Sodium Butyrate Regulates Androgen Receptor Expression and Cell Cycle Arrest in Human Prostate Cancer Cells

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Abstract. *Histone deacetylase (HDAC) inhibitors have been shown to modify the expression of a variety of genes related to cell cycle regulation and apoptosis in several cancer cells. However, the precise mode of action of HDAC inhibitors in prostate cancer cells is not completely understood. This study examined whether an HDAC inhibitor affects cell death in human prostate cancer cells through the epigenetic regulation of androgen receptor (AR) expression. The molecular mechanism of the HDAC inhibitor, sodium butyrate, on the epigenetic alterations of cell cycle regulators was evaluated in androgen-dependent human prostate cancer LNCaP cells. The expression levels of acetylated histone H3 and H4 increased significantly after 48 h treatment with sodium butyrate. Sodium butyrate induced the expression of AR after 48 h treatment. In addition, immunofluorescence assay revealed the nuclear localization of the AR after sodium butyrate treatment. Sodium butyrate also significantly decreased the expression of the cell cycle regulatory proteins (cyclin D1/cyclin dependent kinase (CDK)4, CDK6, and cyclin E/CDK2) in the LNCaP cells after 48 h treatment. Furthermore, p21$^{Waf1/Cip1}$ and p27^{Kip1} were up-regulated as a result of the sodium butyrate treatment. These results suggest that sodium butyrate effectively inhibited cell proliferation and induced apoptosis of human prostate cancer cells by altering the expression of cell cycle regulators and AR. This study indicated that sodium butyrate may be a potential agent in prostate cancer treatment.*

Prostate cancer is the most common malignancy and the second leading cause of cancer-related death in the United States. Although the disease is relatively uncommon in

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Korea (1), it is estimated that prostate cancer is the sixth most common cancer with about 900 prostate cancer patients dying in 2005. The incidence of prostate cancer in Korea is still much lower than in Western countries but has been rapidly increasing over the past 10 years (2).

The androgen receptor (AR) is a member of the nuclear receptor superfamily functioning as a ligand-regulated transcription factor. It is activated by androgens to regulate the development of the prostate gland and prostate cancer progression (3). Androgen binding to the AR induces a conformational change in the AR that leads to dissociation from the chaperone complex and phosphorylation of the receptor (4). This conformation change also facilitates the dimerization of the AR. The AR activated by androgen translocates to the nucleus where it binds to the androgen response elements (ARE) of the target genes and modulates transcriptional factors (5). Androgen deprivation therapy is the most common primary treatments for prostate cancer (6). Although most prostate cancer patients initially respond to these treatments, the cancer eventually progresses as an androgen-, or hormone-refractory cancer. Several studies have reported that the androgen refractory condition in prostate cancer is caused by mutations and overexpression of the AR (7, 8). These changes can allow the AR to be activated by low levels of androgens, other steroids, and even antiandrogens or ligand-independent mechanisms. This means hormone-refractory prostate cancer cells still depend on the AR for their growth and proliferation. Therefore, the regulation of AR would be a better therapeutic strategy than the ablation of androgen (9).

Acetylation and deacetylation of the nuclear histones are reversible epigenetic phenomena that occur through two types of enzymes, histone acetyltransferases (HATs) and histone deacetylase (HDAC). HATs acetylate at the lysine residues of the histone (H) and HDAC reverse this process (10). HDAC regulates a variety of transcription pathways by forming complexes with transcriptional repressors, co-repressors and other transcriptional factors, including unliganded nuclear hormone receptors (11).

Several studies have reported that HDAC inhibitors can suppress cell proliferation and block the G1/M or G2-phase of the cell cycle in various cancer cells (12-14). Moreover, it has been reported that HDAC inhibitors could cause apoptotic cell death in different cancer cell lines (15, 16). The transcription of the p21$^{Waf1/Cip1}$ gene has been shown to be activated through the Sp1 site of the *p21*$^{Waf1/Cip1}$ promoter by HDAC inhibitors (17). The HDAC inhibitors also increase the level of p27^{Kip1} expression (18). The inductions of *p21*$^{Waf1/Cip1}$ and *p27*^{Kip1} inhibit the formation of the cyclin E/cyclin-dependent kinase (CDK)2 complex. The cyclin E/CDK2 complex is essential for promoting cell cycle progression through G1- into the S-phase because it phosphorylates the retinoblastoma (Rb) protein on additional sites. Therefore, increased levels of p21$^{Waf1/Cip1}$ and p27^{Kip1} may induce cell cycle arrest at the G1-phase in several cancer cell lines (19, 20).

Sodium butyrate is a short-chain fatty acid that is generated from the fermentation of dietary fiber by anaerobic bacteria in the lumen of the intestine (21). It is also present in fruits, vegetables and milk fat (22). Sodium butyrate may induce cell cycle arrest and apoptosis (23). The effect of sodium butyrate might result from its function as an HDAC inhibitor (17). However, the precise mechanism of how sodium butyrate inhibits prostate cancer cell growth is not completely understood. Therefore, this study examined the effect of sodium butyrate on the regulation of AR expression and cell cycle regulatory proteins in human prostate LNCaP cancer cells.

Materials and Methods

Materials. Sodium butyrate was purchased from Sigma-Aldrich Biotechnology Incorporated (St. Louis, MO, USA). The medium and its supplements containing antibiotics, Dulbecco's Phosphate Buffer Saline (DPBS) and fetal bovine serum (FBS) were purchased from Gibco® Invitrogen Corporation (Carlsbad, CA, USA). The Western blot detection reagents were purchased from Amersham Bioscience Corporation (Little Chalfont, Bucks, UK). The polyvinylidene difluoride (PVDF) membranes were acquired from Bio-Rad (Hercules, CA, USA). All other chemical reagents were obtained from Sigma-Aldrich.

