

Transcriptional Activation and Repression of Cell Cycle Regulatory Molecules by Trichostatin A

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The dihydrofolate reductase (*dhfr*) promoter contains cis-acting element for the transcription factors Sp1 and E2F. Transcription of *dhfr* gene shows maximal activity during the G1/S phase of cell cycle. The member of the Sp1 transcriptional factor family can act as both negative and positive regulators of gene expression. There was a report that Sp1-Rb and E2F4-p130 complexes cooperate to establish stable repression of *dhfr* gene expression in CHO400 cells. Here, we examined the role of HDAC in *dhfr*, cyclin E, and cyclin A gene regulation using the histone deacetylation inhibitor, trichostatin A (TSA) in U2OS and C33A cells, a Rb-positive human osteosarcoma cell line, and a Rb-negative cervical carcinoma cell line, respectively. When the *dhfr* promoter constructs were applied in U2OS cells, TSA markedly stimulated over 14-fold of *dhfr* promoter activity through *dhfr*-Sp1 sites by the deletion of an E2F element. In contrast, the deletion of *dhfr*-Sp1 binding sites completely abolished promoter stimulation by TSA. The *dhfr* promoter activity including *dhfr*-Sp1 sites increased only 2-fold in C33A cells. Promoter activity containing only *dhfr*-E2F site did not have much effect by the treatment of TSA in both U2OS and C33A cells. On the other hand, treatment with TSA induced significantly mRNA expression of *dhfr* and cyclin E, whereas levels of cyclin A decreased in U2OS cells, but had no effect in C33A cells. These results indicate that TSA have contradictory effect, activation of *dhfr* and cyclin E genes on G1 phase, and down-regulation of cyclin A on G2 phase through transcriptional regulation in U2OS cells.

Key words – Trichostatin A, Sp1, E2F, Osteosarcoma, Cervical *dhfr*

There is a close association between histone deacetylase (HDAC) activity and repression of transcription in tumors, therefore the inhibitors of HDAC are emerging as an new important class of potential anticancer agents [20,24,35,41]. Trichostatin A (TSA) is a potent specific inhibitor of HDAC activity through the mechanism that involves Zn⁺ ion chelation [13]. In general, HDAC is inhibited by accumulation of acetylated histone protein. In the past several years, studies of HDAC inhibitor have shown that TSA induces cell cycle arrest, differentiation, and apoptotic cell death in a number of cancers [9, 12, 34]. However, inhibition of HDAC activity that leads to cell cycle arrest and differentiation is an area of intense investigation.

Histones are core proteins of nucleosomes and acetylation of nuclear histones is regulated by histone acetyltransferase and HDAC [23, 37]. Binding of transcriptional factors to DNA

recruits histone acetyltransferase proteins that lead to the acetylation of core histone, enhance nucleosomal relaxation, and subsequently induce transcription [28]. On the other hand, several transcriptional factors, such as CCAAT binding factor (CBF) [21], hormone-dependent nuclear receptors [15], and Mad [22] can bind to HDAC, which stabilize nucleosomal structure and represses transcription [8]. Thus, histone acetylation is known to be associated with transcriptional activity in eukaryotic cells. For example, HDAC inhibitors induce the expression of cyclin-dependent kinase inhibitor p21WAF1/Cip1 [27, 30, 17, 14], which causes cell growth arrest in various tumor cell lines. Dihydrofolate reductase (*dhfr*) catalyzes the synthesis of purines, thymidylate and glycine, therefore it is necessary for the replication of DNA in the S phase of the cell cycle. Although transcription of the *dhfr* gene increases dramatically at the G1/S phase boundary [10, 11, 31], *dhfr* mRNA is present throughout the cell cycle [16, 31], accounting for a steadily increasing *dhfr* enzymic activity [19]. Considerable efforts have been made to identify the constitutive factors that

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control the *dhfr* promoter, and the factors that confer cell-cycle regulation on this gene. In this regard, the *dhfr* promoter contains transcriptional binding site for the transcription factors Sp1 and E2F. It has been demonstrated that distinct GC boxes, responsible for Sp1 binding, direct transcription at two principal transcriptional initiation sites in rodents [3,7]. Only the most proximal GC box, at least in hamster cells, is needed to correctly initiate transcription at the major proximal site. Deletion of this GC box abolishes transcription of the *dhfr* gene [6], despite the presence of the E2F-binding element just downstream of the major transcriptional start. It has also been shown that E2F from human cells, either in nuclear extracts or purified, is able to binds to its recognition sequence in the murine [26] and hamster [2,36] *dhfr* genes, and that the E2F site is required for growth regulation [4,25]. Transcription of the *dhfr* is low in quiescent cells and is activated after pRb family protein phosphorylation during growth stimulation [38,39]. In some settings the overlapping *dhfr* E2F sites alone are sufficient for induction of transcription after the G1 restriction point. Other studies suggested that the reiterated Sp1 sites, which are required for selection of transcriptional start sites and which regulate basal levels of expression, contribute to activation of *dhfr* during growth stimulation [1,18,29].

It was demonstrated that repression of *dhfr* gene expression by E2F and Sp1 in the presence of low serum can be temporarily alleviated by TSA [5]. HDAC1 was recruited to the *dhfr* gene promoter during withdrawal from the cell cycle, and they showed that complexes of Sp1/pRB and E2F4/p130 act together over time to mediate repression of *dhfr* gene expression in CHO400 cells .

