34]. Briefly, the reaction mixture contained: NH_4Cl, 5 μmol; α-keto-glutarate, 1 μmol; NAD^+, 1 μmol; 15(*S*)-[15-^{3}H]PGE_2, 1 nmol, 30,000 cpm; glutamate dehydrogenase, 100 μg and crude enzyme extract in a final volume of 1 ml of 50 mM Tris–HCl, pH 7.5. The reaction was allowed to continue for 10 min at 37 °C and terminated by the addition of 0.3 ml of 10% aqueous charcoal suspension. The radioactivity in the supernatant after centrifugation ($1000 \times g$, 5 min) was determined by liquid scintillation counting. Calculation of the amount of PGE_2 oxidized was based on the assumption that no kinetic isotope effect was involved in the oxidation of 15(*S*)-hydroxyl group of 15(*S*)-[15-^{3}H]PGE_2 as a substrate.

2.6. Western blotting

To determine the expression of various proteins in the lung cancer cells following various stimulations, Western blot analysis was performed as described previously [25]. Briefly, cells were harvested by trypsinization and lysed in lysed buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA, 1 mM DTT, 0.1% SDS, 1% Nonidet P-40, 1 μg/ml leupeptin and soybean trypsin inhibitor, 0.5 mM PMSF) for 30 min on ice. Approximately 50–150 μg of protein extracts were loaded on a 12% polyacrylamide gel. Next, the separated proteins were electroblotted from the gel onto PVDF membrane and then blocked with a blocking buffer (5% non-fat dry milk in $1 \times$ TBST, i.e. 20 mM Tris–HCl, pH 7.6 containing 0.8% NaCl and 0.1% Tween-20) at room temperature for 1 h. The membrane was incubated with the primary antibodies in blocking buffer, followed by incubation with HRP-labeled second antibodies. Bands were visualized using the ECL Western blotting system.

2.7. Statistical analysis

Each enzyme assay was performed twice. Each experiment was repeated at least three times ($n = 3$). The data were expressed as the mean ± S.E. Statistical significance was assessed by Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

3. Results

A variety of HDAC inhibitors were examined for their effect on the induction of 15-PGDH expression in NSCLC cells. These inhibitors stimulated the expression and activity of 15-PGDH

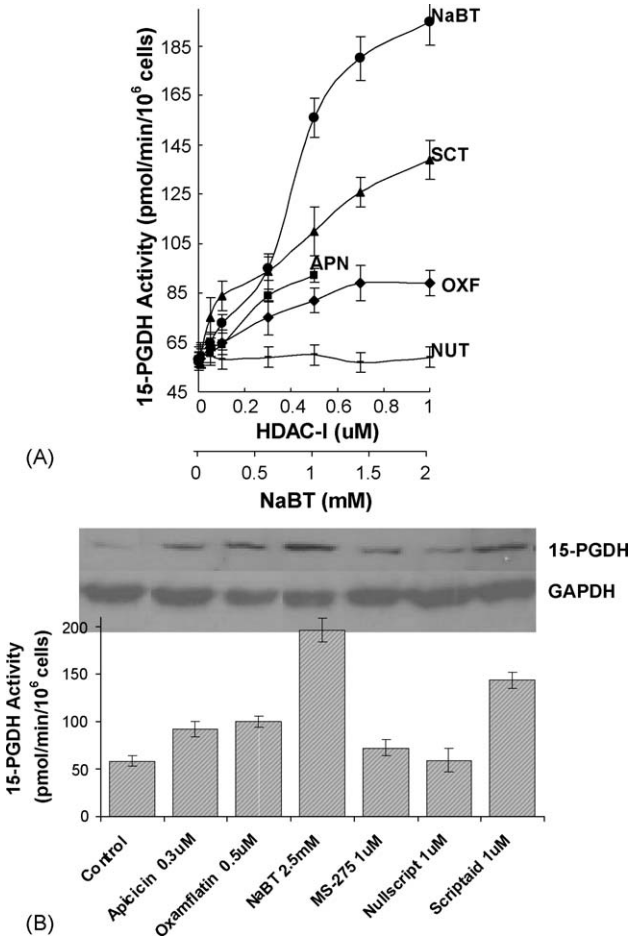

Fig. 1 – Concentration dependent induction of 15-PGDH expression by HDAC inhibitors (HDAC-Is) in A549 cells. (A) A549 cells were treated with an increasing concentration of HDAC-Is as indicated for 48 h. Cells were washed, lysed and assayed for 15-PGDH activity as described in Section 2. (●) Sodium butyrate (NaBT); (▲) scriptaid (SCT); (■) apicidin (APN); (◆) oxamflatin (OXF); (—) nullscript (NUT). (B) Induction of 15-PGDH expression by various HDAC-Is at their optimal concentrations. A549 cells were treated with the indicated concentrations of HDAC-I and assayed for 15-PGDH activity (lower panel) by enzyme assay and for immunoreactivity (upper panel) by Western blot as described in Section 2.