Cell lines and culture condition. The LNCaP cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells between the 10th and 30th generation were grown in Minimum Essential Medium (MEM) supplemented with 10% heat-inactivated FBS, 2.2 g/L NaHCO$_3$, 1 mM sodium pyruvate and 100 U/ml antibiotics. The cells were maintained at 37˚C in a humidified atmosphere containing 5% CO$_2$. The cells were then plated on plastic dishes and the culture medium was replaced every 2 days. During the experiments, the cells were maintained in MEM containing 5% charcoal-dextran treated FBS (CD-FBS).

Preparation of nuclear and cytosolic extracts. Nuclear and cytosolic extracts from the LNCaP cells were prepared using the following

procedure. Briefly, cells were harvested by trypsinization, washed with ice-cold PBS, centrifuged at 3,000 rpm for 5 min and incubated for 20 min in 50 μl of lysis buffer *I* (10 mM HEPES, pH 7.9, 1.5 mM MgCl$_2$, 10 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 0.5 mM dithiothreitol). The mixture was incubated for 10 min on ice and then centrifuged at 12,000 rpm for 10 min. Following collection of the supernatant which contained the cytosolic fraction, the pellet was resuspended by syringe/needle in 30 μl of ice-cold buffer II (2.75% lysis buffer *I* and 0.05% Nonidet P-40) and incubated for 20 min on ice. 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 dithiothreitol, 0.5 mM PMSF and 26% glycerol) for 40 min. After final centrifugation at 12,000 xg for 40 min, the supernatant (nuclear extract) was collected. The protein content from extracts was determined using the protein assay reagent (Bio-Rad).

MTT assay. Cell viability was determined using the microculture tetrazolium technique (MTT). The cultures were initiated in 96-well plates at a density of 2,500 cells per well. The cells were allowed to reattach for 48 h and were then exposed to various concentrations of sodium butyrate. At the end of the 48 h treatment period, 15 μl of a 5 mg/ml MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide in PBS) were added to each well. The plates were incubated in the dark for 4 h at 37˚C. The supernatant was aspirated and formazan crystals dissolved in 100 μl DMSO at 37˚C for 10 min with gentle agitation. The absorbance at 540 nm of each well was measured using a VERSA@max Microplate Reader (Molecular Devices Corp., Sunnyvale, CA, USA).

Immunofluorescence staining. The LNCaP cells were seeded on 6-well dishes, so that on the day of the experiment the cells were 50% confluent. After 48 h, the medium was replaced with fresh MEM containing 5% CD-FBS and 5 mM sodium butyrate. The cells were fixed with 4% paraformaldehyde in PBS for 30 min and then permeabilized with 0.1% Triton X-100 for 20 min at room temperature. After washing three times in PBS, the cells were blocked for 1 h with 10% goat serum in PBS and were incubated overnight with the anti-AR polyclonal antibody (diluted 1:50, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at 4˚C. After removal of the primary antibody, the cells were rinsed three times with PBS. The cells were then incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG (diluted 1:100, Invitrogen, Carlsbad, CA, USA) for 30 min in the dark. For nuclei staining, the cells were incubated with 2.5 μg/ml propidium iodide (PI) at room temperature for 3 min. The fluorescence-localized patterns of AR were visualized using a fluorescence microscope (Axiovert 200 Carl Zeiss Inc., Jena, Thurigen, Germany).

Western blot analysis. The LNCaP cells were harvested and washed twice with cold PBS. Total proteins were prepared using a PRO-PREP™ protein extract solution (iNtRON, Seongnam, Korea) and assayed using a protein assay reagent (BioRad). Fifty micrograms 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). Each sample was separated by 10-15% SDS polyacrylamide gel (PAGE), and transferred to a PVDF membrane at 100 V for 1 h in a transfer
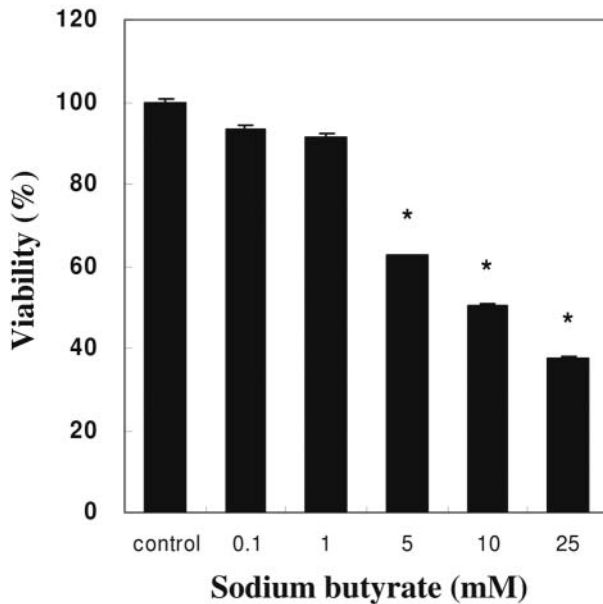
Figure 1. *Effect of sodium butyrate on the cell viability measured by the MTT assay. The LNCaP cells were cultured in 96-well dishes for 48 h and then treated with various concentrations of sodium butyrate for 48 h. Statistical significance: *p<0.05.*