In this study, we investigated the potential function between cell cycle regulatory molecules and HDAC as transcriptional repressor during the growth arrest in retinoblastoma (Rb)-positive human osteosarcoma, U2OS and Rb-negative human cervical carcinoma, C33A cells. Using the *dhfr* promoter as a model system of the transcriptional regulation, we have shown that HDAC1 can mediate transcriptional repression through the Sp1 binding site in *dhfr* promoter in both cells. Although it is not the related proteins of the whole cell cycle, the results of this study indicate that TSA causes an up-regulation of the gene expression such as G1 function genes, *dhfr* and cyclin E, but down-regulation of the gene expression of G2 function gene, cyclin A.

Material and methods

Cell culture

Human osteosarcoma (U2OS) and human cervical carcinoma (C33A) cells were cultured in Dulbecco' modified Eagle' medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotic-antimycotic ($1 \times$) at 37°C under humidified conditions of 95%air/5%CO₂. Trichostatin A (TSA, Sigma) was added to the culture medium of U2OS, or C33A, in what we define hereafter

Trichostatin A was added to the culture medium at 0, 10, 50 and 100 nM concentrations for 24 h.

Plasmids

The pGL3-DHFR-WT, the reporter plasmid containing 230 bp of upstream sequence of *dhfr* promoter was constructed using PCR amplification as previously described [5]. Briefly, the pGL3-DHFR-WT reporter plasmid reporter contains 230 bp of upstream sequence of the *dhfr* promoter that includes the transcription start site, the overlapping E2F elements, and the Sp1 binding sites (GC boxes I-IV). The pGL3-DHFR-Sp1 luciferase reporter includes nucleotides -230 to -85, which contain GC boxes I to IV, but not the E2F sites. The pGL3-DHFR-E2F luciferase reporter contains the two overlapping E2F sites alone. The pCMV-Sp1, pCMV-E2F1, pCMV-DP1 and pCMV-HDAC-Flag plasmids were kind gift of Dr. P. Farnham [32].

Flow cytometry analysis

The cell cycle progression of U2OS and C33A cells following TSA treatment was analyzed by flow cytometry. Approximately 2×10^6 cells were trypsinized, collected, and washed with phosphate buffered saline (PBS) containing 2% FBS. The cells were suspended in 100 μ l PBS and then 200 μ l of 95% ethanol was added to the cells while vortexing. The cells were incubated at 4°C for 2 h, washed with PBS, and re-suspended with 12.5 μ g RNase in 250 μ l of 1.12% sodium citrate buffer (pH 8.45). Incubation was continued at 37°C for 30 min before staining of the cellular DNA with 250 μ g propidium iodide (50 μ g/ml) for 30 min at room temperature. The stained cells were analyzed on a FACScalibur system (BD Biosciences, Palo Alto, USA) for relative DNA content, based on increased red fluorescence.

RT-PCR analysis

Total RNA was extracted with RNazolB (Life Technology, Grand Island, NY, USA), and suspended in 50 μ l of Diethyl Pyrocarbonate (DEPC) treated water. For the first strand of cDNA synthesis, 3 μ g of total RNA was transcribed using an oligo-dT primer (Promega, Madison, WI, USA) and SuperScript RNase H-Reverse Transcriptase (Promega, Madison, WI, USA) at 42°C for 1 h. The mRNAs of genes of interest were amplified for 35 cycles from 10% of the total first strand cDNA. PCR primers are described in Table 1. The following primer pairs were used to amplify cDNA for cyclin A, cyclin E, DHFR or GAPDH respectively. PCR products were analyzed by agarose gel electrophoresis and visualized by ethidium bromide.

Immunoblotting

Cellular lysates were prepared by suspending 3×10^5 cells/35 mm dish in 30 ml of lysis buffer [50 mM Tris, 150 mM NaCl, 5 mM EDTA, 1 mM DTT, 0.5% NP-40, 100 mM phenylmethylsulfonyl fluoride, 20 mM aprotinin, and 20 mM leupeptin, adjusted to (pH 8.0)]. The cells were disrupted and extracted at 4°C for 30 min. The proteins were electrotransferred to Immobilon P-membranes (Millipore Corporation, MA, USA). Detection of specific proteins was carried out with an enhanced chemiluminescence Western blotting kit following the manufacturer's instructions (Amersham, NJ, USA).

Table 1. Primer sequences used for RT-PCR of cyclin A, cyclin E and DHFR expression

Cyclin A (466bp)	5'-TCCAAGAGGACCAGGAGAATATCA-3'	sense
	5'-TCCTCATGGTAGTCTGGTACTTCA-3'	antisense
Cyclin E (474bp)	5'-AGTTCTCGGCTCGCTCCAGGAAGA-3'	sense
	5'-TCTTGTGTCGCCATATACCGGTCA-3'	antisense
DHFR (300bp)	5'-CAGAGAACTCAAGGAACCTCCAC-3'	sense
	5'-TTAATGCCTTTCICCTCTGGAC-3'	antisense
GAPDH (983bp)	5'-TCGGAGTCAACGGATTGG-3'	sense
	5'-AACTGTGAGGAGGGGAGATTACAG-3'	antisense

Ten microliter aliquots of each 50 μ l PCR product was resolved on a 2% agarose gel and the gel was stained with ethidium bromide. DNA fragments were visualized on a UV-illuminator.

