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Differential effects of histone deacetylase inhibitors on cell cycle regulation and apoptosis in human prostate cancer cells

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ABSTRACT

Objectives: Histone deacetylase (HDAC) inhibitors may suppress the proliferation of cancer cells and induce apoptosis. This study examined the molecular mechanism of how HDAC inhibitors modulate the cell cycle regulators and tumor suppressor genes in prostate cancer cells. **Methods:** HDAC inhibitors were evaluated according to the following parameters and techniques: (1) cell viability determined by a microculture tetrazolium assay (MTA); (2) induction of apoptosis according to 4'-6-diamidine-2'-phenylindole dihydrochloride (DAPI) staining; (3) the expression of cell cycle related proteins such as CDK2, CDK4, cyclin D1, and cyclin E by Western blot analysis; (4) p16 mRNA expression by RT-PCR; (5) the acetylation of the histone protein by nuclear protein extraction; and (6) cell cycle analysis by flow cytometry. **Results**: The HDAC inhibitors decreased the level of cell proliferation and induced apoptosis in human prostate cancer cell lines. Trichostatin A (TSA) caused a significant decrease in cyclin D1/CDK4 expression in LNCaP cells. Cell cycle analysis showed that the TSA treatment increased the proportion of LNCaP cells in the G1 phase and decreased the proportion of cells in the S phase. The TSA treatment resulted in the induction of apoptotic cell death which was confirmed by DAPI staining. TSA increased the level of p16 and WT1 expression in the androgen-dependent LNCaP or androgen-independent PC3 and DU145 cells. **Conclusions**: HDAC inhibitors may prevent the proliferation of prostate cancer cells by altering the expression of the cell cycle regulators and tumor suppressor genes, which might be associated with epigenetic regulation.

Key words

Apicidin; Apoptosis; Cell cycle regulator; Histone deacetylase; Trichostatin A

RÉSUMÉ

Objectif: Les inhibiteurs de l'histone-désacétylase (HDCA) peuvent réduire la prolifération de cellules cancéreuses et induire une apoptose. Nous avons examiné dans cette étude les mécanismes moléculaires impliqués par les inhibiteurs de l'HDAC dans la modulation du cycle cellulaire et des gènes répresseurs des tumeurs dans les cellules du cancer de la prostate. **Méthodes:** Les effets des inhibiteurs de l'HDAC ont été étudiés par les techniques suivantes: (1) la viabilité cellulaire par le TTM (test de tétrazolium sur microculture), (2) l'induction de l'apoptose cellulaire par marquage avec le DAPI (4'-6-diamidine-2'-phénylindole dihydrochlorure), (3) l'expression des protéines liées au cycle cellulaire telles que les CDK2, CDK4, cycline D1 et cycline E par immunotransfert, (4) l'expression de l'ARN messager p16 par RT-PCR, (5) l'acétylation des histones par extraction des protéines nucléaires, et (6) l'analyse du cycle cellulaire par cytométrie en flux. **Résultats:** Les inhibiteurs de l'HDAC réduisent la prolifération et induisent l'apoptose des cellules du cancer de la prostate. La trichostatine A (TSA) produit une réduction significative de l'expression de cycline D1/CDK4 dans les cellules LNCaP. L'analyse du cycle cellulaire montre une proportion augmentée de cellules LNCaP en phase G1 et une diminution des cellules en phase S suite an traitement par la TSA. Ce traitement induit la mort cellulaire par apoptose confirmée par marquage par le DAPI. La TSA augmente le taux de p16 et l'expression de WT1 tant dans les cellules androgéno-dépendantes LNCaP que dans les cellules androgéno-indépendantes PC3 et DU145. **Conclusions:** Les inhibiteurs

de l'HDAC peuvent prévenir la prolifération des cellules du cancer de la prostate par la modulation du cycle cellulaire et des gènes répresseurs des tumeurs vraisemblablement par régulation épigénétique.

Mots clés

Apicidine; Apoptose; Régulateur du cycle cellulaire; Histone- déacétylase; Trichostatine A

