

Compound K, a metabolite of ginseng saponin, inhibits colorectal cancer cell growth and induces apoptosis through inhibition of histone deacetylase activity

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Abstract. In this study, we investigated the molecular mechanisms underlying the anti-proliferative effects of Compound K, with specific reference to histone modification. Exposure of HT-29 human colon cancer cells to Compound K resulted in time-dependent inhibition of histone deacetylase (HDAC) activity, mRNA and protein expression. Compound K treatment induced unmethylation of the RUNX3 promoter region such as TSA treatment and an accumulation of acetylated histones H3 and H4 within the total cellular chromatin, resulting in an enhanced ability of these histones to bind to the promoter sequences of the tumor suppressor gene Runt-related transcription factor 3 (RUNX3). Treatment of cells with Compound K increased the mRNA and protein expression of RUNX3, as well as p21, a downstream target of RUNX3. These alterations were consistent with cell cycle arrest at the G₀/G₁ phases and induction of apoptosis. Our results provide new insights into the mechanisms of Compound K action in human colorectal cancer cells and suggest that HDAC inhibition presents a novel approach to prevent or treat colorectal cancer.

Introduction

Post-translational modifications of core histone proteins play a critical role in the regulation of gene expression (1). Acetylation of core histones by histone acetyltransferases is linked to chromatin opening and transcriptional gene activation; in contrast, histone deacetylases (HDACs) remove the

acetyl group from histones and repress gene transcription (2). HDACs regulate many important biological processes, including cell cycle progression, differentiation and development (3).

Most studies performed to date have reported that, relative to adjacent normal mucosa, expression of HDAC1, HDAC2, HDAC3 and HDAC8, which all belong to the class I family of HDACs, is increased in colorectal carcinoma (4-9). Class I HDACs are frequently overexpressed in various human cancers, including colorectal cancer. Furthermore, their differential expression often correlates with drug resistance and poor prognosis, which makes them an attractive target for cancer therapeutics (10,11). Studies have demonstrated a role for class I HDACs in promoting colon cell proliferation and survival (4,7). Knockdown of HDAC1, 2 and 3 reduces the growth of several colon cancer cell lines including HCT-116, HT-29 and SW-480 (4,7,12). Mechanistically, the proliferative effects of HDACs in colon cancer cells have been linked to transcriptional repression of the cyclin-dependent kinase inhibitor, p21 (4,12). Knockdown of HDAC1, HDAC2 and HDAC3 induces p21 expression in colon cancer cell lines, while overexpression of these HDACs represses HDAC inhibitor-mediated p21 induction (13).

Recently, HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) have emerged as a promising class of therapeutic drugs. Crystallographic analysis has shown that SAHA and TSA interact directly with the catalytic site of an HDAC-like protein and inhibit its enzymatic activity (14). Inhibition of HDAC activity by SAHA and related agents alters gene expression, causing cell cycle arrest and apoptosis in cancer cells, primarily by the induction of p21 and Bax, a pro-apoptotic protein (15). However, phase I and II studies have demonstrated that pan-HDAC inhibitors may have numerous side effects, such as bone marrow depression, diarrhea, disordered clotting and cardiac arrhythmias (16). Therefore, there is a clinical need for HDAC inhibitors that are both effective and minimally toxic.

Runt-related transcription factor 3 (RUNX3) belongs to the RUNX family of genes, which are important in mamma-

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lian development and neoplasia (17-19). RUNX3 cooperates with Smad- and Mad-related protein 3 (Smad3) and Smad4 to activate transforming growth factor- β (TGF- β)-dependent growth inhibition and apoptosis by inducing p21 and Bim (20). The RUNX3 gene is localized to a locus at chromosome 1p36 and is linked with gastric epithelial homeostasis and gastric carcinogenesis. The 1p36 region is thought to harbor at least one tumor suppressor gene because this region exhibits frequent loss of heterozygosity in colon, gastric, breast and ovarian cancers (21). In addition, the introduction of a normal human 1p36 chromosome fragment into colon cancer cells suppresses their tumorigenicity (22). The RUNX3 gene is frequently inactivated through histone modification during the development of various carcinomas, including gastric and colon (20,22-25). Fujii *et al* demonstrated that enhancer of Zeste Homolog 2, a histone methyltransferase, binds to the RUNX3 promoter, resulting in the upregulation of H3K27 methylation and concomitant downregulation of RUNX3 expression in colon cancer (26). Lee *et al* extended this finding by demonstrating that hypoxia-induced upregulated expression of HDAC1 and G9a, a histone methyltransferase, also causes epigenetic RUNX3 silencing through H3K9 methylation and decreased histone H3 acetylation at the RUNX3 promoter (27).