Transfection and reporter gene assays.

Cells were plated at 3×10^5 cells per 60 mm-diameter culture dishes, and transfection was performed by calcium phosphate coprecipitation method as described previously [5], or with Lipofectamine™ (molar ratio DNA:lipid=1:3) (Gibco BRL, NY, USA) according to manufacturer's instruction. Each transfection contained a total of 4.8 μ g of DNA, including 2.4 μ g of reporter plasmid and 2.4 μ g of salmon sperm carrier DNA per dish, for calcium phosphate coprecipitation method, and 2 μ g DNA per dish in the Lipofectamine. To analyze luciferase expression, cells were washed twice with PBS and lysed with 200 μ l of 1 \times Reporter lysis buffer (Promega, Madison, WI, USA). Each lysate (50 μ l) was examined for luciferase activity.

Antibodies

Primary antibodies to cyclin A (C-19), cyclin E (HE111), cyclin B1 (GNS1), cdc25C (C-20), Sp1 (Pep-2), HDAC1 (H-51) and actin (C-11) were purchased from Santa Cruz Biotechnology.

Results

TSA blocks cell growth and proliferation at G2/M phase of human osteosarcoma, U2OS and human cervical carcinoma, C33A cells

Proliferation of both U2OS and C33A cells were significantly inhibited by TSA during 2 days. After addition of 100 nM TSA, U2OS and C33A cells underwent a remarkable time-dependent morphological change, U2OS cells showed cytoplasmic extensions and elongation and C33A cells showed rounding shape (Fig. 1). To confirm the effect of TSA on cell cycle, FACS analysis was performed. After treatment for 24 h culture with various concentrations of TSA ranging 0, 10, 50 and 100 nM, the population of U2OS cells in G2/M phase was increased from 15% to 66.8% by 100 nM TSA, while that in G1 phase was decreased from 61.6% to 10% at this content (Fig. 2A). FACS analysis results showed that the TSA treated cells progressed through G1/S phase, but then encountered a G2/M phase block. In the case of C33A cells, cell population increased from 17.1% to 46.5% in G2/M phase and remained accumulation of that in G1 after 24 h of treatment with 100 nM TSA (Fig. 2B).

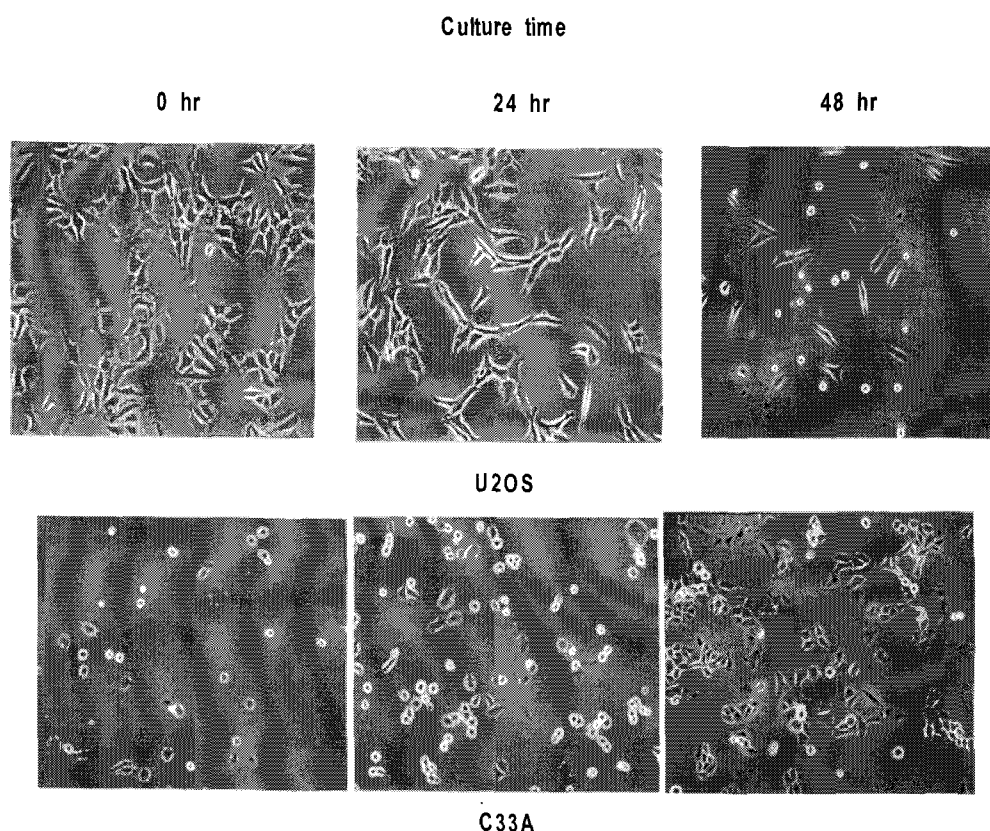


Fig. 1. Morphological change of U2OS and C33A cells by TSA treatment.