Figure 2. *Effect of sodium butyrate on the acetylation of histone proteins in nuclear extracts. Nuclear extracts were isolated from the LNCaP cells treated with the indicated concentration of sodium butyrate for 48 h. Western blot analysis was performed using anti-acetylated histone H3 and anti-acetylated histone H4 antibodies. The quantitative results were represented from one of the three independent experiments.*

buffer. The membrane was blocked for 1 h with the TNT buffer (10 mM Tris-HCl, pH 7.6, 100 mM NaCl and 0.5% Tween 20) containing 5% non-fat dried milk at room temperature. The membrane was then incubated at 4°C overnight with the specific primary antibodies including anti-AR polyclonal (1:200; Santa Cruz), anti-acetylated H3 polyclonal (1:5,000; Upstate, Lake Placid, NY, USA), anti-acetylated H4 polyclonal (1:1,000; Upstate), anti-cyclin D1 polyclonal (1:1,000; Santa Cruz), anti-cyclin E polyclonal (1:200; Santa Cruz), anti-CDK2 mAb (1:200; Santa Cruz), anti-CDK4 polyclonal (1:200; Santa Cruz), anti-CDK6 polyclonal (1:200; Santa Cruz), anti-prostate-specific antigen (PSA) mAb (1:200; Santa Cruz), anti-p21 mAb (1:200; Santa Cruz), anti-p27 polyclonal (1:1,000; Santa Cruz), anti-Rb polyclonal (1:500; Santa Cruz), and anti-pRb polyclonal (1:1,000; Santa Cruz). After washing for 1 h with the TNT buffer, the membrane was incubated with horseradish peroxidase-conjugated anti-mouse antibody or anti-rabbit antibody (diluted 1:10,000, Santa Cruz) at room temperature for 30 min, and then washed for 60 min with TNT buffer. Antibody labeling was detected using enhanced chemiluminescence (ECL) plus (Amersham) detection system.

Immunoprecipitation. For immunoprecipitation, the cells were lysed in a buffer containing 150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM EGTA, pH 8.0, 0.2 mM sodium orthovanadate, 1% Triton X-100, 0.5% Nonidet P-40, 0.2 mM PMSF, 1 mM pepstatin A, and 1 mM aprotinin. Five hundred μg of protein from cell lysates were pre-cleared with protein A/G plus-Agarose (Santa Cruz), followed by immunoprecipitation using polyclonal anti-cyclin E antibodies (Santa Cruz). Immune complexes were harvested with protein A/G Agarose beads, and analyzed by SDS-PAGE. Immunodetection was carried out using
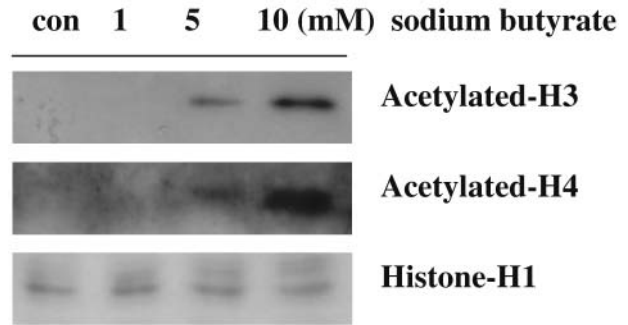
monoclonal anti-CDK2 antibody (Santa Cruz). Antibody conjugated with horseradish peroxidase was used for the secondary antibodies (Santa Cruz). Visualization of membrane-bound proteins was performed by ECL plus (Amersham) detection system.

Flow cytometry analysis. The LNCaP cells were treated with various concentrations of sodium butyrate for 48 h. The cells were then washed with PBS and harvested by trypsinization, washed in 1% bovine serum albumin (BSA) and fixed with 75% ethanol containing 0.5% Tween 20 for at least 1 h at 4°C. The cells were washed in 1% BSA and resuspended in a cold PI staining solution (100 μg/ml RNase A and 10 μg/ml PI in PBS) 1 ml for 40 min at 4°C. Data acquisition and analysis were carried out using a flow cytometry system (Beckman Coulter, Inc., Fullerton, CA, USA).

Statistical analysis. The data were represented as means ± SD of determinations from either 3 or 4 independent experiments. Statistical differences between the untreated control and the treated groups were determined using one-way analysis of variance (ANOVA).

Results

Cytotoxicity. As shown in Figure 1, low concentrations of sodium butyrate had only a minor effect on the viability of the LNCaP cells. The viability of the LNCaP cells decreased significantly at sodium butyrate concentrations of 5 and 10 mM. Based on these results, 1, 5, and 10 mM of sodium butyrate were selected as the concentrations for further experiments.

