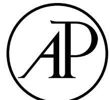
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Restoration of p53 tumor suppressor pathway in human cervical carcinoma cells by sodium arsenite

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Abstract

In most cervical cancer cells, p53 and Rb are disrupted by human papillomaviruses (HPVs) E6 and E7, respectively. Restoration of p53 or Rb function by blocking E6/p53 or E7/Rb pathway might be a potential therapeutic purpose for these cancer cells. Treatment with sodium arsenite (SA) resulted in significant repression of E6 and E7 mRNA levels in SiHa cells. After E6 and E7 repression, p53 was dramatically induced and accumulated in cellular nuclei and Rb was also induced. Two p53-responsive genes, $p21^{waf1/cip1}$ and mdm2, were induced after SA treatment. Furthermore, SA also reduced the expressions of Cdc25A and cyclin B, blocked cell cycle progression at G2/M phase, and induced apoptosis in SiHa cells. SA-induced apoptosis was greatly reduced by expression of a dominant-negative mutated p53. In this study, we have first demonstrated that SA did repress E6 and E7 oncogenes, restore the p53 tumor suppressor pathway and induce apoptosis in SiHa cells. Therefore, it would be a potential strategy to promote SA as therapeutic purpose for HPV-positive cancer cells. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Sodium arsenite; Human papillomavirus; E6; E7; p53; Rb; Apoptosis

High-risk human papillomaviruses (HPVs), such as HPV-16 and HPV-18, are associated with the development of cancers, of which the most serious is cervical cancer, the second leading cause of death from cancer in women worldwide [1]. The high-risk HPVs E6 protein binds to p53, promoting its degradation through the ubiquitin pathway and thus abrogates its function [2–4]; the high-risk HPVs E7 protein binds to hypophosphorylated members of Rb (retinoblastoma) family, resulting in their destabilization and the disruption of Rb/E2F repressor complexes [5–8]. The p53 tumor suppressor gene is critically involved in cell cycle regulation, DNA repair, and programmed cell death (reviewed in [9]); Rb and its family regulate the activity of E2F transcription factors, which control transcription of various genes required for cell cycle progression (reviewed in [10,11]). Thus, the high-risk HPVs E6 and E7 deregulate cell cycle control by neutralizing cellular p53 and Rb tumor suppressor proteins, respectively (reviewed in [12]).

P53 increases in cells responded to different stress signals, such as DNA damage agents, decreased oxygen, oncogenic stimuli, cell adhesion, altered ribonucleotide pool, and redox stress, by unique pathways (reviewed in [13]). P53 turns on the transcription of $p21^{waf1/cip1}$, which inhibits the cyclin-dependent G1 kinase and the G2/M-specific cdc2 kinase, and thus controls both the G1 and the G2/M checkpoints [14]. Furthermore, p53 also mediates apoptosis by Myc or E1A [15,16]. Previous reports showed that HPV E6 could inhibit p53 transcriptional activity and cellular responses and thus disrupted the activation of $p21^{waf1/cip1}$, cell cycle arrest, and apoptosis [17–19].

Rb is a nuclear phosphoprotein that regulates the cell cycle progression by repressing transcription of genes required for the G1–S transition [20]. In early G1 phase, Rb exists in a hypophosphorylated form. As cells proceed to late G1 phase, Rb is hyperphosphorylated by the cyclin-dependent kinases (cdk) and the level of phosphorylation of Rb increases during S phase, and peaks in G2/M. In late M phase, Rb is dephosphorylated by a protein phosphatase [21]. On the other hand, Rb also plays some roles in apoptosis. Loss of Rb function leads to release free E2F, which then triggers apoptosis by

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activating ARF (the alternative reading frame protein) and perhaps other pro-apoptotic proteins [22].

Arsenic, a well-documented human carcinogen, was recently used to cure acute promyelocytic leukemia (APL) and approved by FDA (Food and Drug Administration) in USA in September 2000 [23]. We had previously reported that sodium arsenite (SA) induced apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species (ROS) [24]. The arsenic compound, As_2O_3, presently used to cure APL patients was also reported through apoptosis [25,26]. In addition, arsenic was also applied to treat androgen-independent prostate cancer cells [27].

In contrast to many other human tumor forms, most HPV-positive cervical cancer cells possess wild type p53 and Rb genes [28,29]. But their normal functions are disrupted by expression of HPVs E6 and E7, respectively. Therefore, restoration of p53 or Rb function by blocking E6/p53 or E7/Rb pathway might be a potential therapeutic purpose for these cancer cells. In the present study, we treated HPV-positive SiHa cells with SA and examined whether SA would regulate E6 or E7 oncogene expression and thus affect the p53 or Rb tumor suppressor pathway. We also examined the cell cycle progression and apoptotic responses after SA treatment in SiHa cells.