U2OS and C33A cells were grown in culture dishes in the presence of TSA (100 ng/ml; 24 h) and then followed for 2 days

Effect of TSA on the expression of cell cycle regulators

We examined the effect of TSA on the protein expression of various cell cycle regulators in U2OS and C33A cell lines. The dose dependent effect of TSA on the expression of various cell cycle regulators at the protein levels is shown in Figure 3. Western blot analysis demonstrated that TSA enhanced the protein expression of cyclin E in U2OS and C33A cells, while it reduced the expression of cyclin A, cyclin B, and cdc25C proteins in U2OS cells. However, the protein expression of cyclin B and cdc25C were not altered, whereas expression of cyclin A decreased by TSA in C33A cells. Thus, the expression changes of cyclin A, cyclin B and cdc25C by TSA in the U2OS cells may attribute to a block in cell cycle progression. On the other hand, TSA did not affect the protein expression of cyclin B and cdc25C in C33A cells. No change was observed in the expression of Sp1 and HDAC1 protein in both of U2OS and C33A cells.

TSA significantly increases the *dhfr* and cyclin E mRNA level but decrease cyclin A in U2OS cells.

We examined the effects of TSA on mRNA levels of *dhfr* and cell cycle-regulated genes including cyclin A and cyclin E. RT-PCR assays were performed to examine changes of *dhfr*, cyclin E and cyclin A mRNA expression which observed at 24 h after TSA treatment (Fig. 4). TSA treatment (100 nM) led to a significant induction of *dhfr* and slight induction cyclin E mRNA expression in U2OS cells but no change in C33A cells. In contrast, the mRNA level of cyclin A was decreased in U2OS cells whereas expression of *dhfr* and cyclin E concomitantly increased after TSA treatment. In C33A cells, the same treatment did not affect the level of cyclin A expression. The mRNA levels of cyclin E and cyclin A were consistent with the results of protein expression level in U2OS cells (Fig. 3), We are found down-regulated gene expression by TCA in endogenous cell cycle proteins

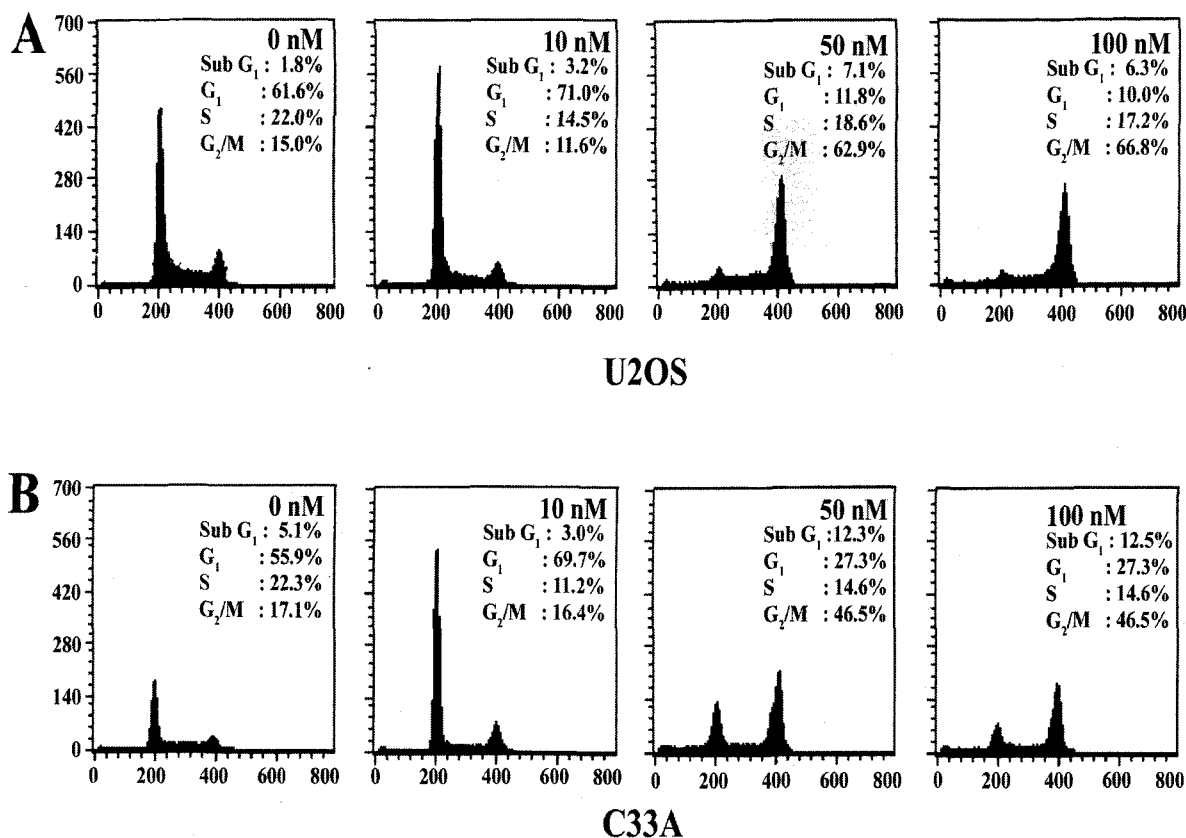


Fig. 2. Effect of TSA on cell cycle distributions in U2OS and C33A cells.

U2OS and C33A cell lines were cultured in the presence of 0, 10, 50 and 100 nM TSA for 24 h. The DNA content of cells was analyzed by a FACScan flow cytometer. Data were confirmed in three independent experiments.

