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Original Research Article

Dietary isothiocyanate sulforaphene induces reactive oxygen species, caspase -9, -8, -3-dependent apoptosis and modulates PTEN/PI3Kinase in human cervical cancer cells

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Abstract

Purpose: *To investigate the apoptotic activity, cell proliferation inhibition and different signaling protein expressions after treatment with a new isothiocyanate, sulforaphene, in human cervical cancer (HeLa) cells.*

Methods: *Cytotoxicity was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after sulforaphene treatment for 3, 6, 12 and 24 h. Apoptosis assay, cell cycle analysis, intracellular oxygen species (ROS) measurement, mitochondrial membrane depolarization and western blot analysis were performed in four time-intervals to explore sulforaphene activity.*

Results: *HeLa cell viability was reduced by sulforaphene dose and time dependently. ROS plays a causative role in sulforaphene induced cytotoxicity and apoptosis during which stimulation of Bax and blocking of Bcl2 were involved. Mitochondrial membrane potential depletion and cytochrome C, AIF modulation suggest mitochondrial pathway for the apoptosis. Activation of caspase -9, -8 and -3 in treated HeLa cells demonstrated caspase-dependent apoptosis by sulforaphene. Again, sulforaphene induced HeLa cell proliferation inhibition was evidenced by cell cycle arrest and PTEN/PI3Kinase modulation.*

Conclusion: *Dietary sulforaphene induces HeLa cell apoptosis by enhancing intracellular ROS levels, thereby activating multiple apoptotic signal cascades. Therefore, sulforaphene is a potential candidate for anticancer therapy.*

Keywords: *Sulforaphene, HeLa cells, Apoptosis, ROS, Caspase activation, PTEN, PI3Kinase*

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INTRODUCTION

Cervical cancer ranked third in female cancer worldwide and this cancer related death was in fifth position among Korean females [1]. Although surgery, chemo-radiotherapy and HPV vaccines are being used in cervical cancer treatment, however complications like bone marrow suppression, nerve injury, gastrointestinal adverse reactions, and renal impairment are also

matters of concern [2-5]. Therefore recent research is interested in the validation of some natural anticancer agents to provide an effective cancer remedial measure without any treatment related complications [6,7].

Dietary cruciferous vegetables have shown substantial protective role against various cancers and received much attention to the scientist in cancer management [8-11]. Traditionally radish has long been used as carminative, diuretic, expectorant, laxative and stomachic agents, especially as anti-cancer and/or anti-inflammatory agents in Asian countries [12]. Anticancer efficacy of radish extract in different solvents has been mentioned in some previous studies. This anticancer efficacy is mainly due to the presence of several isothiocyanates (ITC) in radish [11,13]. 4-Isothiocyanato-4R-(methylsulfinyl) -1-butene widely known as sulforaphene (PubChem CID 6433206) is one of the major ITC present in radish (*Raphanus sativus* sp.). Sulforaphene was reported to have antimicrobial and antiviral activities with antioxidant efficacy in rat carcinogens [14,15]. Again sulforaphene inhibits food-derived heterocyclic amine-induced bacterial mutagenesis in TA100 [16]. In previous studies, sulforaphene was found to induce apoptosis in different colon cancer cell lines such as LoVo, HCT-116 and HT-29, H3-T1-1 followed by reduction in cancer cell proliferation [17]. Recently, sulforaphene was reported to have human breast cancer SUM159 cell migration inhibition efficacy by the down-regulation of Hedgehog signaling [18]. However, the molecular signaling pathways for sulforaphene activity in cancer cells have not extensively been studied yet.

Previous studies reported that inhibition of phosphatase and tensin homolog (PTEN) and thereby overexpression phosphatidylinositol 3-kinase (PI3Kinase) in cervical cancer tissues was found very common. Activation of PI3kinase in cancer cells is associated with increased tumorigenesis which includes cancer cell proliferation and cell cycle progression. On the other hand PTEN negatively regulates PI3kinase activity and inhibits cancer cell proliferation [19,20]. Thus, effectively downregulation of PI3Kinase by upregulated PTEN, is considered as one of the novel therapeutic strategy for human cervical cancer management.

Therefore, in the present study our objectives were (i) to investigate the cytotoxicity and apoptosis inducing activity of dietary isothiocyanate sulforaphene on HeLa cells (ii) to analyze the ROS-production-based signaling protein expressions preliminarily associated with sulforaphene treated HeLa cell apoptosis (iii) to check whether sulforaphene has the ability to cease HeLa cell proliferation by increasing PTEN and decreasing PI3Kinase expression levels.