Materials and methods

Materials. Sodium arsenite (SA) and propidium iodide (PI) were purchased from Merck (Schuchardt, Germany). DMEM was obtained from Gibco (Grand Island, NY, USA). P53 (Do-1), MDM2 (SMP14), HPV-16 E7 (ED17), Rb (IF8), Cdc25A (F-6), cyclin B (GNS1), and cyclin E (HE12) monoclonal antibodies were from Santa Cruz Biotechnology; $p21^{waf1/cip1}$ monoclonal antibody was from BioLab; human β-actin monoclonal antibody was from Boehringer Mannheim (Germany); anti-mouse IgG horseradish peroxidase linked antibody was from Amersham Life Science. FITC-conjugated anti-mouse IgG antibody and Hoechst 33258 were purchased from Sigma.

Cells and culture conditions. SiHa cells are human cervical carcinoma cells containing an integrated human papillomavirus type 16 (HPV-16) genome (ATCC number: HTB-35). They were grown in DMEM supplemented with 10% heat-inactivated (56 °C, 30 min) fetal bovine serum, 0.2% sodium bicarbonate, 0.03% L-glutamate, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified incubator at 37 °C and with 5% CO_2.

RNA extraction and reverse transcription polymerase chain reaction (RT-PCR). This method was performed as previously described [30]. Total cellular RNA was extracted by REzol solution from PROtech Technologies, according to manufacturer's instruction. Ten microgram of total RNA was used to synthesize cDNA in a reaction mixture containing 400 U MML-V reverse transcriptase (USB) at 37 °C for 30 min. The reaction mixture was stopped by denaturing the enzyme at 75 °C for 15 min. Then, the synthesized cDNA was used in PCR amplification for 35 cycles. Each cycle of PCR was set as follows: 1 min at 95 °C, and 48 °C, and 30 s at 72 °C using a programmable thermal controller (MJR PTC-100). RNA concentrations and PCR cycle were titrated to establish standard curves, to document linearity, and to permit semi-quantitative analysis of single strength as previously described [31]. The PCR products were electrophoresed in 2% agarose gel, and then visualized with ethidium bromide staining under UV light. The primer sequences and sizes of amplified products are as follows: HPV-16 E6 sense 5′-ATGCACCAAAAGAGAA-CTGCAATG-3′, and antisense 5′-TTACAGCTCCCTTTCTCTACGTGT-3′, amplified PCR fragment, 477 bp [32]; HPV-16 E7 sense 5′-GCATGGAGATACA-CCTACATTG-3′, and antisense 5′-TGGTTTCTGAGAACAGATGG-3′, amplified PCR fragment, 292 bp; human β-actin sense 5′-TGACTGACTACCTCATGAAG-3′, and antisense 5′-AAGGCTGGAAGAGTGCCTCA-3′, amplified PCR fragment, 239 bp. The PCR product of human β-actin is as an internal control.

Western blotting. Cells were washed twice with ice-cold PBS (phosphate-buffered saline) and lysed on ice in lysis buffer containing 100 μg/ml phenylmethylsulfonyl fluoride (PMSF), 50 mM Tris–base at pH 8.0, 150 mM NaCl, 0.02% NaN_3, 1% NP-40, and 1 μg/ml aprotinine. After centrifugation at 10,000 rpm for 30 min, the supernatant containing proteins was collected and protein concentration was determined by Bio-Rad DC Protein Assay kit (Bio-Rad laboratories, Richmond, CA, USA). Equal amounts of proteins were electrophoresed on 10% SDS–polyacrylamide gel and electrotransferred onto PVDF membrane. Membranes were probed with appropriate antibody and the signals were detected by an enhanced chemiluminescence (ECL) system [33].

Immuno-fluorescence staining of cellular p53. This method was performed and modified as previously described [34]. SiHa cells were seeded onto glass slides. After treatment, cells were washed twice with PBS and then fixed in situ with 4% paraformaldehyde at 4 °C for 1 h. The slides were washed twice with PBS, blocked with 10% serum in 2% Triton X-100 in PBS, and incubated with anti-p53 (Do-1) monoclonal antibody at 4 °C for 1 h. The unbound antibody was removed by extensively washing with PBST (PBS containing 1% Triton X-100). The slides were further incubated with FITC-conjugated secondary antibody at room temperature in the dark for 30 min. Cell nuclei were counterstained with 1 μg/ml PI. The slides finally were observed under fluorescence microscopy.

Flow cytometric analysis. Cells were collected and washed twice with cold PBS and fixed with 70% ethanol at 4 °C overnight. After removing ethanol, the cell pellets were stained with mixture containing 8 μg/ml PI and 50 μg/ml RNase A at room temperature in the dark for 30 min and applied to FACScan flow cytometry (Becton Dickinson) analysis. The percentage of cells in each cell cycle stage was analyzed by ModFit program (Becton Dickinson).