Acetylation of histone proteins. No acetylated histone H3 or H4 proteins were detected in the control culture, whereas 48 h treatment with sodium butyrate significantly increased the levels of acetylated histone H3 and H4 in a dose-dependent manner (Figure 2).
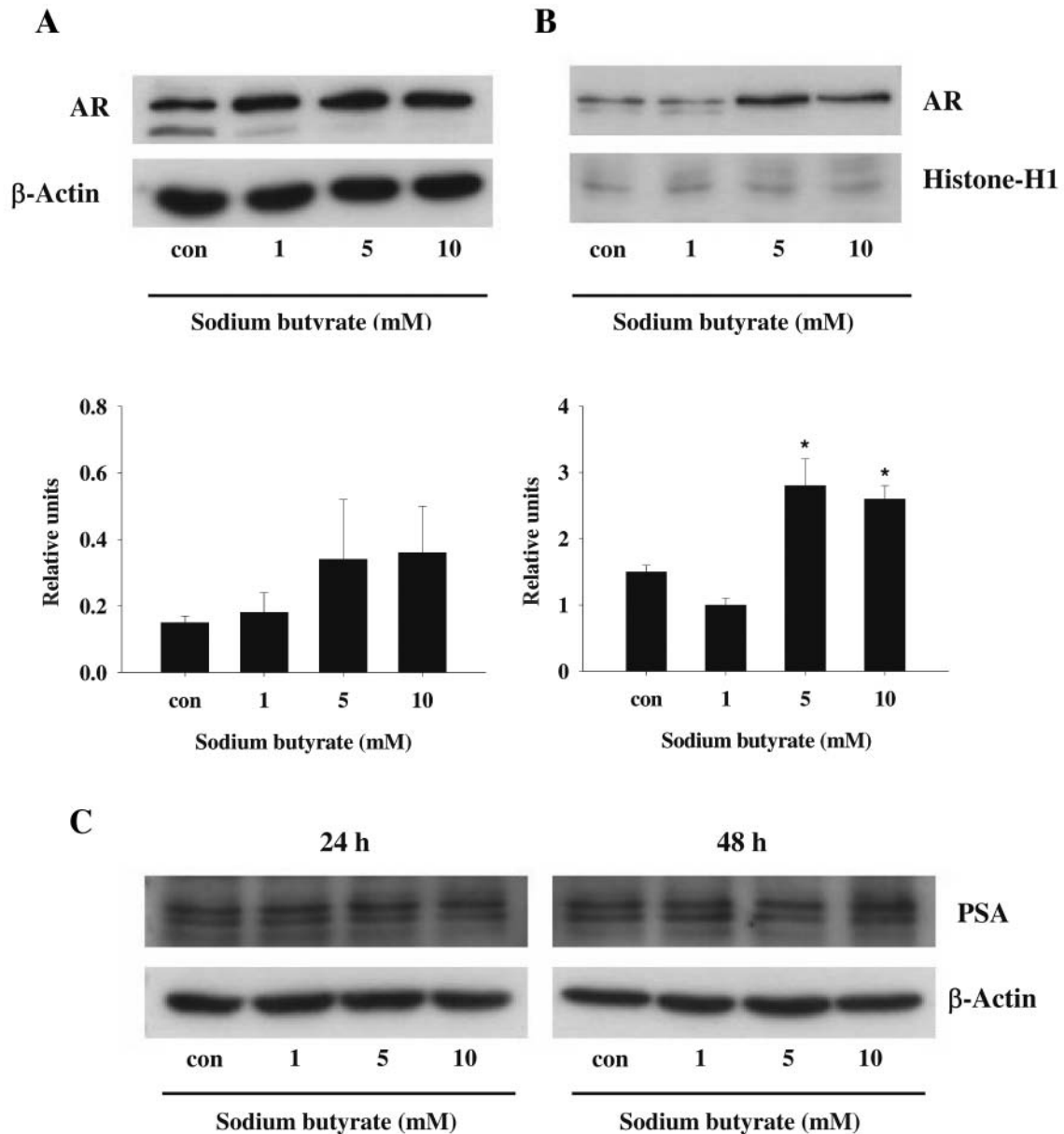
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C

Figure 3. *Effect of sodium butyrate on the expression of AR and PSA. The level of AR was measured in the cytoplasm (A) and nucleus (B) by Western blot analysis. Cells were treated with sodium butyrate for 48 h and then proteins were extracted from the cytoplasm and nucleus separately. The level of PSA was measured in the whole protein after 24 or 48 h treatment (C). The quantitative results were represented from one of the three independent experiments. Diagrams show the relative units (mean±SD) of three independent experiments. Statistical significance: *p<0.05.*

Expression of AR and PSA. The levels of AR and PSA expression were measured using Western blot analysis. In the cytoplasmic proteins AR expression levels were increased dose-dependently by sodium butyrate, but not significantly (Figure 3A). However, high concentrations (5 and 10 mM) of sodium butyrate significantly increased the levels of nuclear AR expression (Figure 3B). However, the levels of the PSA proteins were relatively constant after sodium butyrate treatment (Figure 3C).

Nuclear localization of AR. As shown in Figure 4, the AR was mainly located in the cytoplasm in the absence of sodium butyrate. After treatment with 5 mM sodium butyrate for 48 h, the AR moved into the nucleus.

Cell cycle distribution. Figure 5 shows representative histograms. Sodium butyrate caused the apparent accumulation of cells in the G1-phase. In the control culture, the cells remaining at the G1-stage of cell cycle were 60%. At 1 mM sodium butyrate, the
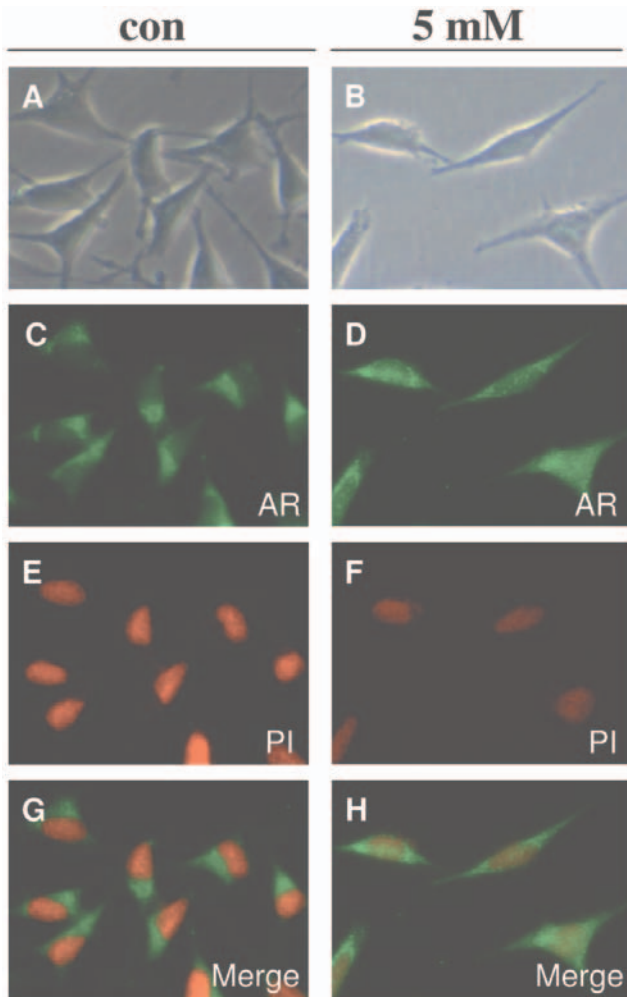
Figure 4. *Effect of sodium butyrate on cellular localization of AR. The LNCaP cells were seeded on 6-well dishes and treated with 5 mM sodium butyrate for 48 h followed by immunofluorescence staining. The green signal represents the AR and the red PI staining shows the localization of nuclei. (A, C, E and G) control, (B, D, F and H) 5 mM sodium butyrate.*

proportion of cells at the G1-phase had increased slightly, up to 68%. This profile, which is typical for cell cycle arrest at the G1-phase, was enhanced by treatment with high concentrations (5 and 10 mM) of sodium butyrate when the majority of cells were in the G1-phase (90~93%).