RESUMEN

Objetivos: Los inhibidores de la histona desacetilasa (HDAC) suprimen la proliferación de las células cancerígenas e inducen la apoptosis. Este estudio examinó los mecanismos moleculares de los reguladores del ciclo celular y los genes supresores tumorales por los inhibidores de la HDAC, en las células prostáticas cancerígenas. **Métodos:** Para evaluar el efecto de los inhibidores de HDCA, nosotros estudiamos (1) la viabilidad celular determinada mediante la prueba de microcultivo con tetrazolium; (2) la inducción de la apoptosis de acuerdo a la tinción de cloruro de 4'-6-diamino-2'-fenilindol (DAFI); (3) la expresión de CDK2, CDK4, ciclina D1, y ciclina E, mediante análisis de Western blot; (4) la expresión de p16 RNA mediante RT-PCR; (5) la acetilación de la histona mediante extracción proteica nuclear; y (6) el ciclo celular mediante citometría de flujo. **Resultados:** Los inhibidores de la HDAC disminuyeron los niveles de proliferación celular e indujeron la apoptosis en las células de cáncer de próstata. La tricostatina A (TSA) disminuyó significativamente la expresión de ciclina D1/CDK4 en las células LNCaP. El tratamiento con TSA incrementó la proporción de células LNCaP en la fase G1, disminuyó la proporción de células en la fase S del ciclo celular, y resultó en la inducción de la muerte celular por apoptosis confirmada mediante la tinción de DAFI. La TSA incrementó los niveles de p16 y la expresión de WT1 en las células dependientes de andrógenos LNCaP así como en las células independientes de andrógenos PC3 y DU145. **Conclusiones:** Los inhibidores de la HDAC pueden prevenir la proliferación de las células del cáncer de próstata mediante la alteración de la expresión de los reguladores del ciclo celular y de los genes supresores tumorales, los cuales podrían estar asociados con regulaciones epigenéticas.

Palabras clave

Apicidina; Apoptosis; Reguladores del ciclo celular; Histona deacetilasa; Trichostatina A

INTRODUCTION

Prostate cancer is one of the most common malignancies and the second leading cause of death among men in the United States [1] [2]. Although the incidence of prostate cancer in Korea is still much lower than in Western populations, it has increased rapidly over the past 10 years [3]. Epidemiological studies have shown that a high incidence of prostate cancer may be related to dietary factors. Excessive consumption of polyunsaturated fats has been associated with prostate cancer in humans [4]. Recent studies suggest that additional factors, including environmental agents (endocrine disrupting chemicals), aging, and oxidative stress are causally related to the development of prostate cancer [5]. In the initial stages, prostate cancer cells depend on androgens for their survival. Therefore, the main form of systemic treatment is androgen-ablation [6]. However, within a few years, most patients progress to an androgen-refractory state, which is capable of growing without androgens [7]. Hence, there is a need for alternative therapeutic strategies for the treatment of hormone-refractory prostate cancer with several chemotherapy trials being carried out for this purpose [8].

DNA methyltransferases (DNMTs) and HDACs are potential targets for regulating these epigenetic changes. DNA methylation and histone modification, which are regulated by several classes of enzymes, play an important role in the epigenetic mechanisms of gene regulation in various tumor initiation and progression stages. Epigenetic changes are defined as heritable changes in gene expression that occur without any changes in DNA sequence. The importance of an epigenetic alteration to cancer has probably been understated, since there has been more than ten years of remarkable advances in the knowledge of the mutational events that lead to the activation of protooncogenes or the inactivation of tumor suppressor genes [9]. Therefore, DNMT and HDAC inhibitors are believed to be one of the most promising classes of new anticancer agents in current clinical trials.

Recently, either naturally derived or synthetic compounds exhibiting HDAC inhibitory activity were shown to have anti-proliferative, pro-differentiating, and pro-apoptotic properties in a variety of cancer cells [10] [11] [12] [13]. TSA, which was originally developed as an antifungal agent, is a potent and reversible HDAC inhibitor. TSA has HDAC inhibitory effect at low concentrations; it targets the cell cycle progression of several cell types, induces cell growth arrest at both the G1 and G2/M phases, and in some cases, induces apoptosis [14] [15].

Apicidin [cyclo(N-O-methyl-L-tryptophanyl-L-iso leucinyl-D-pipecolinyl-L-2-amino-8-oxodecanoyl)] is a fungal metabolite shown to inhibit both mammalian and protozoan HDACs. It has also been reported to inhibit the proliferation of variety of tumor cells and selectively up-regulate the expression of p21WAF/Cip1, which allows tumor cells to undergo cell cycle arrest at the G0/G1 phase and cause morphological changes [16] [17]. However, the precise

mode of actions of these agents in prostate cancer cells is unclear.

Therefore, this study examined the molecular mechanism for the epigenetic alterations of the cell cycle regulator proteins and tumor suppressor genes in prostate cancer cells by DNMT and HDAC inhibitors.

METHODS

Materials

The TSA and apicidin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The medium and its supplements containing antibiotics, fetal bovine serum was obtained from Gibco Invitrogen Corporation (CA, USA). Western blot detection reagents were obtained from Amersham Bioscience Corporation (Bucks, United Kingdom). The polyvinylidene difluoride (PVDF) membranes were obtained from Bio-Rad (CA, USA). The RT-PCR reagents were supplied by Invitrogen Corporation. All other chemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Cell lines and culture media

The LNCaP, PC3 and DU145 cells, which are human prostate cancer cells, were obtained from ATCC (American Type Culture Collection, Rockville, MD, USA). The cells were grown in Minimum Essential Medium (MEM), pH 7.2, containing 2.2 g L^{-1} $NaHCO_3$, 1 mM sodium pyruvate, 100 U mL^{-1} antibiotics, and 10% heat-inactivated fetal bovine serum (FBS). The cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. The cells were plated on plastic dishes. The culture medium was replaced every 2 days. Apicidin and TSA were dissolved in dimethyl sulfoxide (DMSO) and absolute ethanol, respectively. These agents were diluted to the appropriate concentrations with the culture medium containing 5% charcoal-dextran treated FBS (CD-FBS). The final concentration of DMSO or ethanol was < 0.1% (vol vol^{-1}).