Dual-parameter flow cytometric analysis of normal, apoptotic, and necrotic cells. The method was according to the one previously described [35]. In brief, after treatment, cells were washed twice with PBS, harvested, and then incubated with 2 μg/ml Hoechst 33258 at 37 °C for 15 min. After staining, cells were centrifuged, re-suspended in 1 μg/ml PI at 4 °C and analyzed with FACScan flow cytometry (Becton Dickinson) within 30 min of PI-staining. Data were analyzed by CellQuest program (Becton Dickinson).

Apoptotic cells staining. Cells were collected, washed twice with cold PBS and fixed with 70% ethanol at 4 °C overnight. After removing ethanol, the cells were fixed on glass slides with methanol:acetic acid (3:1), stained with Hoechst 33258 and then observed under fluorescence microscopy.

DNA fragmentation assay. Briefly, harvested cells (1×10^7) were centrifuged after being washed twice with cold PBS. The cell pellet was lysed in a buffer containing 10 mM Tris–HCl, 10 mM EDTA, and 0.2% Triton X-100 (pH 7.5). After 10 min on ice, the lysate was centrifuged (13,000*g*) for 10 min at 4 °C. Then, the supernatant (containing RNA and fragmented DNA, but not intact chromatin) was extracted first with phenol and then with phenol–chloroform:isoamyl alcohol (24:1). The aqueous phase was brought to 300 mM NaCl and nucleic acids were precipitated with 2 vol of ethanol. The pellet was washed with 70% ethanol, air-dried, and then dissolved in 20 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5). Following digestion of RNA with RNase A (0.6 mg/ml, at 37 °C for 30 min), the sample was electrophoresed in 2% agarose gel. DNA

was then visualized with ethidium bromide staining under UV light [36].

Plasmid construction and transfection. Plasmid containing dominant negative mutated p53 gene (with amino acid R273H substitution), pSVXR1, was kindly provided by Dr. Liu, Y.C., National Tsing Hua University, Taiwan. The dominant negative mutated p53 gene was then subcloned into neo marker-containing pCDNA3 vector (Invitrogen, USA) by *Hin*dIII digestion. Plasmid transfection was performed as follows. The day before transfection, 5×10^5 SiHa cells were seeded into 60-mm cell culture dish. For each dish, 3 μg of pCDNA3 plasmid (without genes insertion) or pCDNA3-p53^m plasmid (with dominant negative mutated p53 gene) and 15 μg of LipofectAMINE (Life Technologies) were used according to manufacturer's instruction. The cells were incubated for 5 h in serum-free medium (1.5 ml/dish) containing DNA and LipofectAMINE; an equal volume of growth medium containing 20% serum was then added without removing the transfection mixture and incubation allowed to continue for 24 h. Stably transfected cells were selected by 0.4 mg/ml G418 for 2 months.

Results

HPV-16 E6 mRNA levels were down-regulated by SA, but not by IR

Although SiHa cells have normal p53 gene, E6 in these cells abrogates its function. The HPV-16 E6 mRNA levels significantly decreased in these cells during