Differential sensitivity of the *dhfr* Sp1 sites to TSA in U2OS and C33A cells.

In contrast to the other E2F-dependent promoters studied in Figure 3 and 4 (e.g., cyclin E, cyclin A, and cdc25C), *dhfr* is unique in that it contains binding sites for Sp1. To investigate the role of E2F and Sp1 in *dhfr* transcription to the sensitivity of TSA, we used a series of model promoters linked to luciferase reporter genes. Schematics of the wild-type and mutated *dhfr*-luciferase reporter constructs containing Sp1 and E2F elements of the wild-type position are shown in Figure 5. Cells were transfected with luciferase reporter plasmid containing the wild-type promoter (*dhfr*-wt-luc), the four upstream Sp1 sites only (*dhfr*-Sp1-luc), or two overlapping and inverted E2F sites only (*dhfr*-E2F-luc) and then treated with TSA at 100 nM for 24 h. Although these reporters expressed relatively low levels of luciferase activity when transiently expressed in log phase cells, TSA treatment led to about 14-fold more activation of transcriptional activity of containing the re-

iterated *dhf*-Sp1 sites and even the *dhfr*-WT promoter exhibited 10-fold activation of transcriptional activity. Otherwise, two overlapping and inverted *dhfr*-E2F sites promoter showed low basal activity and only a 1.3-fold enhancement was observed in cells treated with TSA (Fig 6A). These results suggested that the Sp1 sites were responsible for the majority of *dhfr* promoter activity, and that the *dhfr* promoter was negatively regulated by TSA-sensitive HDAC activity in U2OS cells. These data also indicated that E2F acts to repress Sp1-dependent transcription. In contrast to U2OS cells, the effect of TSA on the activation of *dhfr* promoter was marginal in C33A cells. Figure 6B showed that Luciferase activity increased 1.8 fold in C33A with *dhfr*-Sp1 and *dhfr*-WT promoter cells but *dhfr*-E2F promoter did not affect the luciferase activity by TSA. To examine the *dhfr* promoter specificity of TSA regulation, pGL3-Basic reporter plasmids was tested for TSA regulation. Transcriptional activity of pGL3-Basic was not affected by treatment with TSA, suggesting that this

regulation is promoter specific (data not shown).

HDAC activity is required for only Sp1 to repress DHFR gene expression in standard condition

We examined the ability of Sp1 to recruit HDAC1. U2OS and C33A cells were transfected with *dhfr*-Sp1-luc or *dhfr*-E2F-luc together with pCMV-Sp1, pCMV-E2F1, pCMV-DP1, or pCMV-HDAC-Flag and we analyzed repression and activation of *dhfr* transcriptional activity. HDAC1 alone had over 2.5-fold repressive effect on the *dhfr*-Sp1 promoter activity by transient transfection, while Sp1 expression increased about 8-folds of the *dhfr*-Sp1 promoter activity in U2OS cells (Fig. 7A). On the other hand, Sp1 expression increased *dhfr*-Sp1 promoter activity only 2-3 fold in C33A cells, while HDAC1 expression did not change the promoter activity (Fig. 7C). These results showed that HDAC1 can mediate Sp1 transcriptional activity of the *dhfr* promoter in U2OS cells. Otherwise, when the *dhfr*-E2F reporter was cotransfected with expression vectors for E2F1 and DP1, luciferase activity of *dhfr*-E2F increased about 2.7-fold in U2OS cells, showing that the con-

struct was responsive to E2F. However, *dhfr*-E2F promoters containing only two overlapping and inverted E2F binding site had no effect on the level of luciferase activity by HDAC1, suggesting that HDAC1 binds to Sp1 than major factor, E2F in the regulation of the *dhfr* promoter in U2OS cells, *dhfr* promoter activation appears to require Sp1 elements (Fig. 7B). On the other hand, in Rb null C33A cells, the luciferase activity of cotransfected *dhfr*-E2F reporter with E2F1 and DP1 significantly increased over 4.5 fold but not changed by HDAC1 expression (Fig. 7D). In support of this interpretation, others have shown that ectopic expression of Rb from a tetracycline-regulated promoter for 24 h down-regulates *dhfr* transcription in cycling U2OS cells and this inhibition is relieved by TSA [40]. We also examined whether the Rb affects the transcriptional regulation mediated by Sp1/HDAC1 complexes through *dhfr*-Sp1 site. The expression of Rb did not change the *dhfr*-Sp1 promoter activity suggesting that in combination with Sp1, HDAC1 and Rb in *dhfr*-Sp1 site may not cooperate the transcription in at least U2OS cells (data not shown). The expression levels of Sp1, E2F, DP1 and Flag-tagged HDAC1 were monitored by western blot analysis with the antibody for Sp1 and the Flag-specific antibody for HDAC1 (data not shown).