Microculture tetrazolium assay

The cell viability was determined using an MTA. The cultures were initiated in 96 well plates at a density of 2500 cells per well. Cells were allowed to reattach for 48 hr and then exposed to the test compounds. At the end of the treatment period, 15 μL of a 5 mg mL^{-1} MTT reagent [3-(4,5-dimethyl- thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide in PBS] was added to each well. The plates were incubated again for 4 h at 37°C in the dark. The supernatant was aspirated, and formazan crystals were dissolved in 100 μL DMSO at 37°C for 10 min with gentle agitation. The absorbance per well was measured at 540 nm using a VersaMax Microplate Reader (Molecular Devices Corp, CA, USA).

Nuclear protein preparation

Nuclear protein from the cells was prepared using the following procedure. All solutions, tubes, and centrifuges were maintained at 4°C. The cells were harvested by tripsinization, washed with cold PBS, centrifuged at 3,000 rpm for 5 min, and incubated in 50 μL of lysis buffer I (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT and 0.5 mM PMSF) for 20 min. After centrifugation at 12,000 rpm for 10 min, supernatant was removed and the pellet was resuspended in 30 μL of a lysis buffer II (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 0.5 mM PMSF and 0.05% NP-40) for 20 min. After centrifugation at 12,000 rpm for 10 min, the pellet was resuspended in 40 μl of lysis buffer III (5 mM HEPES, pH 7.9, 300 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF and 26% glycerol) for 30 min. After the final centrifugation at 12,000 rpm for 30 min, the protein concentration was quantified using a Bio-Rad protein assay reagent.

Western blot analysis

The cells were harvested and washed twice with PBS at 4°C. The total proteins were prepared using a PRO-PREP protein extract solution (Intron, Seongnam, Korea), and quantified using the protein assay reagent (Bio-Rad). Fifty microgram of the proteins were denatured by boiling at 96°C for 5 min in a sample buffer (0.5 M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 0.1% bromphenol blue, 10% β-mercaptoethanol) at 1:1 ratio. Each sample was separated using a 10-15% polyacrylamide gel (SDS-PAGE), and transferred to a PVDF membrane at 100 V for 1 h in a transfer buffer. The membrane was blocked using a blocking buffer containing 5% non-fat dry milk in a TNT buffer (10 mM Tris-Cl, pH 7.6, 100 mM NaCl and 0.5% Tween 20) at room temperature for 1 h. The membrane was then incubated overnight with the primary antibodies specific to CDK4, cyclin D1, CDK2, cyclin E, Rb, p-Rb, WT1 (Santa Cruz Biotechnology Inc., CA, USA), and Ac-H3 (Upstate Biotechnology) at 4°C.

After washing for 1 hr with TNT buffer, the membrane was incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies (Santa Cruz Biotechnology Inc., 1:10,000) at room temperature for 30 min, and then washed for 60 min with the TNT buffer. Antibody labeling was detected using ECL plus Western blotting detection reagents (Amersham Bioscience Corporation).

Assay of RT-PCR

The total RNA was extracted using Trizol Reagent (Gibco). cDNA was generated using the Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's protocol. cDNA was synthesized using a reverse transcription method. Briefly, 2 μg of the total RNA was reverse transcribed for 50 min at 42°C in a 20 μL reaction mixture (containing 1 μL of oligo primer (0.5 g), 2 μL of 10 mM dNTP mix, 2 μL of 10x RT buffer, 4 μL of 25 mM $MgCl_2$, 2 μL of 0.1 M DTT, 40 U of RNase Out inhibitor, and 100 U of Superscript II), followed by denaturation at 70°C for 15 min. RNase inhibitor (2 U) was then added and incubated at 37°C for 20 min. cDNA was used for the subsequent PCR reaction, 2 μL of cDNA was amplified in a 25 μL reaction mixture containing 2.5 μL of 10x PCR buffer, 0.75 μL of 50 mM $MgCl_2$, 0.5 μL of 10 mM dNTP mix, 1 U Taq polymerase, and 0.25 μL of sense and anti-sense primer (20 μM).