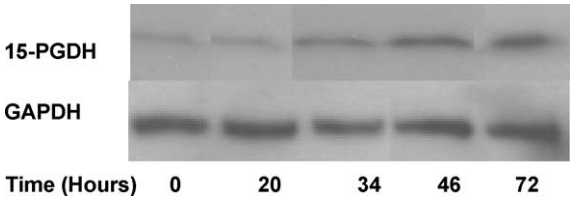

Fig. 2 – Time dependent induction of 15-PGDH expression by scriptaid. A549 cells were treated with scriptaid (1 μM) for the indicated length of time. Cells were washed, sonicated and assayed for 15-PGDH activity (upper panel) and for immunoreactivity (lower panel) as described in Section 2.

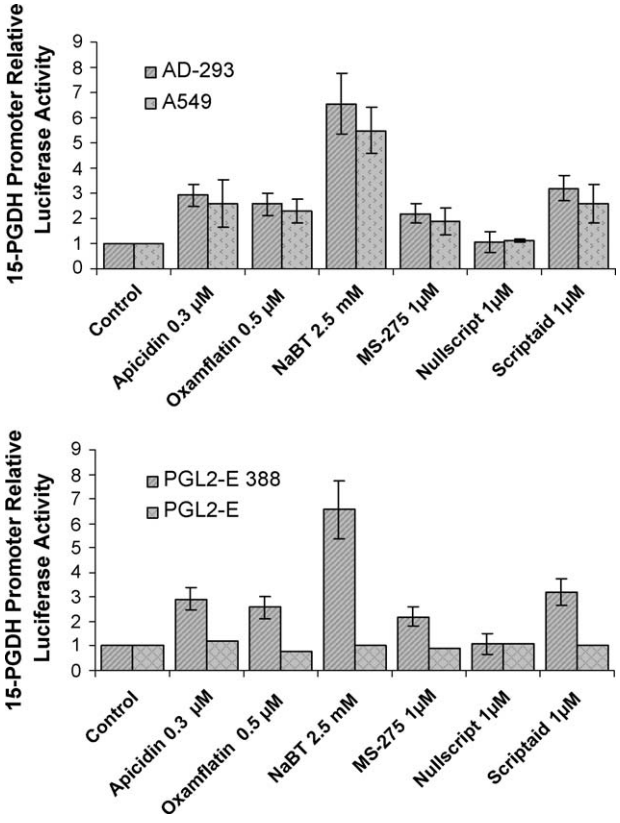

Fig. 3 – Activation of the 15-PGDH promoter by HDAC-Is. A549 or AD293 cells were transiently transfected with the 388-bp 15-PGDH promoter-luciferase reporter construct (upper panel) or AD293 cells were transiently transfected with the promoter construct or the vector control (lower panel) for 18 h and then various HDAC-Is at the indicated concentrations were added for 48 h. Cells were washed, lysed and assayed for luciferase activity as described in Section 2.

in A549 cells in a dose dependent manner as shown in Fig. 1A. Sodium butyrate appeared to stimulate the expression to a highest degree although it took much higher concentrations to induce the expression. Nullscript which is a known inactive control of scriptaid was not able to stimulate the expression as expected. A summary of various HDAC inhibitors at their optimal concentrations to stimulate the expression and activity of 15-PGDH in A549 cells is shown in Fig. 1B. A similar stimulation of 15-PGDH expression by a HDAC inhibitor, scriptaid, was also observed in H1435 cells (data not shown). Trichostatin A was also examined and found to be very toxic to A549 cells at routinely used concentration of 1 μM. Induction of 15-PGDH expression was also time dependent as demonstrated by the use of scriptaid (Fig. 2). Maximal induction can be achieved in two days following the addition of scriptaid. To confirm that HDAC inhibitors regulates 15-PGDH expression at the transcriptional level, AD293 or A549 cells were transfected with the 388-bp 15-PGDH promoter-luciferase reporter construct followed by treatment with vehicle or with HDAC inhibitors (upper panel) or AD293 cells were transfected with the 388-bp 15-PGDH promoter construct or with the pGL2-