A number of saponins have been isolated from ginseng and their possible antitumor activity has been extensively investigated in various cancer cell lines (28-30). Among these saponins, 20-O-(β -D-glucopyranosyl)-20(S)-protopanaxadiol, or Compound K, is the main metabolite of protopanaxadiol-type ginsenoside, formed in the intestine after oral administration (31-33). Our laboratory recently reported that Compound K exhibits cytotoxicity by inducing apoptosis, arrest of growth at the G₁ phase of the cell cycle and inhibition of telomerase activity in human leukemia cells (34,35). In addition, combined treatment with Compound K and gamma irradiation enhances the death of human lung cancer cells (36) and Compound K induces apoptosis in MCF-7 breast cancer cells by modulating AMP-activated protein kinase (37). In addition, we recently demonstrated that Compound K induces RUNX3 reactivation by DNA methyltransferase 1 inhibition (38).

The aims of this study were to determine whether Compound K suppresses HDAC activity and expression and to elucidate the molecular mechanisms by which Compound K induces cell cycle arrest and apoptosis. The results demonstrate that Compound K can downregulate HDAC1, leading to the accessibility of the RUNX3 promoter region in colorectal cancer HT-29 cells via increase of acetylation of histones H3 and H4. Downregulation of HDACs represents a novel mechanism underlying the ability of Compound K to induce cell cycle arrest and apoptosis.

Materials and methods

Cell culture. Human colorectal cancer HT-29 cells were obtained from the Korean Cell Line Bank (Seoul, Republic of Korea). Cells were maintained in an incubator at 37°C with a humidified atmosphere of 5% CO₂. The cells were cultured in RPMI-1640 medium containing 10% fetal calf serum, streptomycin (100 μ g/ml) and penicillin (100 U/ml).

Cell proliferation assay. Cell proliferation was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded into a 96-well plate at a density of 1x10⁵ cells/ml and were treated with 8, 16, 32 and 64 μ mol/l of Compound K or TSA. After incubating for 48 h, 50 μ l of the MTT stock solution (2 mg/ml) was added to each well to attain a total reaction volume of 250 μ l. After 4 h incubation, the supernatants were aspirated; the formazan crystals in each well were dissolved in 150 μ l dimethylsulfoxide and absorbance at 540 nm was measured using a scanning multi-well spectrophotometer (TECAN, Melbourne, Australia).

Reverse transcription polymerase chain reaction (RT-PCR). Total RNA was isolated from cells using TRIzol reagent (Gibco BRL, Grand Island, NY, USA). Complementary DNA (cDNA) (1 μ l) was amplified in a 25 μ l reverse transcription reaction containing primers, dNTPs and 0.5 U of Taq DNA polymerase. The PCR conditions were 5 min at 94°C for initial denaturation, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C and then a final elongation period of 7 min at 72°C. PCR amplification was carried out in a Perkin-Elmer Cetus 9600 thermal cycler (Roche Molecular Systems Inc., NJ, USA). The primers used to amplify the RUNX3 and HDAC1 cDNA were as follows: RUNX3 sense, 5'-GGCAATG ACGAGAACTAC-3' (located in exon 2); RUNX3 antisense, 5'-GGAGAATGGGTTCAGTTC-3' (located in exon 5); HDAC1 sense, 5'-AACCTGCCTATGCTGATGCT-3'; HDAC1 antisense, 5'-CAGGCAATTCGTTTGTGTCAGA-3'; GAPDH sense, 5'-GTGGGCCCGCCCTAGGCACCAGG-3'; and GAPDH antisense, 5'-GGAGGAAGAGGATGCGGCAGTG-3'. The amplified products were resolved on 1% agarose gels, which were stained with ethidium bromide and visualized under ultraviolet light using ImageQuant™ TL analysis software (Amersham Bioscience, Uppsala, Sweden).

Methylation specific (MS)-PCR. The bisulfate modification of DNA was carried out with the Methylamp™ DNA modification kit (Epigentek, Pittsburgh, PA, USA) according to the manufacturer's instructions. For analysis of DNA methylation of RUNX3, MS-PCR carried out using an Epitect MSP kit (Qiagen, Valencia, CA, USA). The PCR products were separated on 6% non-denaturing polyacrylamide gels, stained with ethidium bromide and visualized under UV light. The methylated or unmethylated RUNX3 primer set is as follows: 5'-TTATGAGGGGTGGTTGTATGTGGG-3' and 5'-AAA ACAACCAACACAAACACCTCC-3' for unmethylated RUNX3; 5'-TTACGAGGGGCGGTTCGTACGCGGG-3' and 5'-AAAACGACCGACGCGAACGCCTCC-3' for methylated RUNX3.