The primers were p16 (F; 5'-CAACGCACCGAATAGT TACG-3', R; 5'-ACCAGCGTGTC CAGGAA-3') and b-actin (F; 5'-CCTCGCCTTTGCCG ATCC-3', R; 5'-GGATCTTCATGA GGTAG TCAGTC-3'). The reaction was initiated at 94°C for 5 min and PCR was performed using a variable number of cycles of amplification defined as follows: denaturation at 94°C for 45 sec, annealing at 55°C (p16, 177 bp) or 60°C (b-actin, 626 bp) for 45 sec and extension at 72°C for 45 sec. Electrophoresis was performed in 2% agarose gel containing 1 μg of ethidium bromide (EtBr). The molecular sizes of the amplified products were determined by comparison with the molecular weight markers, a 100 bp DNA ladder (iNtRON) run in parallel with the RT-PCR products.

DAPI staining

Morphological changes in the nuclear chromatin of cells undergoing apoptosis were detected by staining with the DNA binding fluorochrome DAPI. The cells were grown in 6 well plate at a density of 4×10^4 cells per well. After treatment, cells were fixed in absolute methanol, and stained with 300 μL of DAPI solution

(1 g mL^{-1}) at 37°C for 15 min. After removing the staining solution, the cells were examined by fluorescence microscopy (Axiovert 200, ZEISS Inc., Germany).

Flow cytometry analysis

Cells were harvested and washed in 1% BSA before being fixed in 75% ethanol containing 0.5% Tween 20 for at least 1 h at 4°C. The cells were washed in 1% BSA and re-suspended in a cold propidium iodine (PI) staining solution (100 g mL^{-1} RNase and 10 g mL^{-1} PI in PBS) 1 mL for 40 min at 4°C. Data acquisition and analysis was carried out using a flow cytometry system (Becton Dickinson, CA, USA).

Statistical analysis

The results are reported as the means ± S.E. of three independent triplicate measurements. Statistically significant differences between untreated control and treated groups were determined using one-way analysis of variance (ANOVA).

RESULTS

The antiproliferative effect of TSA and apicidin on human prostate cancer cells was determined using a MTT assay and the results are shown in Figure 1. The prostate cancer cell lines (LNCaP, PC3, and DU145 cells) were treated with either TSA or apicidin at the consecutive serial concentrations. TSA and apicidin inhibited the proliferation of LNCaP and DU145 cells in a concentration-dependent manner and significantly decreased the level of cell proliferation after a 48 h treatment at the lowest concentration of 50 nM. In contrast, the PC3 cells were resistant under these conditions (Figure 1, plots A and B).

The effect of apicidin on the level of histone acetylation was then analyzed by Western blotting using the specific antibody to acetylated histone H3. Treatment of the prostate cancer cells with TSA (10 nM) and apicidin (10 nM) for 12 h resulted in a significant increase in acetylated H3 levels (Figure 2).

In order to determine if the decrease in cell viability was related to apoptosis, the cells were treated with 50 nM TSA and 50 nM apicidin for different times. The cells were then stained with DAPI, which enters the cells and binds to the fragmented DNA. The nuclear changes were observed by fluorescence microscopy. TSA and apicidin markedly increased the number of apoptotic bodies after a 24 h treatment (Figure 3). Whole cell protein extracts were made from each cell line after apicidin and TSA treatment for 24 h in order to determine if the cell death observed in prostate cancer cell lines related to cell cycle regulation. In the LNCaP cells treated with TSA at 100 nM, there was a significant decrease in cyclin D1 and CDK4 expression (Figure 4A). When treated with 10, 50, and 100 nM TSA for 24 h, there was a significant decrease in cyclin D1, CDK4, cyclin E, and CDK2 expression in the LNCaP cells but not in the PC3 and DU145 cells (Figure 4B). However, the apicidin treatment had no effect on cyclin E and CDK2 expression (which act in the G1 late stage) in the LNCaP, PC3 and DU145 cells (Figure 4A).