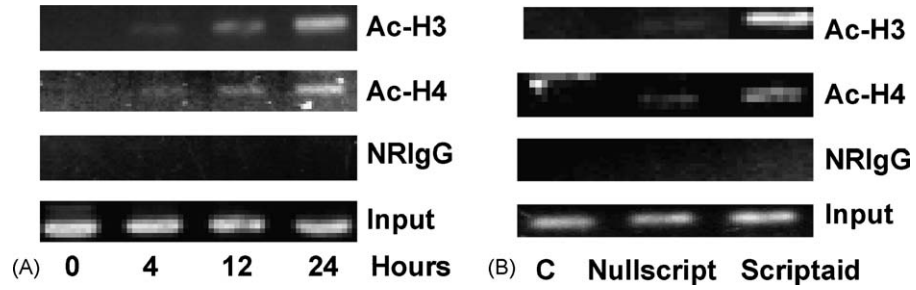

Fig. 4 – Scriptaid induces accumulation of acetylated histones H3 and H4. (A) Time dependency. A549 cells were stimulated with 1 μM scriptaid for the indicated length of time. Crosslinked chromatin from each time point was incubated with acetylated histones H3 (Ac-H3) and H4 (Ac-H4) antibodies or normal rabbit IgG (NRIgG). Immunoprecipitates from each antibody were aliquotted and analyzed by the PCR with primers specific for the 15-PGDH promoter as described in the Section 2. (B) Comparison of scriptaid and nullscript. A549 cells were treated with 1 μM nullscript (N) and 1 μM scriptaid (S) respectively for 4 h, and the DNA–histone complexes were incubated with Ac-H3 and Ac-H4 antibodies or normal rabbit IgG (NRIgG). Immunoprecipitates from each antibody were aliquotted and analyzed by the PCR with primers specific for 15-PGDH promoter as described in Section 2.

enhancer vector (lower panel), and the luciferase activity was determined in cells after treatment with HDAC inhibitors. Fig. 3 shows that the levels of inducibility in response to various HDAC inhibitors in either A549 or AD293 cells are qualitatively comparable to those of 15-PGDH activity demonstrated in Fig. 1. However, the degree of activation of the promoter by HDAC inhibitors was greater than that of the enzyme activity. This is primarily due to relatively high basal enzyme activity before stimulation. Again, a known inactive analog of scriptaid, nullscript, failed to stimulate the induction.