Western blot analysis. Cells were harvested and lysed on ice in 1 ml of lysis buffer (10 mmol/l Tris-HCl, pH 7.9, 10 mmol/l NaCl, 3 mmol/l MgCl₂ and 1% NP-40) for 4 min. After centrifugation for 10 min at 3,000 g, the pellets were re-suspended in 50 μ l of extraction buffer (20 mmol/l HEPES, pH 7.9, 20% glycerol, 1.5 mmol/l MgCl₂, 0.2 mmol/l EDTA, 1 mmol/l DTT and 1 mmol/l PMSF) and then incubated on ice for 30 min and centrifuged at 13,000 g for 5 min. The protein

concentration was measured and supernatants were stored at -70°C . Aliquots of the lysates (40 μg of protein) were boiled for 5 min and electrophoresed on a 10% SDS-polyacrylamide gel. Proteins were transferred onto nitrocellulose membranes, which were subsequently incubated with primary antibodies. The membranes were further incubated with secondary immunoglobulin-G-horseradish peroxidase conjugates (Pierce, Rockford, IL, USA). Protein bands were detected using an enhanced chemiluminescence western blotting detection kit (Amersham, Little Chalfont, UK) and were visualized using a luminescent image analyzer (BMG Labtech, Offenburg, Germany).

HDAC activity. Nuclear extracts were prepared using a nuclear protein extraction kit (Cayman Chemical, Ann Arbor, MI, USA). After measuring the protein concentration, the nuclear fractions were stored at -70°C . HDAC activity was measured using an EpiQuik HDAC activity assay kit (Epigentek, Brooklyn, NY, USA), according to the manufacturer's instructions. In brief, the nuclear extracts were incubated with a specific substrate for 1 h at 37°C , followed by capture antibody for 60 min and then detection antibody for 30 min at room temperature. Absorbance at 450 nm was measured using a microplate spectrophotometer (TECAN). HDAC activity was calculated as $\text{ng/h/ml} = \{[\text{optical density (control-blank)} - \text{optical density (sample-blank)}] / \text{Slope} \times \text{h}\} \times \text{sample dilution}$.

Immunocytochemistry. Cells plated onto coverslips were fixed with 4% paraformaldehyde for 30 min and permeabilized with 0.1% Triton X-100 in PBS for 15 min. Cells were treated with blocking medium (3% bovine serum albumin in PBS) for 1 h and then incubated for 2 h with the RUNX3 antibody in blocking medium. The primary acetylated histone H3, acetylated histone H4 and RUNX3 antibodies were detected by a FITC-conjugated secondary antibody (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h. After washing with PBS, stained cells were mounted onto microscope slides in mounting medium with DAPI (Vector, Burlingame, CA, USA) and imaged using the LSM 510 program on a Zeiss confocal microscope (Carl Zeiss Microscopy Ltd., Cambridge, UK).

Chromatin immunoprecipitation (ChIP) assay. The ChIP assay was performed using a Simple ChIP™ enzymatic chromatin IP kit (Cell Signaling Technology, Danvers, MA, USA), according to the manufacturer's protocol with slight modifications. Briefly, cells were treated with 20 $\mu\text{g/ml}$ of Compound K for 48 h and then cross-linked by the addition of 1% formaldehyde. Chromatin was prepared and digested with nuclease for 12 min at 37°C . ChIP was performed with the acetylated histone H3, acetylated histone H4 and RUNX3 antibodies [Cell Signaling Technology and Abcam (Cambridge, UK)] and normal mouse IgG. Antibodies were added to the chromatin digests and were incubated with constant rotation at 4°C overnight. ChIP-grade protein G magnetic beads were then added to capture the immune complexes. The beads were washed and the immunoprecipitates were eluted with ChIP elution buffer. The cross-links were reversed by incubation at 65°C for 30 min. Proteinase K was added and incubated at 65°C for 2 h. The immunoprecipitated DNA fragments were then purified using spin columns. DNA recovered from the immunoprecipi-

tated complex was subjected to 35 cycles of PCR. The primers for the p21 (RUNX3 binding site) and RUNX3 (acetylated H3, H4 binding sites) gene promoters were as follows: p21 sense, 5'-CACCAGACTTCTCTGAGCCCCAG-3'; p21 antisense, 5'-GCACTGTTAGAATGAGCCCCCTTTC-3'; RUNX3 sense, 5'-GGTTGCAGAAGTCACAGG-3'; and RUNX3 antisense, 5'-AATTTGCTTAGAACGTCCG-3'. The PCR products were separated on 2% agarose gels and DNA bands were visualized using the Image program (NIH, Bethesda, MD, USA).

Flow cytometry. A flow cytometric assay was performed to assess the effects of Compound K or TSA on the cell cycle. Cells were treated with 32 $\mu\text{mol/l}$ of Compound K or TSA for 48 h. Harvested cells were then washed twice with PBS and fixed in 70% ethanol for 30 min at 4°C . Subsequently, the cells were incubated in 50 mg/ml propidium iodide solution and 50 $\mu\text{g/ml}$ RNase A in the dark for 30 min at 37°C . Flow cytometric analysis was performed using a FACSCalibur flow cytometer (Becton-Dickinson, Mountain View, CA, USA). The cell cycle phases were assessed based on histograms generated by CellQuest and Mod-Fit software programs.