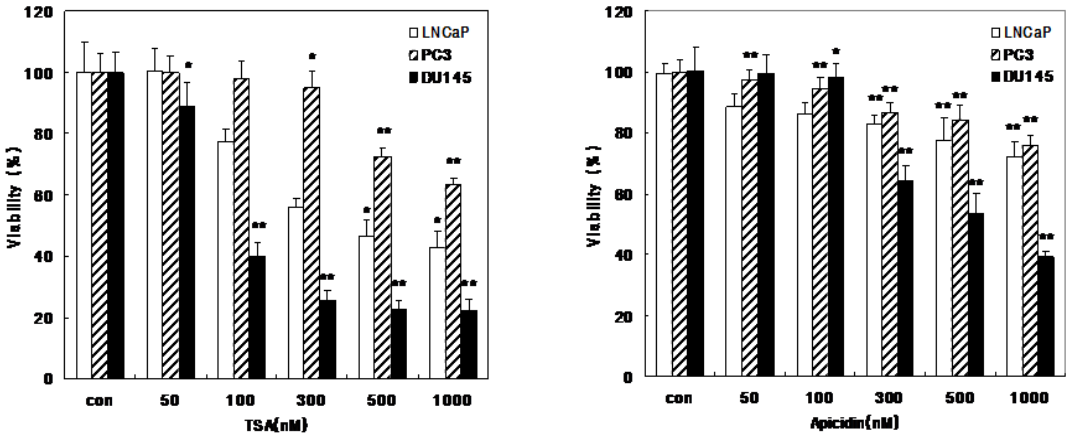

Figure 1 **Growth inhibition of human prostate cancer cell lines by trichostatin A and apicidin.** **The cells were treated with different concentrations of trichostatin A (TSA) and apicidin for 48 h and the cell viability was determined by a MTT assay.** The error bars show a S.D. *P < 0.005, **P < 0.001 as determined by a Student's t test, difference between the treated and the control group.

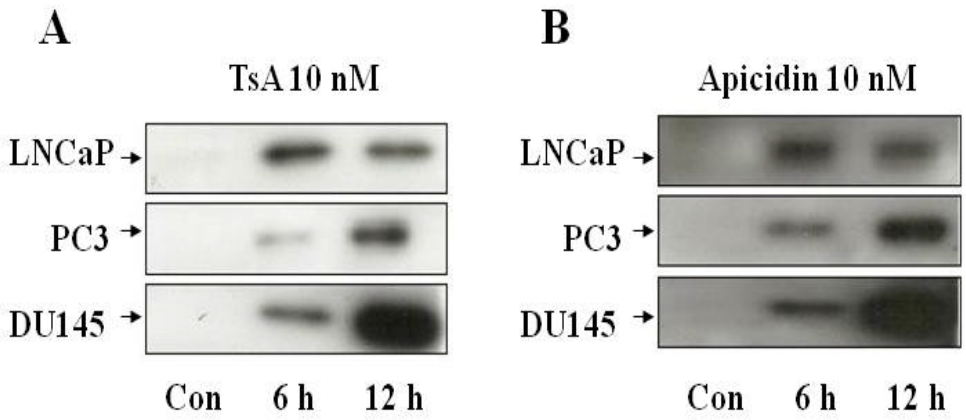

Figure 2 **Accumulation of acetylated histone proteins by treatment with the HDAC inhibitors.** **The cells were treated with 10 nM trichostatin A (TSA) and apicidin for 12 h. The acetylated histone tails were determined by western blot analysis using anti-acetylated H3 antibodies.**

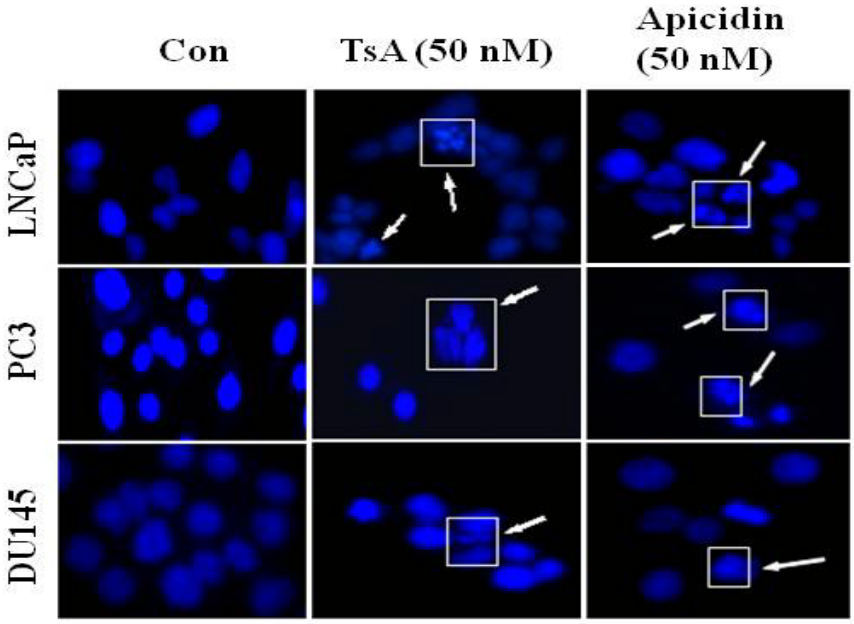

Figure 3 Apoptosis induction in prostate cancer cell lines by trichostatin A and apicidin treatment.
The induction of apoptosis by trichostatin A (TSA) and apicidin in LNCaP, PC-3, and DU-145 was analyzed by fluorescence microscopy after nuclear staining with DAPI. The cells were incubated with or without TSA (50 nM) and apicidin (50 nM) for 24 hr. The cells undergoing apoptosis and nuclear fragmentation are identified with arrows.
The magnification is 400X.