EXPERIMENTAL

Chemicals

S-Sulforaphene was purchased from LKT Laboratories, Inc, USA and dissolved in DPBS to make 50 mg/mL stock solution. From the stock solution, we diluted all respective treatment concentrations in the culture media before the treatment. 3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyl-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), Hoechst33342, propidium iodide (PI), RIPA buffer, protease and phosphatase inhibitors were purchased from Sigma (Saint Louis, MO, USA). H2DCFDA was obtained from Invitrogen (Eugene, OR, United States). Rhodamine123 was purchased from Molecular Probes Inc. (Eugene, OR, United States). Bradford Reagent was supplied by Bio-Rad (Hercules, CA, USA). Poly (ADP-Ribose) polymerase (PARP) and caspase 3 were purchased from Calbiochem (CA, USA). Caspase-9 was purchased from cell signaling technology (Beverley, MA, USA). Cytochrome c, Bax, Bcl2, AIF (apoptosis-inducing factor), caspase 8, PTEN, PI3Kinase and β-actin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Cell culture

Human cervical cancer (HeLa) cells were cultured in DMEM (HyClone, South Logan, UT) supplemented with 10 % fetal bovine serum (FBS) (Equitech-Bio Inc, Texas) and 1 % streptomycin/penicillin (Gibco, BRL). The cells were maintained at 37 ºC in a 5 % CO_2 humidified environment. We used all experimental cell culture dishes from SPL Life Science (Pocheon, South Korea).

Cytotoxicity assay

HeLa cells were inoculated into a 96-well, flat-bottomed microplate at a volume of 100 µl (1 x 10^5 cells/ ml) in complete growth medium. The cells were treated with a series of sulforaphene (0 µg/mL - 100 µg/mL) with new fresh media. Cells were incubated for 3, 6, 12 and 24 h and 50 µL MTT solutions (2mg/ mL) were added to each well. After incubation for 4 h in 5 % CO_2 at 37 ºC, the media of each well was removed and 150 µL DMSO was added to dissolve violet blue crystals. The concentrations were determined by

measuring the absorbance at 570 nm using ELISA reader (Biochrom, UK). Cell cytotoxicity was expressed as the percentage of absorbance of treated cells with respect to that of control cells. The results were obtained from the mean values and standard deviation (SD) from at least three independent experiments. From the MTT assay 12 µg/mL sulforaphene concentration was selected for our further studies.

Apoptosis assay

To evaluate the morphological changes of the nuclear chromatin after apoptosis and necrosis induction in HeLa cells, Hoechst33342 and propidium iodide (PI, Sigma, St. Louis, USA) double staining was performed. Cells were treated with 12 µg/mL sulforaphene and incubated for 3, 6, 12 and 24 h in 5 % CO_2 at 37 ºC. Then the cells were incubated with Hoechst 33342 (1µg/mL) for 30 min and PI (1 µg/mL) for 15 min. The stained cells were observed to assess cellular apoptosis and necrosis using a confocal laser scanning microscope (Zeiss 510 Meta, Germany) under equivalent conditions. Histogram was prepared to evaluate the percentage decrease in normal cells and the percentage of increase in apoptotic cells in different time intervals before and after sulforaphene treatment by counting cells from three different sets of samples.

Analysis of cell cycle progression

To evaluate time dependent sub-G1 DNA content after sulforaphene treatment, cell cycle analysis was performed. Briefly, HeLa cells were treated with 12 µg/mL of sulforaphene and incubated for 3, 6, 12 and 24 h, and were incubated with PI mixed with DPBS for 15 minutes at room temperature. The DNA contents in each treatment were analyzed by flow cytometry (Accuri C6, BD, CA, USA). The percentages of viable and dead cells were determined as 10,000 events per sample using a FL-2 filter. Histogram was prepared and evaluated for the percentage of cell count in different sub populations in different time intervals after sulforaphene treatment from three different sets of samples.

Evaluation of intracellular ROS generation

To analyze intracellular ROS levels after sulforaphene treatment in HeLa cell, H2DCF-DA (2', 7'-Dichlorodihydro fluorescein diacetate, Molecular Probes, OR) was used as the oxidant sensitive fluorescent probe. The cells were treated with 12 µg/ ml sulforaphene as mentioned previously, and incubated for 3, 6, 12 and 24 h. Cells were centrifuged at 1000 rpm for 5 min and incubated with 20 µM of H2DCF-DA at 37 ºC for 30 min. The fluorescent intensities of stained 10,000 cells were subjected to fluorescence-activated cell sorting using FL-1A filter of flow cytometer at different incubation time after sulforaphene treatment. Data was analyzed using Accuri C6 software. Histogram was prepared and evaluated for the percentage of increase in ROS in treated cells in different time intervals after sulforaphene treatment from three different sets of samples.