Nuclear staining with Hoechst 33342. A volume of 1.5 μl of Hoechst 33342 (10 mg/ml), a DNA-specific fluorescent dye, was added to each well and cells were incubated for 10 min at 37°C . To examine the degree of nuclear condensation, the stained cells were observed under a fluorescent microscope, which was equipped with a CoolSNAP-Pro color digital camera (Carsen Group, Markham, ON, Canada).

Statistical analysis. All measurements were performed in triplicate and values are expressed as the mean \pm standard error of the mean (SEM). The results were examined using analysis of variance (ANOVA) and Tukey's test to determine pairwise differences. $P < 0.05$ was considered statistically significant.

Results

Compound K inhibits HDAC activity and expression in HT-29 cells. We recently demonstrated that Compound K acts as a DNA methyltransferase inhibitor in human colorectal cancer cells (38); the present study provides evidence that Compound K also inhibits HDACs in human colorectal cancer HT-29 cells. Compound K and TSA inhibited HT-29 cell growth in a dose-dependent manner at concentrations between 8 and 64 $\mu\text{mol/l}$; the concentration that yielded 50% growth inhibition (IC_{50}) was 32 $\mu\text{mol/l}$ for Compound K and 24 $\mu\text{mol/l}$ for TSA (Fig. 1). As a result, 32 and 24 $\mu\text{mol/l}$ were chosen as the optimal doses of Compound K and TSA, respectively. Treatment of cells with the optimal dose of Compound K decreased HDAC1 mRNA and protein expression in a time-dependent manner (Fig. 2A and B). HDAC enzyme activity was reduced at all time-points in cells treated with TSA and enzyme activity was also decreased in a time-dependent manner in cells treated with Compound K (Fig. 2C).

Compound K increases histone acetylation. We then determined whether the decrease in HDAC expression and activity induced by Compound K affects histone acetylation. Compound K induced a significant increase in the acetylation

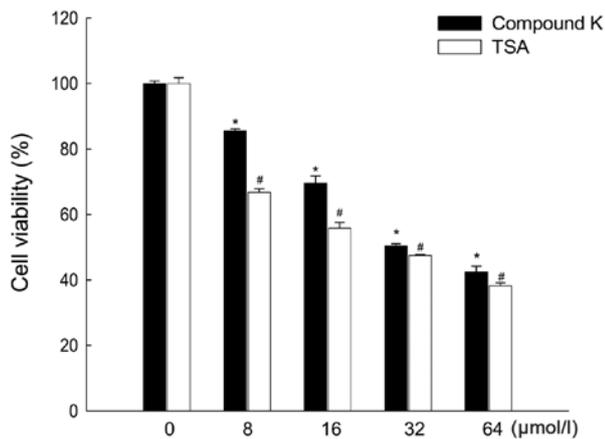


Figure 1. Growth inhibition effects of Compound K and TSA in HT-29 cells. Cell viability was assessed using the MTT test following the treatment of cells with various concentrations of Compound K or TSA for 48 h. *[#]Significantly different from the control of Compound K- or TSA-treated cells, respectively ($p < 0.05$).