The tumor suppressor, Wilm's tumor suppressor gene (WT1), is essential for normal kidney and gonadal development and regulates cell differentiation and apoptosis *in vitro*. The expression of WT1 was examined to determine the effect of TSA on prostate cancer cells. The results showed that the expression of WT1 was induced dose-dependently in androgen-independent PC3 and DU145 cells. In contrast, the androgen-dependent LNCaP cells did not express a detectable level of WT1 (Figure 4B). In general, the p16 gene regulates progression through the G1/S phase of the cell cycle by binding CDK 4/6, which limits the formation of the CDK 4/6-cyclin D complex. The mRNA levels of the tumor suppressor gene, p16, were measured by RT-PCR to determine if the prostate cancer cell death, when treated with TSA, is related to p16 expression. The levels of p16 mRNA in LNCaP cells increased after TSA treatment in a dose-dependent manner, whereas there was no effect observed in the androgen-independent PC3 and DU145 cells (Figure 5, plots A and B). However, apicidin did not affect the levels of p16 expression in the LNCaP cells (Figure 5C).

Finally, flow cytometry analysis was used to examine the cell cycle progression of prostate cancer cells treated with TSA or apicidin for 24 h. TSA induced cell cycle arrest in the G1 phase in the LNCaP and DU145 cells. However, this was not observed in PC3 cells.In PC3 cells, the cell cycle arrest in the G1 phase was induced only after 24 h of treatment (Figure 6A). On the other hand, 100 nM of apicidin for 24 h induced cell cycle arrest in the G1 phase in PC3 cells only. In contrast, apicidin decreased the proportion of LNCaP and DU145 cells in the S phase and increased the proportion of cells in the G2 phase (Figure 6B). Finally, flow cytometry analysis was used to examine the cell cycle progression of prostate cancer cells treated with TSA or apicidin for 24 h. TSA induced cell cycle arrest in the G1 phase in the LNCaP and DU145 cells. However, this was not observed in PC3 cells. In PC3 cells, the cell cycle arrest in the G1 phase was induced only after 24 h of treatment (Figure 6A). On the other hand, 100 nM of apicidin for 24 h induced cell cycle arrest in the G1 phase in PC3 cells only. In contrast, apicidin decreased the proportion of LNCaP and DU145 cells in the S phase and increased the proportion of cells in the G2 phase (Figure 6B).

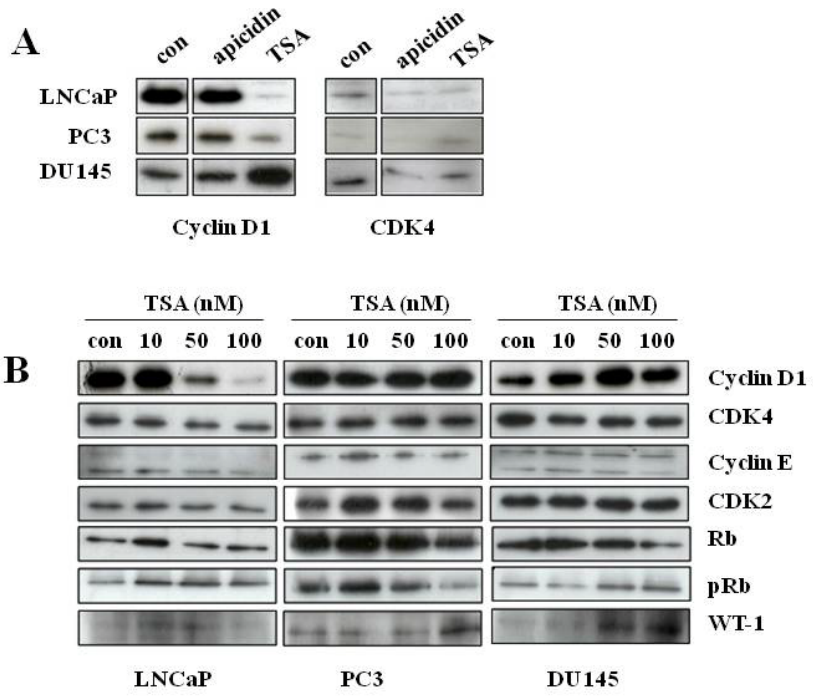

Figure 4 **Effect of the HDAC inhibitors on cell cycle regulators.**
The human prostate cancer cells were treated with 10, 50 and 100 nM trichostatin A (TSA) and apicidin (100 nM) for 24 hr. The proteins were isolated from LNCaP, PC-3, and DU145 cells and lysates (50 μg) of the cells were examined by 10% SDS-PAGE and analyzed by immunoblotting using specific antibodies.