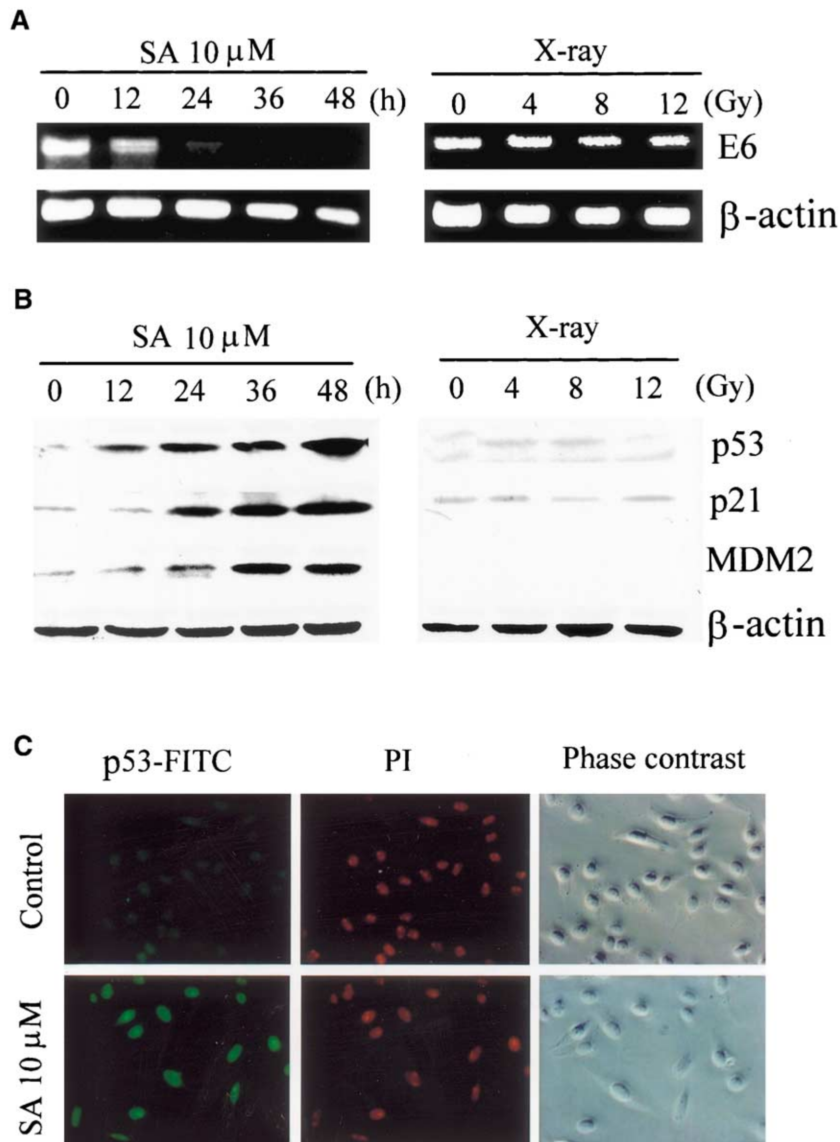

Fig. 1. Alterations of E6 mRNA levels, and p53, p21$^{waf1/cip1}$, and mdm2 protein levels. SiHa cells were treated with 10 μM SA for indicated time or exposed to different doses of X-ray and post-incubated at 37 °C for 2 h. (A) Total cellular RNA was isolated and RT-PCR was performed to determine relative difference of E6 mRNA levels. (B) Total cellular proteins were extracted and applied to Western blotting of p53, p21$^{waf1/cip1}$, MDM2, and β-actin. β-Actin mRNA or protein level was used as internal control. (C) SiHa cells were treated with or without 10 μM SA for 24 h. Immuno-fluorescence staining of p53, nuclear counterstaining with PI, and phase contrast image of cells on slides were shown from left to right columns.

the first 24 h and declined to undetectable level by 36 h after 10 μM SA treatment. However, the HPV-16 E6 mRNA levels had no change after 4, 8, and 12 Gy X-irradiation plus 2 h post-incubation (Fig. 1A). It indicated that SA, but not IR, did suppress the transcription of HPV-16 E6 oncogene in SiHa cells.

SA activated the p53 tumor suppressor pathway and caused p53 accumulation in cellular nuclei

Because high-risk HPVs E6 binds to normal p53 protein, promotes its degradation through the ubiquitin pathway and abrogates its function, the expressions of p53 and two of its downstream genes, $p21^{waf1/cip1}$ and mdm2, in SiHa cells were examined. After 10 μM SA treatment for various time, p53 was dramatically induced in a time-dependent manner, and persisted for at least 48 h. The expressions of $p21^{waf1/cip1}$ and MDM2 were also induced in similar kinetic profile of p53. Nevertheless, neither p53 nor $p21^{waf1/cip1}$ protein was induced after different doses of X-ray exposure (Fig. 1B). To determine the localization of p53 within SiHa cells, immuno-fluorescence staining of p53 was performed. The p53 dramatically increased and accumulated in the nuclei of SiHa cells after 10 μM SA treatment for 24 h (Fig. 1C). It indicated that the SA-induced p53 is functional in SiHa cells, because the expressions of $p21^{waf1/cip1}$ and mdm2 were induced.

SA suppressed HPV-16 E7 oncogene and induced Rb expression

High-risk HPVs E7 binds to pRb to mediate carcinogenesis [37]. A time course study demonstrated that the expressions of HPV-16 E7 mRNA and protein significantly decreased in SiHa cells after 10 μM SA treatment for 36 and 48 h (Fig. 2A and B). The expression of total Rb (ppRb+pRb) showed 1.3–2.4-fold increase in comparison with control cells after SA treatment for 12–48 h. The ratio of ppRb/pRb greatly increased from 0.2 to 0.9 during SA treatment in SiHa cells (Fig. 2B).