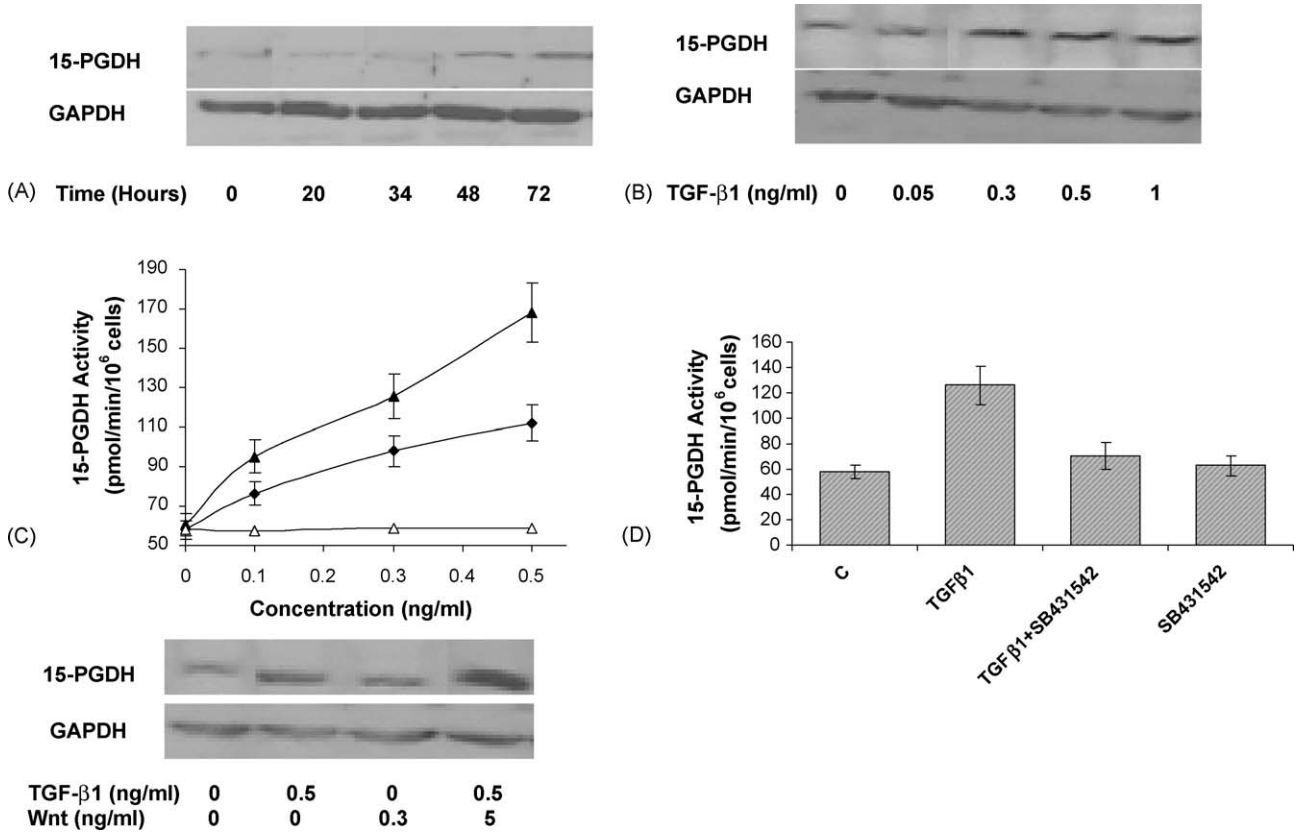

Fig. 5 – Induction of 15-PGDH expression by TGF-β1 in A549 cells. (A) A549 cells were treated with TGF-β1 (0.5 ng/ml) for the indicated length of time. (B) A549 cells were incubated with an increasing concentration of TGF-β1 for 48 h. (C) A549 cells were incubated with an increasing concentration of TGF-β1 (◆) or Wnt3A (△) or both (▲) for 48 h. (D) A549 cells were treated with TGF-β1 (0.5 ng/ml) in the absence and presence of SB-431542 for 48 h. A549 cells were washed, lysed and assayed for 15-PGDH immunoreactivity by Western blot or for 15-PGDH activity by enzyme assay as described in Section 2.

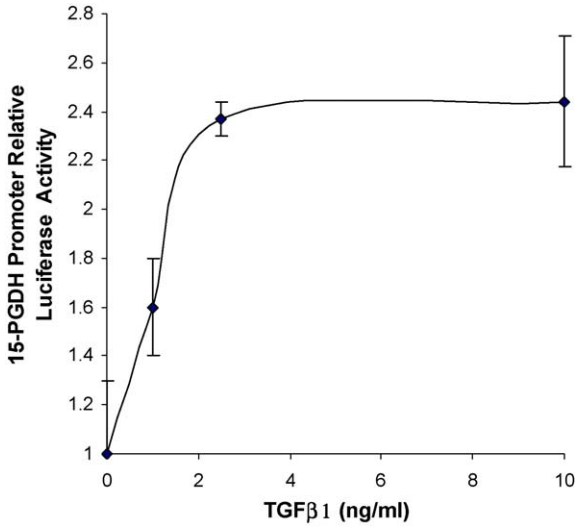

Fig. 6 – Activation of the 15-PGDH promoter by TGF-β1. A549 cells were transiently transfected with the 388-bp 15-PGDH promoter-luciferase reporter construct for 18 h and then TGF-β1 at the indicated concentrations were added for 48 h. Cells were washed, lysed and assayed for luciferase activity as described in Section 2.

Therefore, the proximal 388-bp region appears to contain the minimal promoter needed for the responsiveness of HDAC inhibitors tested.

We next examined the effect of scriptaid and its inactive control nullscript on the acetylation of histones H3 and H4 associated with the 15-PGDH promoter in A549 cells. As shown in Fig. 4A, ChiP assay indicates that following treatment with scriptaid the levels of the PCR product (280 bp) of a fragment of the 15-PGDH promoter that was associated with hyperacetylated histones H3 and H4 precipitated by specific antibodies were increased in a time dependent manner. On the contrary, Fig. 4B shows that the inactive control nullscript did not result in a significant 280-bp fragment after 4 h of treatment as compared to scriptaid. Normal immunoglobulin control showed no specific 280-bp fragment.

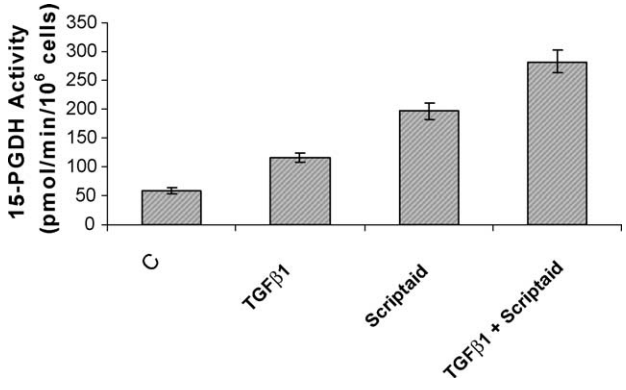

Fig. 7 – Induction of 15-PGDH expression by TGF-β1 and scriptaid. A549 cells were treated either with TGF-β1 (0.5 ng/ml) or with scriptaid (1 μM) or with both for 48 h. Cells were washed, lysed and assayed for 15-PGDH activity as described in Section 2.