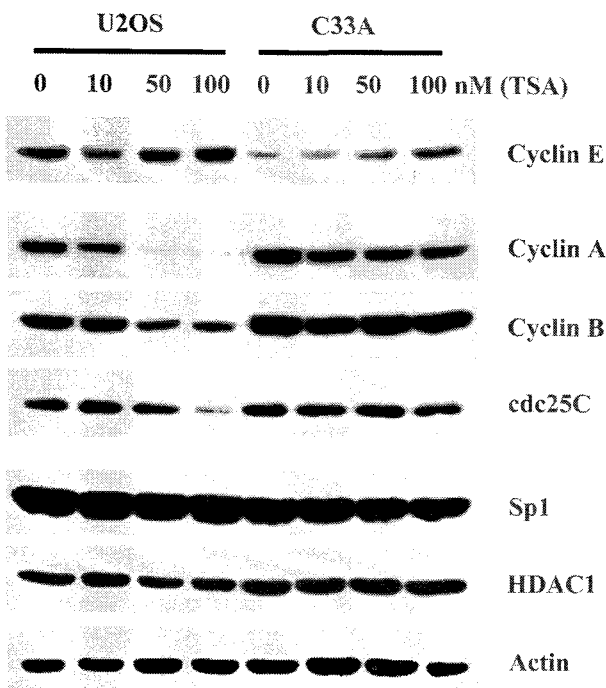


Fig. 3. Effect of trichostatin A on the expression of cell cycle regulatory proteins in U2OS and C33A cells. U2OS and C33A cells were treated with 0, 10, 50 and 100 nM TSA for 24 h. Changes in the amounts of the endogenous cell cycle proteins were analyzed by Western blotting.

Discussion

The promoter of the dihydrofolate reductase (*dhfr*) gene,

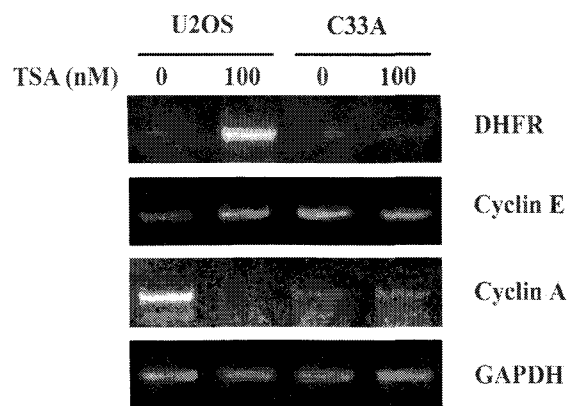


Fig. 4. TSA significantly induced *dhfr* and cyclin E mRNA expression and decreased cyclin A mRNA expression in U2OS but not C33A cells. U2OS and C33A cells were treated with 100nM TSA for 24 h. Changes in the amounts of the endogenous cell cycle mRNA were analyzed by RT-PCR.

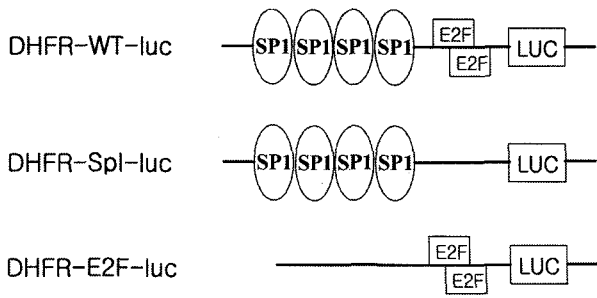


Fig. 5. DHFR-luciferase promoter constructs.

We used a series of model promoters linked to luciferase reporter gene, pGL3-Basic (Promega, USA). The pGL3-DHFR-WT reporter plasmid reporter contains 230 bp of upstream sequence of the *dhfr* promoter that includes the transcription start site, the overlapping E2F elements, and the Sp1 binding sites (GC boxes I-IV). The pGL3-DHFR-Sp1 luciferase reporter includes nucleotides -230 to -85, which contain GC boxes I to IV, but not the E2F sites. The pGL3-DHFR-E2F luciferase reporter contains the two overlapping E2F sites alone.

which has been well characterized in hamster, mouse, and human, contains Sp1 and E2F response elements in the vicinity of its transcription initiation region [18]. The basal transcription of this gene relies on the presence of these sites, because the position of the E2F site influences the transcription initiation position, and because the absence of functional Sp1 protein or Sp1-binding sites in the promoter abolishes transcription of *dhfr* gene. Moreover, other experiment showed that the E2F site of the *dhfr* promoter is involved in promoter silencing in serum-starved conditions, whereas serum responsiveness relies on the Sp1 sites [2]. However, assigning specific roles for Sp1 and E2F in the regulation of *dhfr* gene expression has generated considerable debate.

In the present study, TSA make activated the promoter activity of *dhfr*-WT and *dhfr*-Sp1 luciferase reporter plasmids which has four Sp1 sites and also TSA induced the *dhfr* mRNA in U2OS cells, although weak activity responded, showed less than a two fold increase in promoter activity in C33A cells. On the other hand, we also