SA blocked the cell cycle progression in G2/M phase coinciding with decreased Cdc25A and cyclins

Because p53 and Rb are well known to regulate cell cycle progression, we monitored cell cycle progression and examined the alterations of the proteins involved in cell cycle progression. After 10 μM SA treatment, the expressions of Cdc25A and cyclin B dramatically decreased at 24 and 36 h, and reduced to undetectable level at 48 h in SiHa cells. Nevertheless, the expression of cyclin E only greatly decreased after 48 h treatment (Fig. 3A). These phenomena were in line with the significant increase of the population of SA-treated SiHa

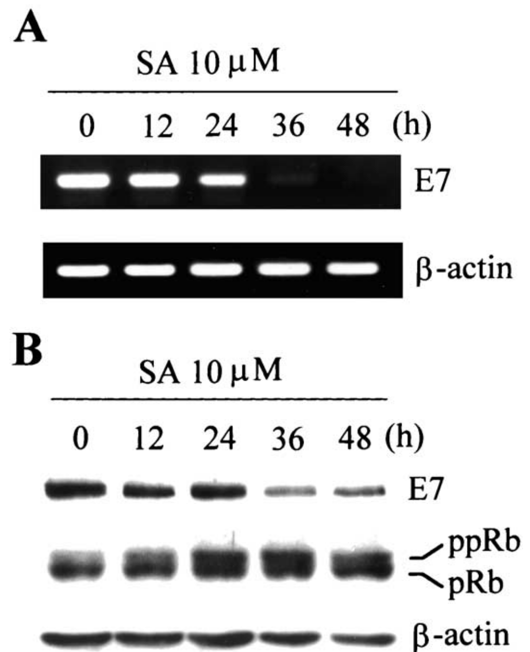

Fig. 2. Alterations of E7 mRNA, E7 and Rb protein levels. SiHa cells were treated with 10 μM SA for different time intervals. (A) Total cellular RNA was isolated and RT-PCR was performed to determine relative difference of E7 mRNA levels. (B) Whole cellular proteins were extracted and applied to Western blotting of HPV-16 E7 and Rb. The hyperphosphorylated and hypophosphorylated forms of Rb were indicated as ppRb and pRb, respectively.

cells in G2/M phase in comparison with that of untreated cells. Additionally, while the G2/M peak declined, sub-G1 apoptotic cells appeared and increased after 24–48 h treatment (Fig. 3B and C). It indicated that SA could block the cell cycle progression at G2/M phase coinciding with the appearance of sub-G1 apoptosis in SiHa cells.

Induction of apoptosis was the major event after SA treatment

To determine the SA-induced apoptotic responses in SiHa cells, the chromatin condensation and DNA fragmentation were further examined. The apoptotic cells after 10 μM SA treatment for 48 h were observed (Fig. 4A). In contrast, no apoptotic cells were observed in control cells (Fig. 4B). Significantly fragmented DNA appeared and increased in a time-dependent manner after 10 μM SA treatment for 24–48 h in SiHa cells (Fig. 4C). In order to discriminate apoptotic cells from necrotic cells, the dual-parameter flow cytometric analysis was used. Apoptotic cells significantly increased in SiHa cells after 10 μM SA treatment for 2 days ($21.24 \pm 6.87\%$) to 3 days ($50.74 \pm 8.04\%$) in a time-dependent manner, but necrotic cells slightly enhanced in the same treated cells (Fig. 5). It indicated that SA mainly induced apoptotic cell death in SiHa cells.