TGF-β1 was previously shown to induce the expression of 15-PGDH in colon cancer cells [9]. Similarly, TGF-β1 was found to stimulate 15-PGDH expression in A549 cells in a time (Fig. 5A) and dose (Fig. 5B) dependent manner as shown by Western blot and activity assay (Fig. 5C). Wnt3A did not appear to induce the expression by itself. However, it stimulated synergistically TGF-β1-induced 15-PGDH expression in A549 cells as shown in Fig. 5B and C. Stimulation of 15-PGDH expression by TGF-β1 appeared to be mediated by TGF-β type I receptor since the inhibitor of type I receptor kinase, SB 431542 [35], attenuated the stimulation as demonstrated in Fig. 5D. Similar stimulation of 15-PGDH expression by TGF-β1 as demonstrated by Western blot was also observed in H1435 cells (data not shown). Promoter analysis also indicated that TGF-β1 induced the expression of luciferase activity in a concentration dependent manner as shown in Fig. 6. Combination of TGF-β1 and a HDAC inhibitor, scriptaid, showed an additive effect in inducing 15-PGDH expression in A549 cells as shown in Fig. 7.

4. Discussion

HDAC inhibitors represent a novel and diverse class of agents that act by promoting the acetylation of several histones including histone H3 and H4 leading to uncoiling and relaxation of chromatin structure [12,13]. Chromatin relaxation permits the expression of diverse genes involving those involved in differentiation, apoptosis and cell cycle arrest [40]. Four different subclasses of HDAC inhibitors have been developed including: (a) short-chain fatty acids such as butyric acid; (b) hydroxamic acids such as scriptaid, trichostatin A and oxamflatin; (c) cyclic tetrapeptides such as apicidin; (d) benzamides such as MS-275. We have examined at least one compound from each subclass and have found that compounds from each subclass induced the expression of 15-PGDH. The expression of 15-PGDH was assessed by several means. Firstly, the expression was determined by an enzyme activity assay which was shown to be specific for 15-PGDH since the only tritium in the substrate [^{3}H]-PGE$_2$ was labeled at C-15 and was transferred to glutamate by coupling with glutamate dehydrogenase following oxidation of the substrate. Secondly, the expression was estimated by 15-PGDH specific antibodies using Western blot. Thirdly, the expression was determined at the promoter level using a 388-bp 15-PGDH promoter-luciferase reporter construct. Fourthly, the expression was assessed by analyzing hyperacetylated histones which were associated with the 15-PGDH gene promoter. All different means confirmed that HDAC inhibitors induced 15-PGDH expression. The present study represents the first to establish that HDAC inhibitors induce 15-PGDH expression in A549 cells.

Induction of 15-PGDH by HDAC inhibitors occurred in a time and dose dependent manner. Sodium butyrate, a classical HDAC inhibitor, induced the greatest degree of 15-PGDH expression at mM concentration, a level comparable to that seen in studies of induction of other tumor suppressive genes, such as p21^{WAF1} [36] and 15-lipoxygenase-1 [37]. Other synthetic HDAC inhibitors induced 15-PGDH expression also at concentrations comparable to those for the induction of

$p21^{WAF1}$ [38,39]. Concentrations higher than the indicated concentration of each synthetic inhibitor shown in Fig. 1B appeared to be toxic to A549 cells. Therefore, maximal induction of 15-PGDH expression could not be reached. The need of the HDAC inhibition in inducing 15-PGDH expression can be best illustrated by the use of scriptaid/nullscript pair. Scriptaid, an improved HDAC inhibitor with lower toxicity than trichostatin A [40], was shown to be effective in inducing 15-PGDH expression. Nullscript which is almost identical in structure to scriptaid except for a three-carbon (rather than five-carbon) linker was reported to be inactive in inhibiting HDAC [40]. It also failed to induce 15-PGDH expression further supporting the notion that the inhibition of HDAC is essential to stimulate 15-PGDH expression by HDAC inhibitors.

Mechanisms that are involved in the induction of 15-PGDH expression by HDAC inhibitors remain to be elucidated. We have demonstrated that a promoter of 388-bp was responsive to various HDAC inhibitors examined indicating that this promoter region contains some needed *cis*-acting elements for interacting with the transcriptional factors involved. It has been suggested that different HDAC inhibitors use distinct mechanisms to regulate gene expression. Either a Sp1 site or a NF-Y site (CCAAT box) has been shown to be involved in HDAC inhibitors-induced target gene expression. Sp1 site has been reported to be the target promoter element for HDAC inhibitor activation of $p21^{WAT1}$, tyrosine hydroxylase, and hTERT genes [41–43]. NF-Y site has been demonstrated to mediate the activation of the genes for TGF-β type II receptor, thioredoxin-binding protein 2, and multi-drug resistance 1 by HDAC inhibitors [16,44,45]. Within the 388-bp of the promoter of 15-PGDH, both a Sp1 site (−125/−116) and a NF-Y site (−273/−268) are identified. Whether any of these two sites or other sites is involved in HDAC inhibitor-induced 15-PGDH gene expression remains to be determined. Further experiments using various promoter mutants at these sites may provide the information.