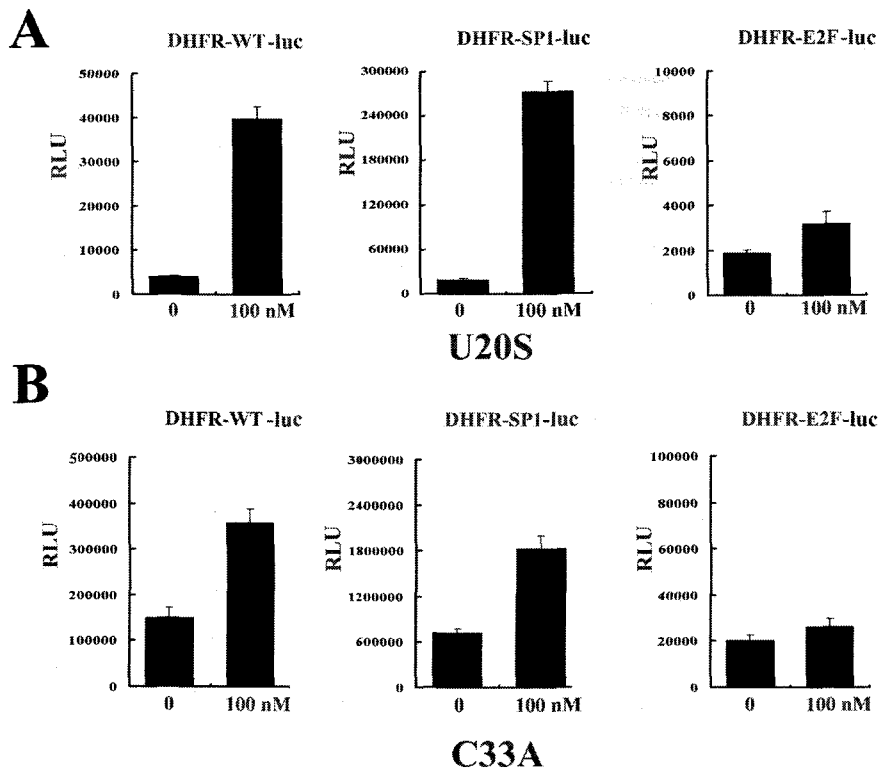


Fig. 6. Transient expression assays to examine the transcriptional *dhfr* promoter by TSA.

U2OS and C33A cells were transfected with luciferase reporter genes *dhfr*-WT-luc, *dhfr*-Sp1-luc, and *dhfr*-E2F-luc and incubated with or without TSA. After 24 h treatment, cells were harvested and luciferase assays were performed. Data represent means and standard deviations from three independent experiments.

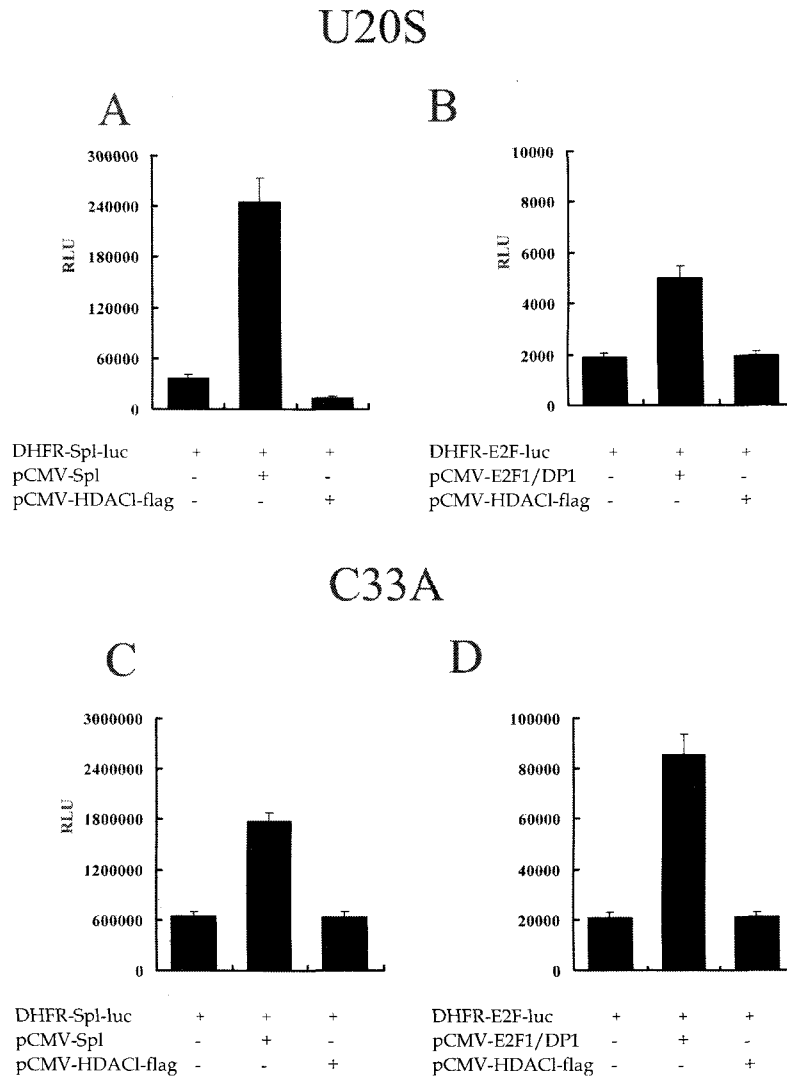


Fig. 7. HDAC1 mediate transcriptional repression through *dhfr* Sp1 binding sites.