Expression of cell cycle regulatory proteins. Sodium butyrate significantly decreased the cyclin D1 levels in the LNCaP cells at 5 and 10 mM concentrations (Figure 6A). As expected, the levels of CDK4 and CDK6 expression were lower in the LNCaP cells treated with sodium butyrate. The expression of cyclin E and CDK2 was down-regulated by sodium butyrate treatment for 48 h. The levels of the expression of CDK inhibitors p21[Waf1/Cip1] and p27[Kip1] were higher in the LNCaP cells treated with sodium butyrate than the untreated control cells (Figure 6A). The levels of the phosphorylated Rb protein were significantly lower, even though the level of Rb did not change (Figure 6A). In addition, sodium butyrate treatment inhibited the formation of the cyclin E/CDK2 complex (Figure 6B).

Discussion

Recently, considerable attention has been focused on the use of HDAC inhibitors as anticancer agents. This is because HDAC inhibitors have the ability to induce either apoptosis or cell cycle arrest (15). HDAC inhibitors maintain the acetylation of the lysine residues of the histones and modulate the transcriptional factors of various genes. Many studies have examined the ability of HDAC inhibitors to induce the expression of genes such as *p21*[Waf1/Cip1], *p27*[Kip1], and *p53* and also induce cell cycle arrest at the G1- or G2/M-phase (24-27). In this study, it was found that sodium butyrate inhibited growth of the LNCaP cells through cell cycle arrest at the G1-phase. Like other HDAC inhibitors, sodium butyrate blocks the deacetylation of histone H3 and H4
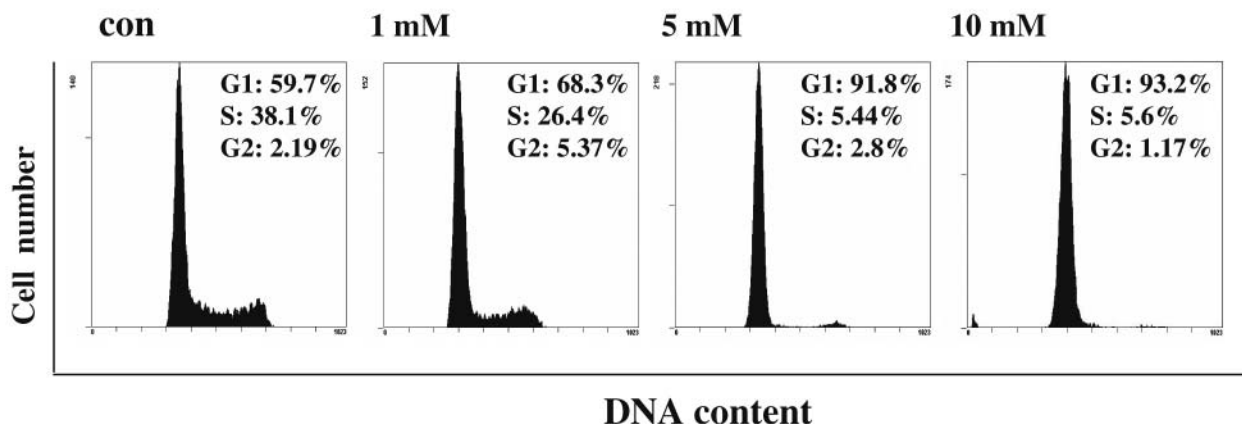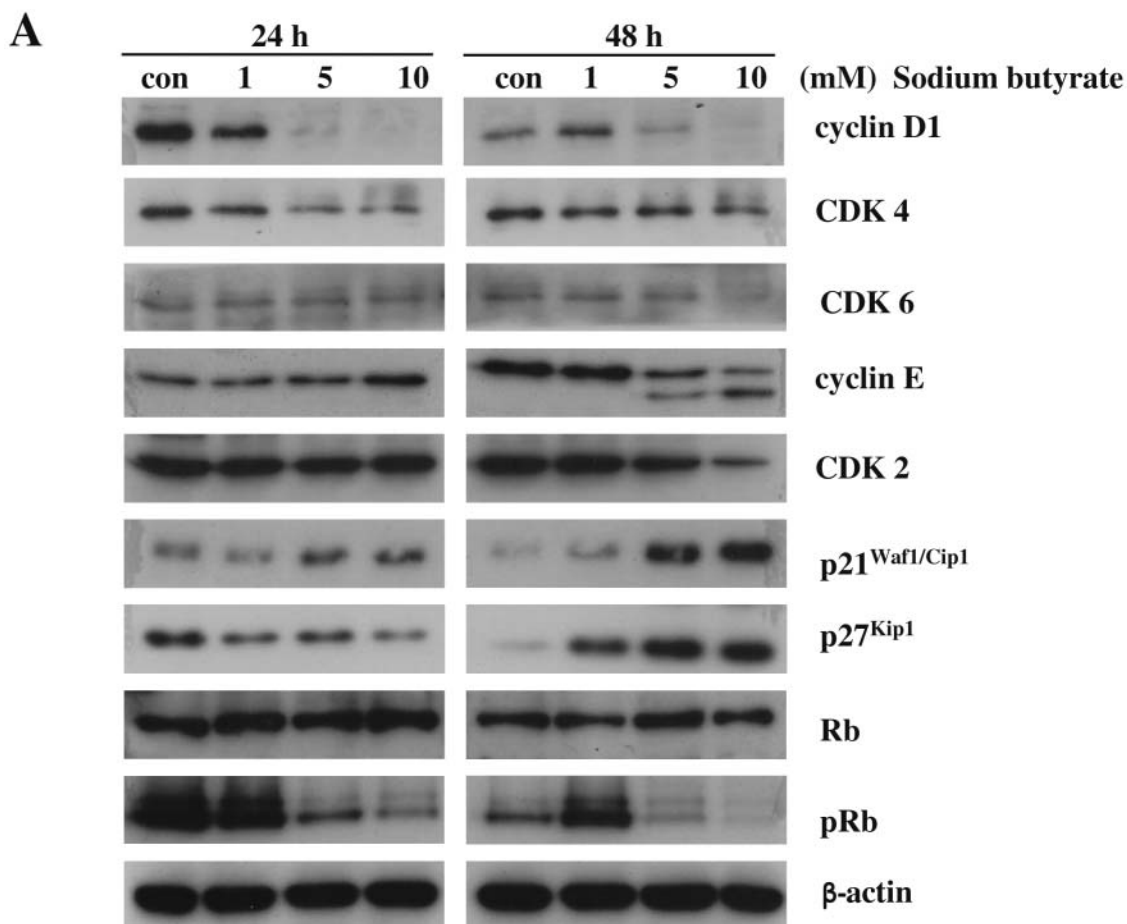