TGF-β plays a critical role in regulating cell proliferation and differentiation [18]. It also possesses tumor suppressive activity in a variety of different human cell types [18]. We have shown earlier that 15-PGDH behaves as a tumor suppressor in A549 cells [8]. It is possible that the tumor suppressive activity of TGF-β may, in part, be due to the induction of 15-PGDH expression by TGF-β. In this report, we demonstrated that TGF-β1 induced 15-PGDH expression in a time and dose dependent manner in A549 cells. Promoter analysis also showed that TGF-β1 stimulated the luciferase activity in a dose dependent manner although the concentrations needed appeared to be higher than those used in stimulating the expression of 15-PGDH in A549 cells. This is probably due to the possibility that the length of the promoter construct used (388 bp) for analysis may not contain all the needed responsive elements. The induction of 15-PGDH expression by TGF-β1 was found to be mediated by type I receptor since its specific kinase inhibitor SB-431542 blocked the induction. We could also show that TGF-β1 stimulated 15-PGDH expression in another NSCLC cell line H-1435. However, we could not show the induction in two other NSCLC cell lines that we examined, namely, H157 and H460. This could be due to the defective or reduced expression of signaling molecules in the TGF-β signal transduction pathway in these cell lines since human cancer cells frequently demonstrate resistance to the normal growth-inhibitory effects of TGF-β [46]. Whether or not this is the potential reason for the non-responsiveness of these two non-responsive lung cancer cell lines remains to be determined. Earlier Markowitz and his co-workers also demonstrated that TGF-β1 induced 15-PGDH expression in some colon cancer cells but not others [9]. One of the defective cell lines became responsive to TGF-β1 after the cells were transfected with type II receptor of TGF-β [9]. Whether we can restore the responsiveness to TGF-β in the two non-responsive lung cancer cell lines still awaits further investigation. The induction by TGF-β1 in A549 cells was amplified by Wnt3A which by itself was inactive. Synergism of TGF-β and Wnt pathways has been demonstrated in the induction of Xenopus homeobox gene twin (Xtwn) [20]. This has been attributed to the formation of an effective transcriptional activation complex consisting of TGF-β relevant Smads and LEF/TCF transcriptional factors and Wnt associated β-catenin and LEF/TCF signaling molecules. We anticipate that such a mechanism may be applicable to the synergistic induction of 15-PGDH expression by TGF-β1 and Wnt3A.

Since HDAC inhibitors are capable of inducing the expression of signaling molecules such as type I and type II receptors of TGF-β in the TGF-β signaling pathway [15,16], we decided to examine if combination of a HDAC inhibitor, scriptaid, and TGF-β might amplify TGF-β-induced 15-PGDH expression. However, we did not find a synergistic activation in the induction. Instead we only observed an additive effect in inducing 15-PGDH expression in A549 cells. This indicates that signaling molecules in the TGF-β pathway might have been expressed in adequate concentration in the resting state. Alternatively, HDAC inhibitors and TGF-β may work through converging mechanisms. For example, chromatin remodeling by the HDAC inhibitors could make SMAD sites accessible to constitutively phosphorylated SMADs, whereas TGF-β could increase the levels of phospho-SMADs. Further experiments are needed to provide more clear-cut answer to the mechanisms of interaction between these two different types of stimulants.

Acknowledgements

This work was supported in part by Grants from the NIH (HL-46296) and the Kentucky Lung Cancer Research Program. We are indebted to Dr. John Yannelli and Dr. Kyungbo Kim of the University of Kentucky for supplying H157 and H460 cell lines, respectively.

REFERENCES

[1] Tai HH, Ensor CM, Tong M, Zhou H, Yan F. Prostaglandin catabolizing enzymes. Prostaglandins Other Lipid Mediat 2002;68/69:483–93.
[2] Ferreira SH, Vane JR. Prostaglandins: their disappearance from and release into the circulation. Nature 1967;216:868–73.
[3] Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN. Modulation of apoptosis and Bc1-2 expression by prostaglandin E_2 in human colon cancer cells. Cancer Res 1998;58:362–6.