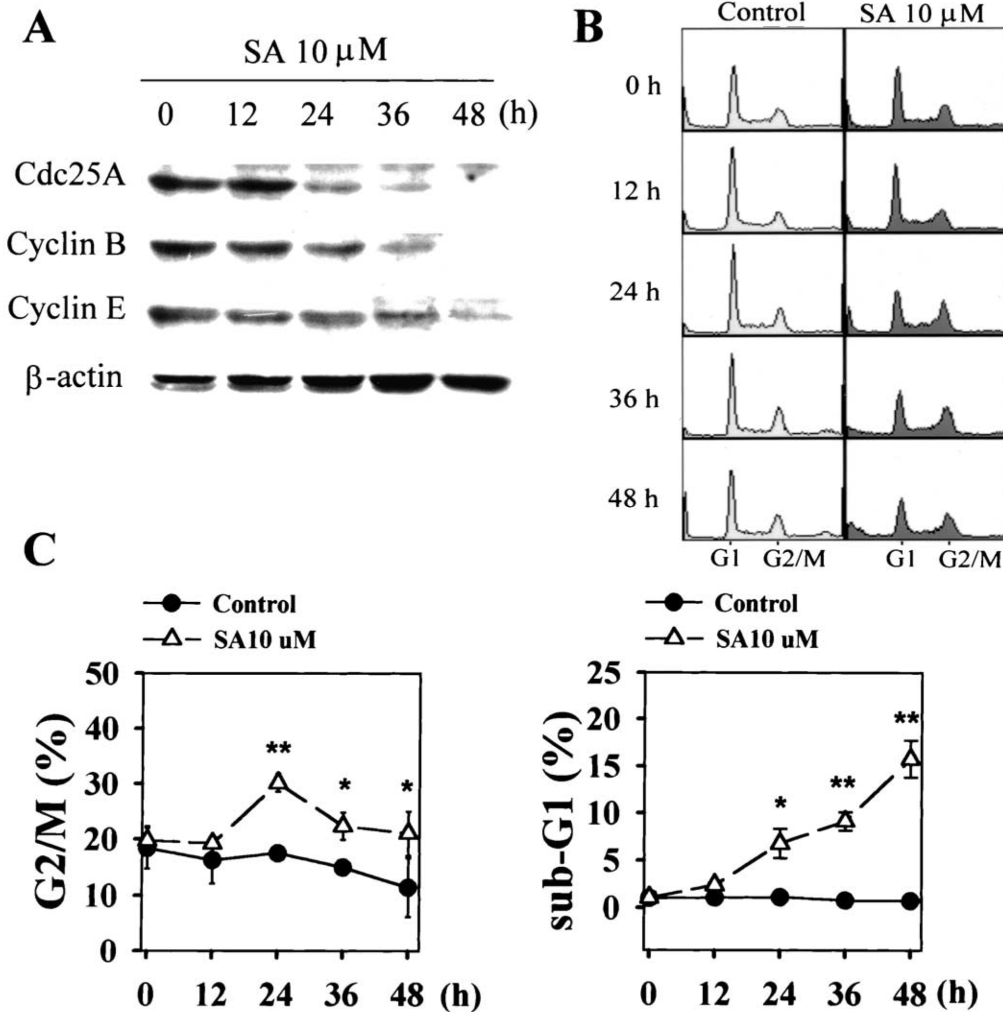

Fig. 3. Alterations of Cdc25A, cyclins, and cell cycle progression. SiHa cells were treated with 10 μM SA for different time intervals. (A) Total cellular proteins were extracted and applied to Western blotting of Cdc25A, cyclin B, cyclin E, and β-actin. (B) Cells were collected, fixed, stained with PI, and then applied to flow cytometric analysis. (C) The percentages of cells in G2/M and sub-G1 phase from three independent experiments were shown. Statistics analysis was performed with t test. The symbols, ** and *, indicate $p < 0.01$ and $p < 0.05$, respectively, in comparison with untreated cells at each time point.

Expression of dominant-negative p53 significantly rescued SiHa cells from SA-induced apoptotic cell death

It was reported that p53 mutant at R273H (arginine to histidine substitution at 273 amino acid of p53) had dominant-negative effects on apoptosis, but not on growth arrest [38]. In order to examine the role of p53 in SA-induced apoptotic cell death, pCDNA3 (mock) or pCDNA3-p53^{m} (containing dominant-negative mutated p53 at R273H) plasmid was transfected into SiHa cells, respectively. SiHa-pCDNA3 and SiHa -p53^{m} cells were named after selection in G418-containing medium for 2 months. SiHa-p53^{m} cells showed significantly less sub-G1 apoptosis than SiHa-pCDNA3 and parental SiHa cells in a dose-dependent manner after 5–20 μM SA treatment for 2 days (Table 1). This finding confirmed that p53 mediated SA-induced apoptotic cell death in SiHa cells. However, the percentage of cells in each cell cycle stage had no difference among these three cells after the same treatment (data not shown).

Discussion

Several approaches have been proposed to control the growth of E6-expressing cancer cells recently. Hietanen et al. [39] used leptomycin B and actinomycin D to restore p53 activity in HPV-positive cervical carcinoma cells. Others applied (i) the bovine papillomavirus E2 gene [40], (ii) antisense strategies [41], and (iii) the variant forms of E6 that interact both with full length E6 protein and with p53 [42], resulting in suppression of E6-mediated degradation of p53. In the present study,