U2OS and C33A Cells were transfected with *dhfr*-Sp1-luc or *dhfr*-E2F-luc together with pCMV-Sp1, pCMV-E2F1, pCMV-DP1, or pCMV-HDAC-Flag. Luciferase activity in whole-cell extracts prepared from transfected cells was measured. Data are means and standard deviations from three independent experiments.

observed that mRNA level of cyclin E regulated by Sp1 was profoundly increased by TSA in U2OS but not C33A cells. These data showed that Sp1 is involved transcriptional activation of the *dhfr* in response to TSA in U2OS cells. These results also suggested that TSA action is specific to U2OS cells and further suggested a mechanism whereby HDACs is involved in *dhfr* repression in U2OS cells. There was a reporter that demonstrating the regulation of *dhfr* promoter by TSA, in which the role of Sp1 remained to be elucidated [5]. These results were consistent with the Sp1 site effects of HDAC inhibitors on the regulation of the gene expression. Sp1 plays a key role in

the activation of a large number of genes, including house-keeping and cell-cycle regulated genes, containing upstream GC Box promoter elements. Furthermore, the Sp1 site has been shown to be responsible for TSA-induced expression of the p21 gene promoters [33]. On the other hand, reported that Sp1 could be a target for HDAC1-mediated transcriptional repression. Moreover, Sp1 was found to make a complex directly with the Histone Acetyl Transferase (HAT) and HDAC1 (15Doetzlhofer et al., 1999).

Finally, results of RT-PCR analysis indicated that in only U2OS cells, expression of G1 phase genes such as *dhfr* and cyclin E, which contain Sp1 and E2F sites in their pro-

motors, were activated by TSA treatment, but S genes such as cyclin A were repressed. In several model, Rb repressed genes normally activated during G1 such as cyclin E, thymidine kinase, and dihydrofolate reductase as well as the S phase genes cyclin A and cdc2 [42]. Based on the above results, we suggested that cooperation of Rb-HDAC activity may be required for Rb to regulate the *dhfr* and cyclin E gene in cell cycle.

In summary, the results compared the effects of HDAC inhibitor on the transcription of cell cycle related gene in both of U2OS and C33A cells. We showed that TSA, a inhibitor of HDAC dramatically induced transcription of *dhfr* in U2OS cells, but not in C33A cells and Sp1 plays a crucial role in this regulation. A series of mutational analyses of the *dhfr* promoter have revealed that the main TSA response element is the Sp1 site in U2OS cells. These findings explain one mechanism of promoter silencing of the *dhfr* gene through Sp1 site in U2OS cell line. We also observed that expression of cyclin A was profoundly suppressed by TSA, despite the presence of Sp1 and E2F elements similar to *dhfr* promoter. Ectopic expression of E2F1 represses promoter activity of human telomerase reverse transcriptase in tumor cells, while it activates the promoter in normal human somatic cells [40]. Differences in the number and the arrangement of binding motifs might influence the factors recruited to the promoter, their mutual interactions, and thus entire promoter activity.

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초록 : Trichostatin A 처리에 의한 세포주기 조절인자들의 전사활성화 및 불활성화

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Dihydrofolate reductase (*dhfr*) promoter에는 전사 인자 Sp1과 E2F가 결합하는 cis-acting 배열을 가지고 있다. *dhfr* 유전자의 전사는 세포 주기 G1/S기 동안 최대의 발현을 나타낸다. 또한 Sp1 전사 인자는 *dhfr* 유전자 발현의 활성화 및 불활성화를 조절하는 다양한 역할에 대한 연구가 보고 되고 있으며, 최근 Sp1-Rb과 E2F4-p130 복합체가 CHO400 세포에서 *dhfr* 유전자 발현에 안정한 형태를 형성하여 *dhfr* 발현을 억제한다는 연구 결과가 보고되었다. 본 연구에서는 Rb-양성 골육종 세포인 U2OS 및 Rb-음성인 자궁경부암 C33A 세포에서 histon deacetylase (HDAC)에 대한 특이적인 저해제인 trichostatin A (TSA)를 처리한 후 세포주기 조절에 중심적 인자들인 *dhfr*, cyclin E 및 cyclin A의 전사활성에 대한 HDAC1의 기능을 조사하였다. U2OS 및 C33A 세포에서 TSA를 처리한 후, *dhfr*, cyclin E, cyclin A에 대한 mRNA 및 단백질 발현을 조사한 결과 U2OS 세포 특이적으로 *dhfr*와 cyclin E의 mRNA 발현과 단백질 발현이 크게 증가하였지만, cyclin A의 발현은 감소하였다. U2OS 세포에서 *dhfr* promoter construct에 대한 전사활성을 검사한 결과, TSA 처리는 *dhfr* promoter 영역으로부터 E2F 결합부위를 제거시킨 DHFR-Sp1-luc를 통하여 *dhfr* promoter 활성이 약 14배 증가되었다, 그러나 *dhfr* promoter 영역으로부터 Sp1 결합부위를 제거시킨 DHFR-E2F-luc 영역을 포함하고 있는 promoter 활성은 TSA 처리에 의해 크게 증가되지 않았다. 본 연구에서 이러한 결과는 HDAC1이 Sp1을 통하여 *dhfr* promoter 활성을 제어한다는 사실을 입증하였다. 한편 TSA는 U2OS 세포에서 HDAC의 활성을 통해서 세포주기 관련 인자들 가운데서 G1 후기부터 활성화되는 대표적인 인자들인 *dhfr*과 cyclin E의 발현을 증가시키지만 G2 기에서 활성화 되는 대표적인 인자인 cyclin A의 발현을 억제하는 상반된 기능을 가지고 있다는 사실을 확인하였다.