[4] Sheng H, Shao J, Washington MK, Dubois RN. Prostaglandin E_2 increases growth and mobility of colorectal carcinoma cells. J Biol Chem 2001;276:18075–81.
[5] Fujino H, Regan JW. FP prostanoid receptor activation of a T-cell factor/β-catenin signaling pathway. J Biol Chem 2001;276:12489–92.
[6] Daniel TO, Liu H, Morrow JD, Creers BL, Marnett LJ. Thromboxane A_2 is a mediator of cyclooxygenate-2-dependent endothelial migration and angiogenesis. Cancer Res 1999;59:4574–7.
[7] Soslo RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, et al. COX-2 is expressed in human pulmonary, colonic and mammary tumors. Cancer 2000;89:2637–45.
[8] Ding Y, Tong M, Liu S, Moscow JA, Tai HH. 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) behaves as a tumor suppressor in lung cancer. Carcinogenesis 2005;26:65–72.
[9] Yan M, Rerko RM, Platzer P, Dawson D, Willis J, Tong M, et al. 15-Hydroxyprostaglandin dehydrogenase, a COX-2 oncogene antagonist is a TGF-β-induced suppressor of human gastrointestinal cancers. Proc Natl Acad Sci USA 2004;101:17468–73.
[10] Backlund MG, Mann JR, Holla VR, Buchanan G, Tai HH, Musiek ES, et al. 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. J Biol Chem 2005;280:3217–23.
[11] Subbaramaiah K, Dannenberg AJ. Cyclooxygenase 2: a molecular target for cancer prevention and treatment. Trends Pharmacol Sci 2003;24:96–102.
[12] Marks PS, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. Nature Rev Cancer 2001;1:194–202.
[13] Somech R, Izrael S, Simon AJ. Histone deacetylase inhibitors—a new tool to treat cancer. Cancer Treatment Rev 2004;30:461–72.
[14] Archer SY, Meng S, Shei A, Hodin RA. $p21^{WAF1}$ is required for butyrate-mediated growth inhibition of human colon cancer cells. Proc Natl Acad Sci USA 1998;95:6791–6.
[15] Ammanamanch S, Brattain MG. Restoration of transforming growth factor-β signaling through receptor RI induction by histone acetylase activity inhibition in breast cancer cells. J Biol Chem 2004;279:32621–5.
[16] Park SH, Lee SR, Kim BC, Cho EA, Patel SP, Kang HB, et al. Transcriptional regulation of the transforming growth factor β type II receptor gene by histone acetyltransferase deacetylase is mediated by NF-Y in human breast cancer cells. J Biol Chem 2002;277:5168–74.
[17] Zhang X, Wharton W, Yuan Z, Tsai SC, Olashaw N, Seto E. Activation of the growth-differentiation factor 11 gene by the histone deacetylase (HDAC) inhibitor trichostatin A and repression by HDAC3. Mol Cell Biol 2004;24:5106–18.
[18] Attisano L, Labbe E. TGF-β and Wnt pathway cross-talk. Cancer Metastatic Rev 2004;23:53–61.
[19] Nishita M, Hashimoto MK, Ogata S, Laurent MN, Uene N, Shibuya H, et al. Interaction between Wnt and TGF-β signaling pathways during formation of Spemann's organizer. Nature 2000;403:781–3.
[20] Labbe E, Letamendia A, Attisano Z. Association of Smads with lymphoid enhancer binding factor 1/T-cell-specific factor mediated cooperative signaling by the transforming growth factor-β and Wnt pathways. Proc Natl Acad Sci USA 2000;97:8358–63.
[21] Xun CQ, Tian ZG, Tai H. Stimulation of de novo synthesis and activity of NAD^+-dependent 15-hydroxyprostglandin dehydrogenase in human promyelocytic (HL-60) cells by phorbol ester. Biochem J 1991;279:553–8.
[22] Xun CQ, Ensor CM, Tai HH. Regulation of synthesis and activity of NAD^+-dependent 15-hydroxyprostglandin dehydrogenase (15-PGDH) by dexamethasone and phorbolester in human erythroleukemia (HEL) cells. Biochem Biophys Res Commun 1991;177:1258–69.
[23] Pichand F, Roux S, Frendo R, Delage-Mourrous R, Maclouf I, de Vernejoul MC, et al. 1,25-Dihydroxyvitamin D_3 induces NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase in human neonatal monocytes. Blood 1997;89:2105–12.
[24] Moreno J, Krishnan AV, Swami S, Nonn L, Peehl DM, Feldman D. Regulation of prostaglandin metabolism by cacitriol attenuated growth stimulation in prostate cancer cells. Cancer Res 2005;65:7917–25.
[25] Tong M, Tai HH. Induction of NAD^+-linked 15-hydroxyprostaglandin dehydrogenase expression by androgens in human prostate cancer cells. Biochem Biophys Res Commun 2000;276:77–81.
[26] Tong M, Tai HH. Synergistic induction of the nicotinamide adenine dinucleotide-linked 15-hydroxyprostaglandin dehydrogenase by an androgen interleukin 6 of forkskolin in human prostate cancer cells. Endocrinology 2004;145:2141–7.
[27] Tong M, Tai HH. 15-Hydroxyprostaglandin dehydrogenase can be induced by dexamethasone and other glucocorticoids at the therapeutic level in A549 human lung adenocarcinoma cells. Arch Biochem Biophys 2005;435:50–5.
[28] Croxtall JD, Flower RJ. Lipocortin1 mediated dexamethasone-induced inhibition of A549 cell growth and eicosanoids release. Proc Natl Acad Sci USA 1992;89:3571–5.
[29] Peehl DL, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. Antiproliferative effects of 1.25-dihydrosyvitamin D_3 on primary cultures of human prostatic cells. Cancer Res 1994;54:805–10.
[30] Bruckheimer EM, Kyprianou N. Dihydrotestosterone enhances transforming growth factor-β-induced apoptosis in hormone-sensitive prostate cancer cells. Endocrinology 2001;142:2419–26.
[31] Greenland KJ, Jantke I, Jenatschke S, Bracken KE, Vinson C, Gellersen B. The human NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase gene promoter is controlled by Ets and activating protein-1 transcriptional factors and progesterone. Endocrinology 2000;141:581–7.
[32] Tai CL, Mak OT, Arai T, Tai HH. Monoclonal antibodies that inhibit the enzyme activity of NAD^+ dependent 15-hydroxyprostaglandin dehydrogenase. Biochem J 1990;267:75–8.
[33] Wu P, Hou WM, Tai HH. NAD^+-dependent glyceraldehydes 3-phosphate dehydrogenase is a novel substrate for cholera and pertussis toxins in human platelet cytosol. Res Commun Cell Biol Biochem 1997;1:21–32.
[34] Tai HH. Enzymatic synthesis of 15(S)-[15-^{3}H] prostaglandins and their use in the development of a simple and sensitive assay for 15-hydroxyprostaglandin dehydrogenase. Biochemistry 1976;15:4586–92.
[35] Laping NJ, Grygielko E, Mathur A, Butter S, Bomberger J, Tweed C, et al. Inhibition of transforming growth factor (TGF)-β1-induced extracellular matrix with a novel inhibitor of the TGF-β type I receptor kinase activity: SB-431542. Mol Pharmacol 2002;62:58–64.
[36] Archer SY, Meng S, Shei A, Hodin RA. p21(WAF1) is required for butyrate-mediated growth inhibition of human colon cancer cells. Proc Natl Acad Sci USA 1998;95:6791–6.
[37] Kamitani H, Tauiura S, Ikawa H, Watanabe T, Kelaukar P, Eling E. Expression of 15-lipoxygenase-1 is regulated by histone acetylation in human colorectal carcinoma. Carcinogenesis 2001;22:187–91.
[38] Richon VM, Sandhoff TW, Rifkind RA, Marks PA. Histone deacetylase inhibitor selectively induces p21 WAF1 expression and gene-associated histone acetylation. Proc Natl Acad Sci USA 2000;97:10014–9.
[39] Kim JS, Lee S, Lee T, Lee YW, Trepel JB. Transcriptional activation of p21 WAF1/c1p1 by apicidin, a novel histone