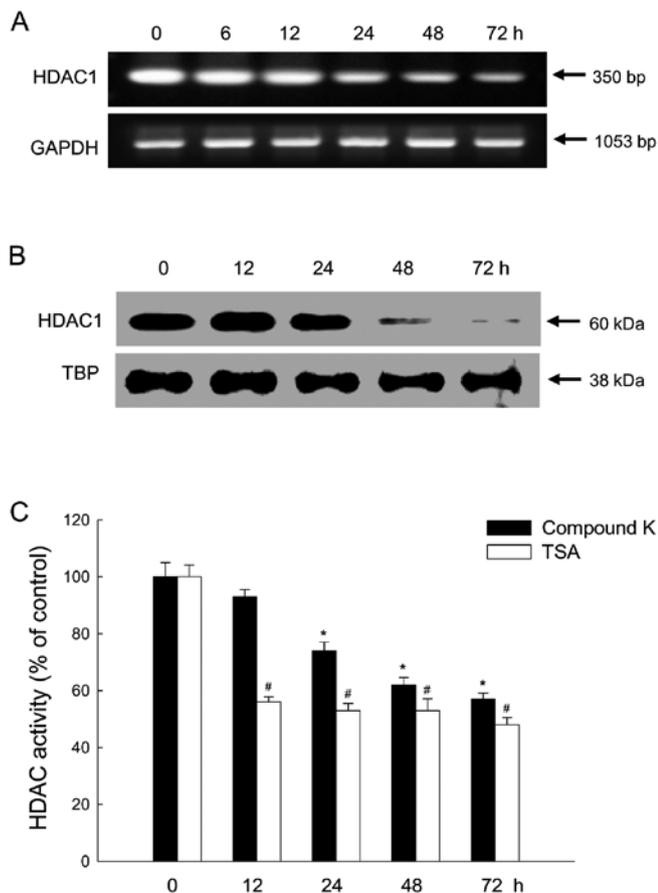


Figure 2. Time-course of the effect of treatment of HT-29 cells with Compound K for 0-72 h on mRNA, protein expression and enzyme activity of HDAC1. (A) HDAC1 mRNA and (B) HDAC1 protein expression were assessed using RT-PCR and western blotting, respectively. (C) HDAC enzyme activity was detected using a histone deacetylase activity assay kit. *[#]Significantly different from the control of Compound K- or TSA-treated cells, respectively ($p < 0.05$).

of histones H3 and H4 in a time-dependent manner (Fig. 3A). Furthermore, direct immunofluorescence analysis revealed

that acetylated histones H3 and H4 are present in the nuclei of HT-29 cells following treatment with Compound K (Fig. 3B).

Compound K reactivates RUNX3 via inhibition of HDAC. The RUNX3 gene in HT-29 cells is reportedly epigenetically silenced (39-43). ChIP analysis revealed acetylation of the RUNX3 promoter in Compound K-treated HT-29 cells (Fig. 4A), which resulted in the time-dependent re-expression of RUNX3 mRNA (Fig. 4B) and protein (Fig. 4C). We then investigated whether the status of DNA methylation in the RUNX3 promoter region changed after treatment of Compound K, as well as TSA (Fig. 4D). Mislocalization of nuclear RUNX3 protein to the cytoplasm was also observed in HT-29 cells; however, treatment of cells with Compound K increased the nuclear localization of the RUNX3 protein (Fig. 4E).

Compound K induces RUNX3-mediated expression of p21. RUNX3 cooperates with Smad3 and Smad4 to activate TGF- β -dependent growth inhibition and also causes apoptosis by inducing p21 and Bim (20,41). Enhanced p21 mRNA (Fig. 5A) and protein (Fig. 5B) expression was observed in HT-29 cells following Compound K treatment. Additionally, ChIP assay data showed that RUNX3 interacted with p21 (Fig. 5C); therefore, Compound K induced p21 expression via the binding of RUNX3 to the p21 promoter region.

Compound K induces cell cycle arrest and apoptosis. Analysis of DNA content by flow cytometry revealed that Compound K and TSA caused a marked increase in the percentage of cells in the G₀/G₁ phases of the cell cycle. Following Compound K treatment of HT-29 cells, the cell population in the G₀/G₁ phase at 6 and 12 h was 64 and 68% comparing to 50 and 52% cell population in the G₀/G₁ phase of control group; following TSA treatment, 69% was in the G₀/G₁ phase at 6 h and 37% in the G₂/M phase at 12 h compared to 50% in the G₀/G₁ phase at 6 h and 21% in the G₂/M phase at 12 h of control group (Fig. 6A). Furthermore, the sub-G₁ population (Fig. 6B) and apoptotic body formation (Fig. 6C) were increased in Compound K-treated cells compared with control cells.

Discussion

Epigenetic gene regulation plays a crucial role in the etiology of cancer. Post-translational histone modifications are important epigenetic events in the regulation of gene expression and maintenance of cellular function, which may contribute to cancer development (44-46). Abnormal histone modification is a hallmark of cancer that often causes silencing of tumor suppressor genes, which in turn leads to cancer development and progression. The anti-carcinogenic effects and preventative action of Compound K, the main metabolite of ginseng saponin, against colorectal cancer have been extensively studied in various tumor models (35-37,47); however, less attention has been given to the epigenetic modifications induced by Compound K and their potential role in reducing the risk of developing colorectal cancer. In this study, Compound K