Figure 5. *Effect of sodium butyrate on the cell cycle distribution. The LNCaP cells were treated with the indicated concentrations of sodium butyrate for 48 h. After treatment, the cells were subjected to cell cycle analysis using flow cytometry.*
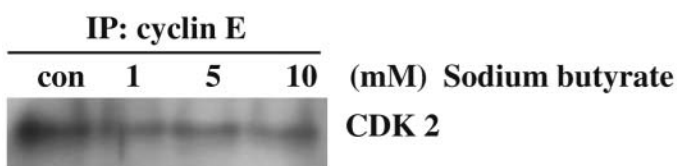
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Figure 6. *Effect of sodium butyrate on cell cycle regulatory proteins. (A) The LNCaP cells were treated with the indicated concentrations of sodium butyrate for 24 or 48 h. Fifty mg of the proteins were separated on 10 ~ 12% SDS-PAGE gels. After electrotransfer, the membranes were incubated with specific antibodies against cyclin D1, CDK4, CDK6, cyclin E, CDK2, p21$^{Waf1/Cip1}$, p27^{Kip1}, Rb, and pRb. The data is representative of at least three independent experiments. (B) The cells were treated with sodium butyrate at the indicated concentrations for 48 h. The cell lysates were immunoprecipitated with anti-cyclin E antibodies, and the protein complexes were separated by electrophoresis and probed with anti-CDK2 antibodies.*

proteins. The increase in acetylated histone proteins may influence the activation of AR-mediated transcriptional factors and cell cycle regulators.

One of the main findings in this study was that sodium butyrate regulated expression and transcriptional activity of the AR gene through an epigenetic mechanism. In general, the interaction between androgen and AR is essential for the growth and survival of primary prostate cancer cells (28). In this study, the level of nuclear AR expression was increased significantly by sodium butyrate treatment. Western blot analysis carried out to compare the subcellular expression of AR in both the cytosolic and nuclear extracts revealed that AR was significantly up-regulated in the nucleus compared with the cytoplasm. Immunofluorescence staining confirmed that sodium butyrate significantly induced the nuclear translocation of AR. This suggested that the hyperacetylation of histones by sodium butyrate might increase the transcriptional activity of AR and its co-regulators. Although many studies have reported that HDAC inhibitors alter AR- and co-regulator-mediated transcription (29-33), the molecular mechanism of HDAC inhibitors on the regulation of AR will require further analysis. In general, the ARE-

bound AR homodimers recruit co-activators, such as p160 and p300/CBP, and activate gene expression (34). The binding sites for the AR have been identified at the promoter and/or enhancer sequences of many genes, such as *PSA* (35), *probasin* (36), and $p21^{Waf/Cip1}$ (37). In particular the $p21^{Wap1/Cip1}$ gene contains a canonical ARE in its proximal promoter region (37). Increased transcription of AR by sodium butyrate has the potential to induce $p21^{Waf1/Cip1}$ expression.

Another finding of this study was that sodium butyrate mediated control of the cell cycle. Previous studies have reported that HDAC inhibitors induce cell cycle arrest at the G1-phase through the induction of $p21^{Waf1/Cip1}$ and $p27^{Kip1}$ (22, 27, 38, 39). The CDK inhibitors, $p21^{Waf1/Cip1}$ and $p27^{Kip1}$, play an important role in regulating the cell cycle through inhibition of cyclin/CDK complexes. It has been proposed that $p21^{Waf1/Cip1}$ and $p27^{Kip1}$ might be among the better candidates for gene regulating cancer chemotherapy because they are silenced in most common cancer cells (40). HDAC inhibitor dramatically increases $p21^{Waf1/Cip1}$ expression in a variety of cancer cells such as lymphoma and colon carcinoma (41,42). Increased $p21^{Waf1/Cip1}$ and $p27^{Kip1}$ levels retard the progression of the cell cycle due to an insufficient amount of cyclin/CDK complexes (20). In addition, the HDAC inhibitors decrease the level of cyclins and CDKs in the majority of cells. In agreement with previous data, the growth inhibition of the LNCaP cells by sodium butyrate was associated with increases in the levels of $p21^{Waf1/Cip1}$ and $p27^{Kip1}$. The formation of the cyclin E/CDK2 complexes also decreased after sodium butyrate treatment. Furthermore, sodium butyrate significantly down-regulated the cell cycle regulatory proteins cyclin D1, CDK4, CDK6, cyclin E, CDK2 and pRb. Overall, sodium butyrate may induce cell cycle arrest at the G1-phase, altering the cell cycle regulators.