deacetylase inhibitor. Biochem Biophys Res Commun 2001;281:866–71.
[40] Su GH, Sohn TA, Ryu B, Kern SE. A novel histone deacetylase inhibitor identified by high-throughput transcriptional screening of a compound library. Cancer Res 2000;60:3137–42.
[41] Sowa Y, Orita T, Minamikawa S, Makano K, Mizuno T, Nomura H, et al. Histone deacetylase inhibitor activated the WAF1/Cip1 gene promoter through the Sp1 sites. Biochem Biophys Res Commun 1997;241:142–50.
[42] Kim HS, Park JS, Hong SJ, Woo MS, Kim SY, Kim KS. Regulation of the tyrosine hydroxylase gene promoter by histone deacetylase inhibitors. Biochem Biophys Res Commun 2000;312:950–7.
[43] Hou M, Wang X, Popov N, Zhang A, Zhao X, Zhou R, et al. The histone deacetylase inhibitor trichostatin A derepresses the telomerase reverse transcriptase (LTERT) gene in human cells. Exp Cell Res 2002;274:25–34.
[44] Butler LM, Zhou X, Xu WA, Scher I, Rifkind RA, Marks PA, et al. The histone deacetylase inhibitors SAHA arrests cancer cell growth, up-regulates their thioredoxin-binding protein-2 and down regulates thioredoxin. Proc Natl Acad Sci USA 2002;99:11700–5.
[45] Jin S, Gorfajn B, Faircloth G, Scotto KW. Ecteinascidin 743, a transcription-targeted chemotherapeutic that inhibits MDR1 activation. Proc Natl Acad Sci USA 2000;97: 6775–9.
[46] Park K, Kim SJ, Bang YJ, Park JG, Kim NK, Roberts AB, et al. Genetic changes in the transforming growth factor β (TGF-β) type II receptor gene in human gastric cancer cells—correlation with sensitivity to growth inhibition by TGF-β. Proc Natl Acad Sci USA 1994;91:8772–6.