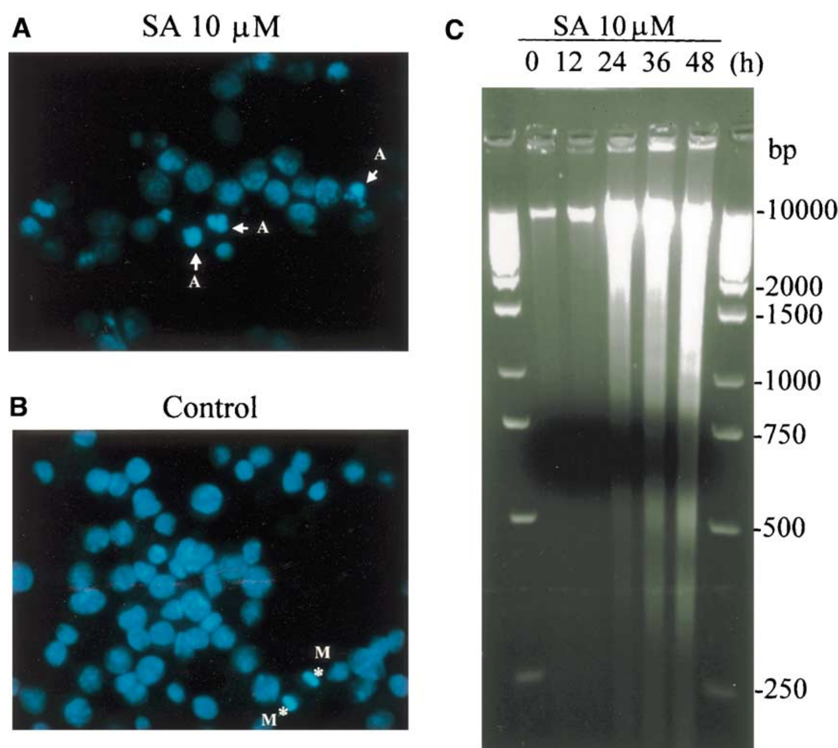

Fig. 4. Induction of apoptosis by SA. (A) SiHa cells were treated with 10 μM SA for 48 h, stained with Hoechst 33258 and analyzed under a fluorescence microscope. Control cells were shown in (B). The apoptotic (A) and mitotic (M) cells were indicated as arrows and stars, respectively. (C) SiHa cells were treated with 10 μM SA for indicated time. Fragmented DNA was isolated and separated by 2% agarose gel electrophoresis. The right and left lanes were DNA molecular weight markers.

we first provide SA for suppressing E6 oncogene and restoring normal p53 function in cervical carcinoma cells (Fig. 1).

Under normal growth conditions, p53 is turned over by the ubiquitin-proteasome system. The p53 regulation under these conditions is mainly via MDM2-mediated degradation. But in cervical cancer cells infected with HPVs, the degradation of p53 is completely switched to HPVs E6-mediated ubiquitination [43]. Therefore, the increase of p53 expression might partially be due to the down-regulation of HPV-16 E6 oncogene in SA-treated SiHa cells (Fig. 1). It would reduce intracellular E6 levels to abrogate p53. On the other hand, SA might directly or indirectly bind the thiol groups of E6 protein to affect its function or activity. Since E6 protein contains 2 zinc finger sequences, cysteine residues at the base of each of the Cys–X–X–Cys motifs [44], it would be possible that SA changes its abrogation ability to p53 protein through binding to cysteine or thiol groups of E6 protein. In fact, arsenite has been shown to bind compounds containing vicinal thiol [45], or bind NF-kB on its critical cysteine, it did affect the function or activity of these proteins [46]. It should not rule out another possibility to find out whether SA also affects another components besides E6 protein itself in E6/p53 degradation pathway. It is reasonable to believe that SA might also affect the integrity of another E6 associated protein or some other factors in E6 mediated p53 degradation pathway. Therefore, E6 loses its abrogating ability to degrade p53, and increases apoptosis. Further evidence is required to support these arsenic–E6 interactions.

Recently, it was reported that p53 prevented G2–M transition by decreasing cyclin B levels and attenuating activity of the cyclin B promoter [47]. The cdc25 tyrosine phosphatase removes the phosphate from Tyr-15 of Cdc2–cyclinA, B dimers to trigger the start of mitosis [48]. As we have shown here, both Cdc25A and cyclin B dramatically decreased in SiHa cells after SA treatment (Fig. 3A), indicating that SA might cause G2/M arrest in SiHa cells through reducing the expressions of Cdc25A and cyciln B. In addition, Rb inactivation (ppRb) to release E2F is a critical step leading to G1–S transition [20]. In this study, SA suppressed HPV-16 E7 oncogene and greatly induced ppRb expression in SiHa cells (Fig. 2). We suggested that SA not only induced Rb expression, but also might affect its post-translational modification to inactivate its function. It is another possible reason why SA blocks cell cycle progression at G2/M phase, but not at G1 phase.

Involvement of Rb in apoptosis has already been reported in several groups. For instance, increasing the expression of pRb (active form) inhibited apoptosis [49–51]. In contrast, loss of pRb function induced both