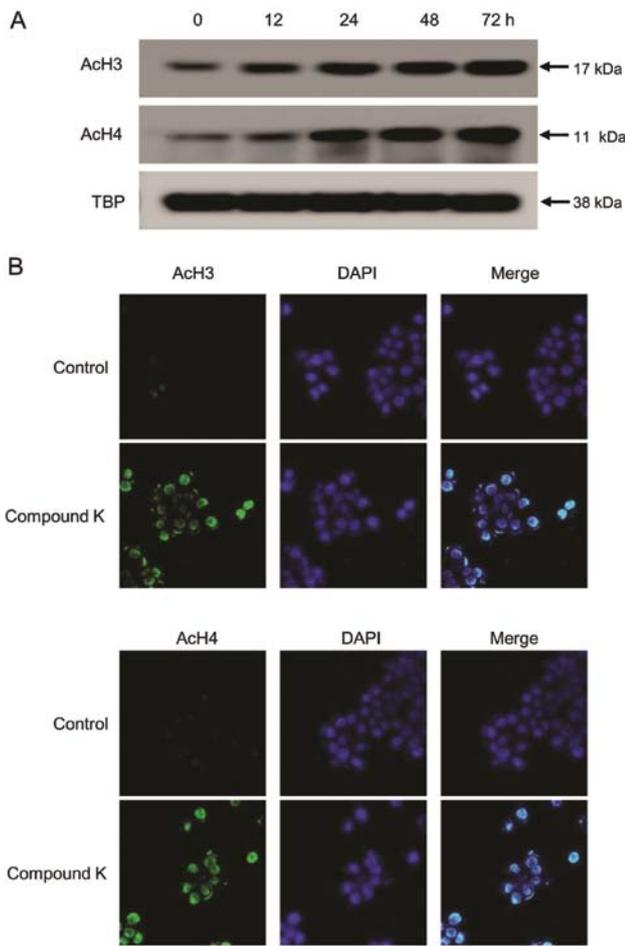


Figure 3. Effect of Compound K on histone H3 and H4 acetylation. (A) Acetylated histone H3 (AcH3) and H4 (AcH4) protein expression assessed by western blotting. (B) Confocal images showing the nuclear location of histones H3 (top panels) and H4 (bottom panels), indicated by green staining. DAPI (blue) staining indicates the location of the nucleus; the merged image indicates the nuclear location of histone H3 and H4 proteins.

induced the transcription of RUNX3 and p21 transcription factors and reduced DNA methylation of RUNX3. Also, this Compound K increased acetylation of histones H3 and H4, leading to cell cycle arrest and apoptosis.

Class I HDACs are overexpressed in human colorectal cancer specimens and colorectal cancer cell lines (5,48); specifically, HDAC1, HDAC2 and HDAC3 are highly expressed in colorectal cancer (11). Overexpression of HDAC1 in prostate cancer cells causes increased cell proliferation and a reduction in cell differentiation markers; HDAC1 may therefore represent a putative therapeutic target for cancer (49,50). HDACs form multi-subunit transcriptional co-repressor

Figure 5. Compound K induces RUNX3-mediated p21 expression in a time-dependent manner. (A) Western blot and (B) RT-PCR analyses were assessed for the detection of p21 protein and mRNA expression, respectively. The numbers below (B) represent densitometric analysis of the ratio of p21 and GAPDH mRNA expression. (C) Analysis of the interaction between RUNX3 and p21 performed by ChIP analysis. The bands indicate the level of RUNX3 in the p21 promoter region, or the level of IgG as a negative control. Input represents amplification of total DNA from whole cell lysates.

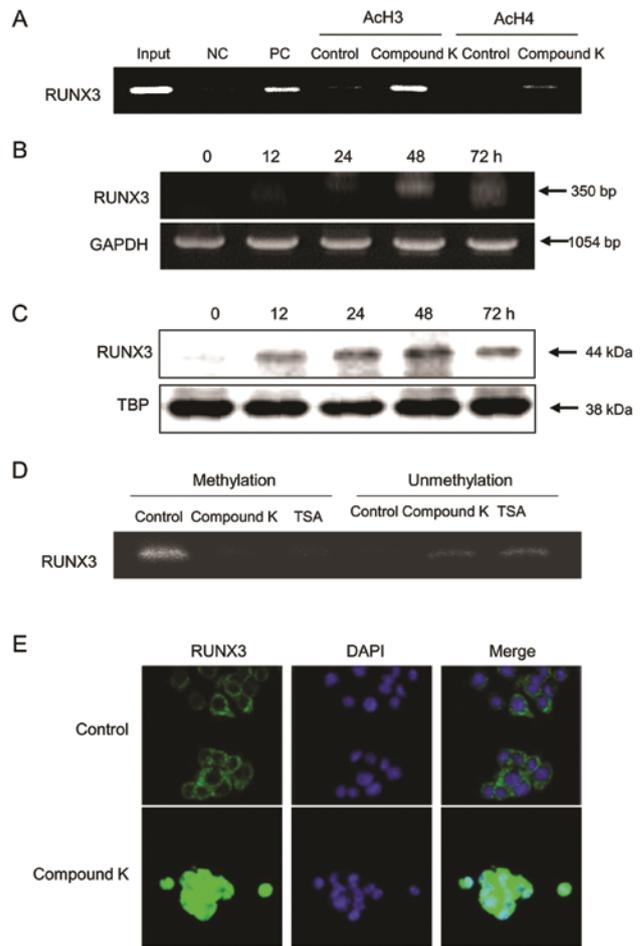
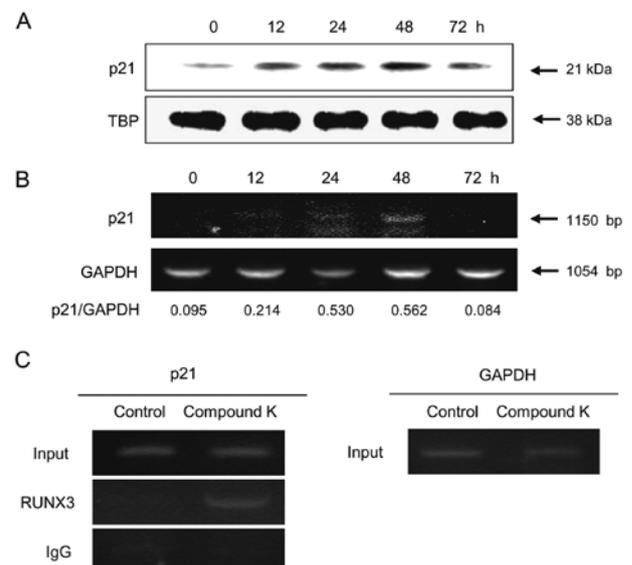