In conclusion, the HDAC inhibitor, sodium butyrate, influences the AR transcriptional activity through the accumulation of acetylated histones. The transactivation of AR might up-regulate the expression of the $p21^{Waf1/Cip1}$ gene. Further studies will be needed to determine the precise mechanism of HDAC inhibitors on the regulation of AR expression. In addition, sodium butyrate induces the expression of the $p21^{Waf1/Cip1}$ and $p27^{Kip1}$ proteins which affects the cell cycle regulatory proteins and inhibits the formation of their complexes. Eventually, sodium butyrate arrests the cell cycle at the G1-phase and effectively inhibits the proliferation of prostate cancer cells. Therefore, sodium butyrate is a potential chemotherapeutic agent for prostate cancer, particularly for hormone-refractory prostate cancer.

Acknowledgements

References

1　Park SK, Sakoda LC, Kang D, Chokkalingam AP, Lee E, Shin HR, Ahn YO, Shin MH, Lee CW, Lee DH, Blair A, Devesa SS and Hsing AW: Rising prostate cancer rates in South Korea. Prostate *66*: 1285-1291, 2006.

2　Korea National Statistical Office, Korean Statistical Information System (KOSIS), Annual report on the cause of death statistics, 1983-2003. Seoul, Korea, 2005. [http://kosis.nso.go.kr:7001/ups/chapterRetrieve.jsp?pubcode=MA&seq=241&pub=3]

3　Culig Z, Hobisch A, Hittmair A, Peterziel H, Cato AC, Bartsch G and Klocker H: Expression, structure, and function of androgen receptor in advanced prostatic carcinoma. Prostate *35*: 63-70, 1998.

4　Wang LG, Liu XM, Kreis W and Budman DR: Phosphorylation/dephosphorylation of androgen receptor as a determinant of androgen agonistic or antagonistic activity. Biochem Biophys Res Commun *259*: 21-28, 1999.

5　Kang Z, Janne OA and Palvimo JJ: Coregulator recruitment and histone modifications in transcriptional regulation by the androgen receptor. Mol Endocrinol *18*: 2633-2648, 2004.

6　Miyamoto H, Messing EM and Chang C: Androgen deprivation therapy for prostate cancer: current status and future prospects. Prostate *61*: 332-353, 2004.

7　Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL and Visakorpi T: Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res *61*: 3550-3555, 2001.

8　Agoulnik IU and Weigel NL: Androgen receptor action in hormone-dependent and recurrent prostate cancer. J Cell Biochem *99*: 362-372, 2006.

9　Zegarra-Moro OL, Schmidt LJ, Huang H and Tindall DJ: Disruption of androgen receptor function inhibits proliferation of androgen-refractory prostate cancer cells. Cancer Res *62*: 1008-1013, 2002.

10　Monneret C: Histone acetylase inhibitors. Eur J Med Chem *40*: 1-13, 2005

11　Roy S, Packman D, Jeffrey R and Tenniswood M: Histone deacetylase inhibitors differentially stabilize acetylated p53 and induce cell cycle arrest or apoptosis in prostate cancer cells. Cell Death Differ *12*: 482-491, 2005.

12　Lee KW, Kim JH, Park JH, Kim HP, Song SH, Kim SG, Kim TY, Jong HS, Jung KH, Im SA, Kim TY, Kim NK and Bang YJ: Antitumor activity of SK-7041, a novel histone deacetylase inhibitor, in human lung and breast cancer cells. Anticancer Res *26*: 3429-3438, 2006.

13　Beklemisheva AA, Fang Y, Feng J, Ma X, Dai W and Chiao JW: Epigenetic mechanism of growth inhibition induced by phenylhexyl isothiocyanate in prostate cancer cells. Anticancer Res *26*: 1225-1230, 2006.

14　Alao JP, Lam EW, Ali S, Buluwela L, Bordogna W, Lockey P, Varshochi R, Stavropoulou AV, Coombes RC and Vigushin DM: Histone deacetylase inhibitor trichostatin A represses estrogen receptor α-dependent transcription and promotes proteasomal degradation of cyclin D1 in human breast carcinoma cell lines. Clin Cancer Res *10*: 8094-8104, 2004.

15　Sawa H, Murakami H, Ohshima Y, Sugino T, Nakajyo T, Kisanuki T, Tamura Y, Satone A, Ide W, Hashimoto I and Kamada H: Histone deacetylase inhibitors such as sodium butyrate and trichostatin A induce apoptosis through an increase of the bcl-2 related protein bad. Brain Tumor Pathol *18*: 109-114, 2001.

16 Jung JW, Cho SD, Ahn NS, Yang SR, Park JS, Jo EH, Hwang JW, Jung JY, Kim SH, Kang KS and Lee YS: Ras/MAP kinase pathways are involved in Ras specific apoptosis induced by sodium butyrate. Cancer Lett 225: 199-206, 2005.

17 Davie JR: Inhibition of histone deacetylase activity by butyrate. J Nutr 133: 2485S-2493S, 2003.

18 Chen JS and Faller DV: Histone deacetylase inhibition-mediated post-translational elevation of p27^{Kip1} protein levels is required of G1 arrest in fibroblasts. J Cell Physiol 202: 87-99, 2005.

19 Ouwehand K, de Ruijter AJ, van Bree C, Caron HN and van Kuilenburg AB: Histone deacetylase inhibitor BL1521 induces a G1-phase arrest in neuroblastoma cells through altered expression of cell cycle proteins. FEBS Lett 579: 1523-1528, 2005.