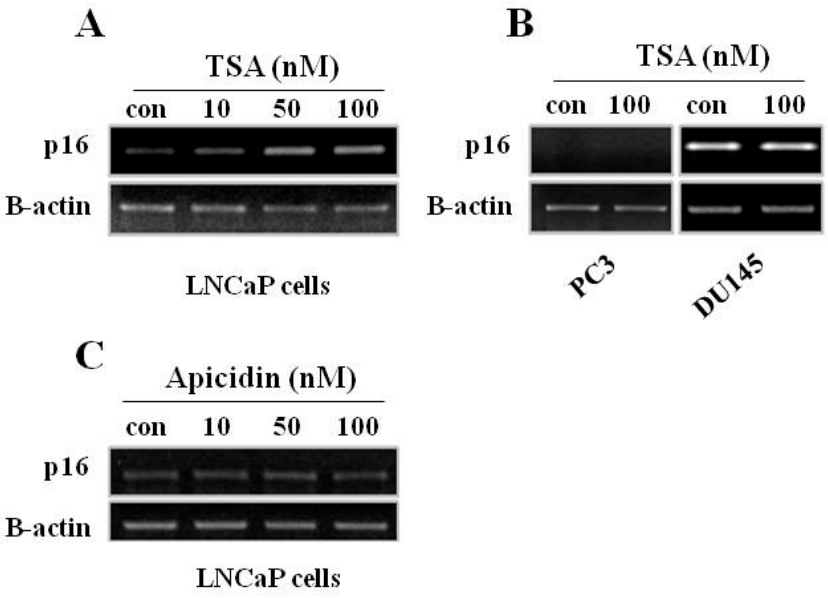

Figure 5 **Effect of HDAC inhibitors on tumor suppressor gene p16 expression in prostate cancer cells.**
The cells were treated with the indicated concentrations of trichostatin A (TSA) and apicidin for 48 h. Total RNA was isolated, and RT-PCR was performed using the specific primers described in the Materials and Methods section. Actin was used as a housekeeping control gene.

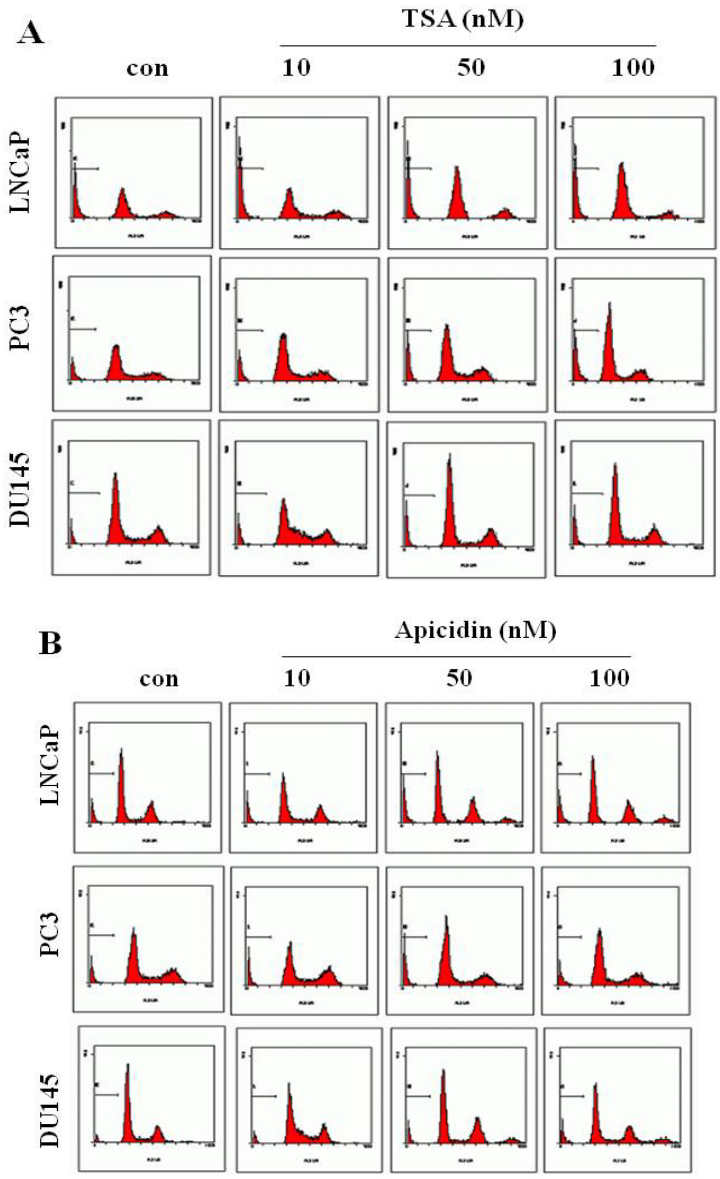

Figure 6 Effect of apicidin on cell cycle distribution. Human prostate cancer cells were treated with trichostatin A (TSA) (A) and apicidin (B) at the indicated concentrations for 24 h. The cells were fixed, permeabilized and stained with propidium iodide to measure DNA content by fluorescence-activated cell sorting (FACS) analysis. The percentages of cells in each phase of the cell cycle (G1, S and G2) are indicated.