Figure 4. Time-course of the effect of Compound K on RUNX3 activation. (A) ChIP analysis performed with the histone H3 and H4 antibodies and primers for amplifying the RUNX3 promoter region; the bands show the level of histone H3 and H4 in the RUNX3 promoter region and GAPDH or TATA-binding protein (TBP) as a positive control. Input represents amplification of total DNA from whole cell lysates. (B) RT-PCR and (C) western blot analyses were assessed for detection of RUNX3 mRNA and protein expression, respectively. (D) MS-PCR analysis of the RUNX3 promoter was assessed by using the methylated or unmethylated RUNX3 primer set. (E) Confocal images indicating the location of RUNX3 (green staining) in control and Compound K-treated cells. DAPI (blue) staining indicates the location of the nucleus and the merged image indicates the nuclear location of RUNX3 protein.



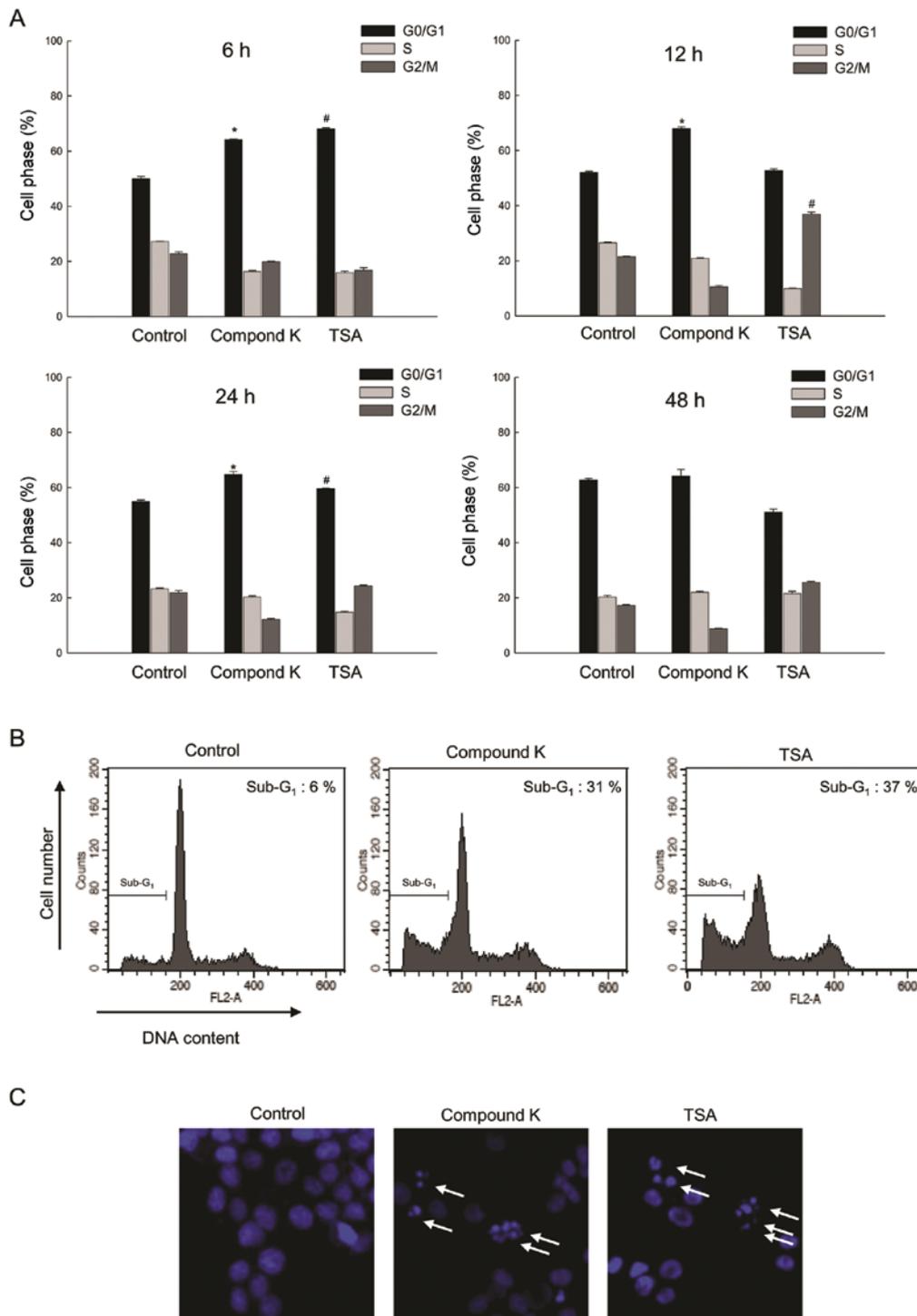


Figure 6. Effects of Compound K and TSA on cell cycle and apoptosis. (A) Cells were treated with Compound K or TSA for 6, 12, 24 or 48 h and the distribution of cells in each phase of the cycle was analyzed using flow cytometry. *Significantly different from the G₀/G₁ phase of control and #significantly different from the G₂/M phase of control, respectively ($p < 0.05$). (B) Apoptotic sub-G₁ cells were detected by flow cytometry after propidium iodide staining. (C) Apoptotic body formation was observed after Hoechst 33342 staining using a fluorescent microscope; apoptotic bodies are indicated by arrows.

complexes that are recruited to promoter regions by sequence-specific transcription factors (51). There are reportedly several co-repressor complexes for distinct promoters, which recruit specific HDAC isoforms that silence their target genes. Class I HDACs are specifically responsible for deacetylation of the catalytic core of different co-repressor complexes, resulting in transcriptional repression. For example, HDAC1 and HDAC2 are present in the CoREST, mi2/NURD and Sin3 complexes

and HDAC3 is responsible for the catalytic activities of the N-CoR and SRMT co-repressor complexes (51). Silencing of RUNX3 by HDACs plays an important role in the regulation of tumor suppression during gastric and colon carcinogenesis (26,27).

It is important to note that Compound K affects HDAC1 and therefore has profound effects on cancer cells; however, the specific mechanisms underlying these effects requires