20 Abramova MV, Pospelova TV, Nikulenkov FP, Hollander CM, Fornace AJ Jr and Pospelov VA: G1/S arrest induced by histone deacetylase inhibitor sodium butyrate in E1A Ras-transformed cells is mediated through down-regulation of E2F activity and stabilization of β-catenin. J Biol Chem 281: 21040-21051, 2006.

21 Mai A, Massa S, Rotili D, Cerbara I, Valente S, Pezzi R, Simeoni S and Ragno R: Histone deacetylation in epigenetics: an attractive target for anticancer therapy. Med Res Rev 25: 261-309, 2005.

22 Chopin V, Toilon RA, Jouy N and Le Bourhis X: P21$^{WAF1/CIP1}$ is dispensable for G1 arrest but indispensable for apoptosis induced by sodium butyrate in MCF-7 breast cancer cells. Oncogene 23: 21-29, 2004.

23 Finzer P, Kuntzen C, Soto U, zur Hausen H and Rosl F: Inhibitors of histone deacetylase arrest cell cycle and induce apoptosis in cervical carcinoma cells circumventing human papillomavirus oncogene expression. Oncogene 20: 4768-4776, 2001.

24 Boutillier AL, Trinh E and Loeffler JP: Selective E2F-dependent gene transcription is controlled by histone deacetylase activity during neuronal apoptosis. J Neurochem 84: 814-828, 2003.

25 Hirsch CL and Bonham K: Histone deacetylase inhibitors regulate p21^{WAF1} gene expression at the post-transcriptional level in HepG2 cells. FEBS Lett 570: 37-40, 2004.

26 Caldon CE, Daly RJ, Sutherland RL and Musgrove EA: Cell cycle control in breast cancer cells. J Cell Biochem 97: 261-274, 2006.

27 Zhao Y, Lu S, Chai G, Wang H, Chen Y, Sun J, Yu Y, Zhou W, Zheng Q, Wu M, Otterson GA and Zhu WG: Acetylation of p53 at lysine 373/382 by the histone deacetylase inhibitor depsipeptide induces expression of p21$^{Waf1/Cip1}$. Mol Cell Biol 26: 2782-2790, 2006.

28 Burnstein KL: Regulation of androgen receptor levels: implications for prostate cancer progression and therapy. J Cell Biochem 95: 657-669, 2005.

29 Korkmaz CG, Fronsda K, Zhang Y, Lorenzo PI and Saatcioglu F: Potentiation of androgen receptor transcriptional activity by inhibition of histone deacetylation-rescue of transcriptionally compromised mutants. J Endocrinol 182: 377-389, 2004.

30 Thelen P, Schweyer S, Hemmerlein B, Wuttke W, Seseke F and Ringert RH: Expressional changes after histone deacetylase inhibition by valproic acid in LNCaP human prostate cancer cells. Int J Oncol 24: 25-31, 2004.

31 Gaughan L, Logan IR, Neal DE and Robson CN: Regulation of androgen receptor and histone deacetylase 1 by Mdm2-mediated ubiquitylation. Nucleic Acids Res 33: 13-26, 2005.

32 Rokhin OW, Glover RB, Guseva NV, Taghiyev AF, Kohlgraf KG and Cohen MB: Mechanisms of cell death induced by histone deacetylase inhibitors in androgen receptor-positive prostate cancer cells. Mol Cancer Res 4: 113-123, 2006.

33 Xia Q, Sung J, Chowdhury W, Chen CL, Hoti N, Shabbeer S, Carducci M and Rodriguez R: Chronic administration of valproic acid inhibits prostate cancer cell growth in vitro and in vivo. Cancer Res 66: 7237-7244, 2006.

34 Kim J and Coetzee GA: Prostate specific antigen gene regulation by androgen receptor. J Cell Biochem 93: 233-241, 2004.

35 Sang Y, Myers M and Brown M: Formation of the androgen receptor transcription complex. Mol Cell 9: 601-610, 2002.

36 Yeung LH, Read JT, Sorenson P, Nelson CC, Jia W and Rennie PS: Identification and characterization of a prostate-specific androgen-independent protein-binding site in the probasin promoter. Biochem J 371: 843-855, 2003.

37 Lu S, Liu M, Epner DE, Tsai SY and Tsai MJ: Androgen regulation of the cyclin-dependent kinase inhibitor p21 gene through an androgen response element in the proximal promoter. Mol Endocrinol 13: 376-384, 1999.

38 Ju R and Muller MT: Histone deacetylase inhibitors activate p21^{WAF1} expression via ATM. Cancer Res 63: 2891-2897, 2003.

39 Wallick CJ, Gamper I, Thorne M, Feith DJ, Takasaki DY, Wilson SM, Seki JA, Pegg AE, Byus CV and Bachmann AS: Key role for p27^{Kip1}, retinoblastoma protein Rb, and MYCN in polyamine inhibitor-induced G1 cell cycle arrest in MYCN-amplified human neuroblastoma cells. Oncogene 24: 5606-5618, 2005.

40 Sanchez-Beato M, Saez AI, Navas IC, Algara P, Sol Mateo M, Miluendas R, Camacho F, Sanchez-Aguilera A, Sanchez E and Piris MA: Overall survival in aggressive B-cell lymphomas is dependent on the accumulation of alterations in p53, p16, and p27. Am J Pathol 159: 205-213, 2001.

41 Chen YX, Fang JY, Zhu HY, Lu R, Cheng ZH and Qiu DK: Histone acetylation regulates p21WAF1 expression in human colon cancer cell lines. World J Gastroenterol 10: 2643-2646, 2004.

42 Wang YF, Chen NS, Chung YP, Chang LH, Chiou YH and Chen CY: Sodium butyrate induces apoptosis and cell cycle arrest in primary effusion lymphoma cells independently of oxidative stress and p21$^{CIP1/WAF1}$ induction. Mol Cell Biochem 285: 51-59, 2006.