DISCUSSION

This study examined whether or not HDAC inhibitors induced apoptotic cell death and cell cycle arrest in various human prostate cancer cell lines. It was demonstrated that the inhibition of histone deacetylation leads to cell death, which is associated with nuclear chromatin condensation and cell cycle arrest in the G1 or G2 phase [18] [19] [20]. In this study, TSA suppressed the growth of prostate cancer cells in a dose-dependent manner. These effects were related to epigenetic regulation of the specific genes associated with cell cycle regulation. In particular, TSA exhibited strong anticancer efficacy against cultured human androgen-dependent and -independent prostate cancer cells. Additionally, TSA inhibited cell growth and induced apoptosis and cell cycle arrest, induced a decrease in cyclin D1 and CDK4 expression in LNCaP cells, and restored the levels of p16 expression in prostate cancer cells. The p16 gene is localized to chromosome 9p21 and encodes the CDK inhibitor. This protein regulates cell cycle progression through the G1/S by binding CDK 4/6, which limits the formation of the CDK 4/6-cyclin

D complex and the phosphorylation of Rb [21]. Recently, aberrant DNA methylation of the 5' promoter region, an epigenetic event, was associated with the transcriptional silencing of p16 expression in several cancer cell lines including lung, bladder, colon, and prostate cancers [22] [23] [24]. Based on these results, the level of p16 expression was measured to determine if its expression is related to the cell cycle regulation of prostate cancer cells treated with TSA. The results showed that p16 expression was induced in androgen-dependent LNCaP cells in a dose-dependent manner, whereas there was no expression observed in the androgen-independent PC3 and DU145 cells. However, apicidin did not affect the expression of p16 in LNCaP cells. Therefore, TSA can restore the expression of p16 by inducing the relaxed chromatin conformation in androgen-dependent prostate cancer cells. This restored p16 expression induces prostate cancer cell death by inhibiting the cell cycle regulators, cyclin D1 and CDK4 in G1 phase of the cell cycle.

Another tumor suppressor gene, WT1 is essential for normal kidney and gonadal development and regulates cell differentiation and apoptosis in vitro [25] [26]. WT1 represses the androgen-signaling pathway. Moreover, WT1 expression in prostate cancer cell lines is limited to those cell lines lacking functional AR [27] [28]. PC3 and DU145 cells express high levels of WT1 mRNA and protein, whereas the androgen-dependent LNCaP cell did not express WT1 [29]. Furthermore, WT1 modulates the expression or activity of apoptotic genes, such as the prostate apoptosis response gene, par-4 [30] [31] or bcl-2 [32] [33]. In this study, the WT1 protein level was measured to determine the anticancer effect of TSA on human prostate cancer cells. The results showed that expression of WT1 was induced in androgen-independent PC3 and DU145 cells in a dose-dependent manner, whereas androgen-dependent LNCaP cells did not express detectable levels of WT1. Therefore, the HDAC inhibitor TSA would restore WT1 expression by inducing a relaxed chromatin conformation in androgen-independent prostate cancer cells. In addition, the intracellular level of acetylated histone H3 in prostate cancer cell lines induced by HDAC inhibitors was measured. Low levels of histone acetylation were observed in the absence of TSA, whereas hyperacetylated H3 was observed in all prostate cancer cells tested. This suggests that the induction of histone hyperacetylation by TSA due to the inhibition of HDAC and the activation of the transcriptional factors for WT1 gene expression.

TSA was found to dramatically induce cell cycle arrest in the G1 phase and increase the level of apoptosis in LNCaP cells. The transition from the G1 to S phase is believed to be triggered mainly by CDK4 after binding to its regulatory subunit, cyclin D1 [27] [28]. In general, G1 arrest depends on the ability of p16 to activate the transcription of specific genes, while G2/M arrest involves the repression of the transcription of CDK1/cyclin B1 complex [30]. This shows that TSA decreases the expression of cyclin B1 and CDK1 at both the mRNA and protein levels, and apicidin also increased the expression p21 and p27, which play a key role in the cell cycle arrest at the G2/M phase and apoptosis. In conclusion, HDAC inhibitors suppress the proliferation of prostate cancer cells by altering the expression of cell cycle regulators and inducing apoptosis. In particular, TSA may have different mechanism of action in either androgen-dependent or androgen-independent prostate cancer cells by altering the tumor suppressor genes, which might be associated with the epigenetic regulations.

AUTHORS' PARTICIPATION

HS Kim and HJ Kim conceived of and designed the study. WS Choi and JY Han analyzed and interpreted the data. H.J. Kim drafted the paper and J Lee and JY Han revised it for important intellectual content. All authors gave final approval of the version to be published.

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CONFLICT OF INTERESTS/DISCLAIMERS

HS Kim is member of the Editorial Board of the journal.

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