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

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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

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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