further clarification, specifically with regard to understanding the differential response of colorectal cancer cells to Compound K. Repression of tumor suppressor genes involves deacetylation of various transcription factors by class I HDACs (32,33). In addition, evidence to date indicates that specific HDACs are consistently overexpressed in colon cancer. The effect of overexpression and depletion of multiple class I HDACs on the growth and survival of colon cancer cells has been investigated *in vitro* in a number of studies; for example, deficiency of HDAC1, 2 and 3 reduces the growth of several colon cancer cell lines (4,7,12). In this study, we observed a differential response of Compound K-mediated cell death, probably due to significant inhibition of class I HDACs at the protein and mRNA levels. The results provide additional evidence that Compound K inhibits class I HDACs by altering the acetylated histone status and enhancing the expression of tumor suppressor genes and pro-apoptotic proteins. These effects were accompanied by corresponding increases in cell growth inhibition and apoptosis.

Tumor suppressor gene repression involves deacetylation of various transcription factors by class I HDACs. Class I HDAC-mediated deacetylation has been shown to decrease the DNA-binding activity of sequence-specific transcription factors. For example, covalent modifications of several transcription factors, including E2F, SP1/ SP3, p53, GATA1, TFIIF and RUNX3 by class I HDACs, have been reported (51-53). HDAC inhibitors reduce HDAC activity, altering the dynamic balance between HDAC and histone acetyltransferase activity, which results in increased acetylation of non-histone proteins, including RUNX3. Deacetylation by HDAC1 and HDAC2 results in the silencing of RUNX3 and HDAC inhibitors have been shown to reverse this process (54,55). Inhibition of HDACs results in the acetylation of RUNX3, thereby increasing both its half-life and binding to the p21 promoter (54). Our chromatin immunoprecipitation assays confirmed an increase in the expression of acetylated histones H3 and H4 associated with the RUNX3 promoter in the nuclei of HT-29 cells.

In conclusion, this study demonstrates that Compound K can decrease HDAC enzyme activity and may be effective in inhibiting cancer cell growth. The degree of HDAC inhibition and the alterations in downstream gene expression induced by Compound K were similar to those induced by TSA, a pharmacological inhibitor of HDACs. Our data suggest that epigenetic regulation of tumor suppressor genes by Compound K may play a role in colorectal cancer chemoprevention or therapy. These findings are important for understanding the anticancer mechanisms and clinical applications of Compound K.

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