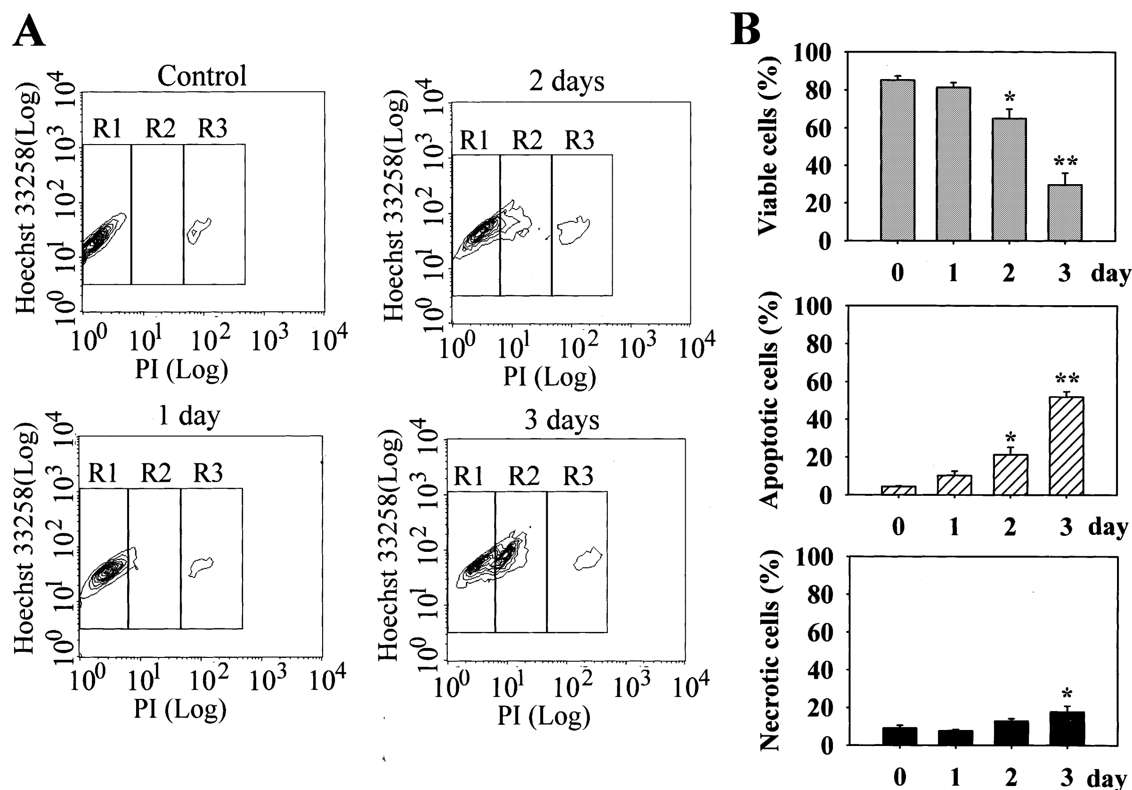

Fig. 5. Dual-parameter flow cytometric analysis of normal, apoptotic, and necrotic cells after SA treatment. SiHa cells were treated with 10 μM SA for 0–3 days. (A) Cells were collected, incubated with Hoechst 33258 and PI, and applied to flow cytometric analysis. The regions, R1, R2, and R3, indicate viable, apoptotic, and necrotic cells, respectively. The percentage of cells in each region was analyzed by CellQuest program. (B) The results from 3–6 independent experiments were shown, and statistics analysis was performed with *t* test. The symbols, ** and *, indicate $p < 0.01$ and $p < 0.05$, respectively, in comparison with untreated cells.

Table 1
Flow cytometric analysis for detection of apoptotic cells

SA (μM)	Sub-G1 (%)		
	SiHa	SiHa-pCDNA3	SiHa-p53^m
0	1.98 ± 0.53	1.82 ± 0.50	1.89 ± 0.59
5	9.34 ± 1.16	10.38 ± 2.61	4.86 ± 0.91**
10	19.59 ± 2.85	19.91 ± 2.21	8.96 ± 0.50**
20	35.32 ± 1.38	36.10 ± 5.56	15.50 ± 1.61**

Cells were treated with different concentrations of SA for 2 days. After treatment, cells were collected, fixed, stained with PI, and then applied to flow cytometric analysis. The percentages of cells in sub-G1 population were analyzed by CellQuest program (Becton Dickinson). Data shown here were expressed as means ± SD from three independent experiments and statistics analysis was performed with *t* test.

** $p < 0.01$ in comparison with SiHa cells at each concentration point.

p53-dependent and p53-independent cell death [52]. Previous evidence showed that phosphorylation of pRb by cdc2 and cdk2 triggered TGF-β1 (transforming growth factor-β1)-induced apoptosis [53]. In this study, pRb (activated form) was greatly phosphorylated to ppRb (inactive form) by SA (Fig. 2B), suggesting that SA-induced apoptosis was partially due to inactivation of pRb. On the other hand, expression of a dominant-negative p53 mutant significantly rescued SiHa cells from SA-induced apoptosis (Table 1). Here we provided direct evidence that SA-induced apoptosis was correlative with restoring normal p53 functions in SiHa cells.

In conclusion, we have provided first evidence to show that SA could restore normal p53 tumor suppressor pathway (p53, p21$^{waf1/cip1}$, and mdm2), and induce G2/M growth arrest and apoptosis in SiHa cells. And induction of p53 expression by SA has been correlated with repression of E6 oncogene. SA also suppressed E7 oncogene and greatly induced ppRb expression in SiHa cells. Thus, SA sensitized SiHa cells through restoring p53 function and might partially through inactivating pRb function. Therefore, SA might be promoted as another potential candidate to restore normal p53 function and induce apoptosis in HPV-positive cervical cancer cells for therapeutic purpose.

Acknowledgments

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