Determination of changes in mitochondrial membrane potential ($\Delta\psi_m$)

The potential-dependent accumulation of fluorescent cationic dye rhodamine123 is used to analyze the changes in mitochondrial membrane potential in HeLa cells. Briefly, the cells were treated with 12 µg/ mL of sulforaphene and incubated for 3, 6, 12 and 24 h, then. 10 mM rhodamine123 in pre-incubated media were added to each well and incubated for 30 minutes at 37 ºC. The intensities of green fluorescence of cells were observed by laser scanning confocal microscope (Zeiss 510 Meta, Germany) under equivalent conditions. A histogram was prepared by measuring the fluorescence intensities from three different sets of samples at different intervals of time. The quantitative changes in rhodamine123 fluorescence were determined by flow cytometry. Cells were then centrifuged at 1000 rpm for 5 min and then incubated with 10µM of rhodamine123 for 30 min. The fluorescent intensities of cells were subjected to fluorescence-activated cell sorting using FL-1A filter of flow cytometer at different incubation times after sulforaphene treatment. Data were analyzed using BD Accuri C6 software.

Assessment of caspase 3 activity

To study the time-dependent activation of caspase 3 by sulforaphene treatment CaspGLOW Red Active Caspase 3 Staining kit was purchased from BioVision, USA. 12 µg/mL sulforaphene was to HeLa cells were treated with 12 µg/mL sulforaphere for 3, 6, 12 and 24 h and subjected caspase 3 staining to according to the manufacturer's protocol. Relative fluorescence intensities of individual samples in different time intervals were measured using a confocal microscope (Zeiss 510 Meta, Germany).

Western blot analysis

The expressions of apoptotic signaling and cell proliferation proteins were analyzed by Western blot. HeLa cells were treated with 12 µg/mL

sulforaphene and incubated for 3, 6, 12 and 24 h. The whole protein was extracted using RIPA buffer (50mM Tris-HCl, pH 8.0, 1 % NP-40, 0.5 % sodium deoxycholate, 150 mM NaCl, and 0.1 % sodium dodecyl sulfate with protease and phosphatase inhibitor cocktail (Sigma, MO) and centrifuged at 15,000 rpm for 30 min at 4 ºC. The protein concentration was determined using BCA Reagent. Equivalent amounts of protein from each sample were loaded onto polyacrylamide gels and separated by electrophoresis. Then the proteins were transferred to PVDF membranes (Immuno-Blot PVDF, BioRad Laboratories, Hercules, USA). Both electrophoresis and blotting were performed using a PowerPac200 electrophoresis system (BioRad Laboratories, Hercules, USA). The membranes were then blocked for 2 h at room temperature in Tris-buffered saline containing 0.1 % Tween-20 and 5 % skim milk. Then membrane was incubated overnight at 4 ºC with the primary antibody diluted with 3 % skim milk. The membranes were probed with horseradish peroxidase-conjugated to anti-mouse IgG or anti-rabbit IgG antibody for 2 h. The protein bands were developed by ECL Western Blotting detection reagents (GE Healthcare, Buckinghamshire, UK) and the pictures were taken Kodak in vivo image analyzer (Eastern Kodak, Rochester, NY, USA).

Statistical analysis

All the data are expressed as mean ± SEM (n = 3). Statistical analysis was performed and differences between two groups were analyzed using the two-tailed Student's t-test. Differences between control and treated groups were analyzed using the Newman-Keuls one-way ANOVA test. $P < 0.05$, 0.01 or < 0.001 was considered as statistically significant.

RESULTS

Sulforaphene induces concentration- and time-dependent cytotoxicity in HeLa cells

Sulforaphene was found to be cytotoxic and inhibited the growth of human cervical cancer HeLa cells. Figure 1(a) shows concentration and time dependent significant HeLa cell proliferation inhibition efficacy of sulforaphene. Based on MTT assay results, inhibitory concentration (IC_{50}) values are 83.45 ± 2.52 µg/mL, 61.15 ± 2.24 µg/mL, 22.64 ± 1.93 and 10.95 ± 1.73 after 3, 6, 12 and 24 h respectively after sulforaphene treatment. With increase in incubation time after sulforaphene treatment the viability of HeLa cell decreases.

Sulforaphene induces apoptosis in HeLa cells

The trigger of apoptosis by sulforaphene in HeLa cells was confirmed in confocal microscopic images after staining with Hoechst 33342 and PI. Figure 1(b) clearly shows the early and late apoptotic cells with bright fluorescence of condensed nucleus. In our experiment, as the treatment time increases, the percentage of normal cells decreases and percentage of apoptotic cells increases. At 12 h and 24 h incubation, maximum cells were found to be apoptotic and at 24 h few necrotic cells were also observed. Histogram indicates significant decrease in percentages of normal cells and significant increase in percentages of apoptotic cells after sulforaphene treatment at different intervals of time.

Sulforaphene inhibits cell cycle progression and increases sub-G1 DNA content

The flow cytometric analysis indicates that sulforaphene induced cytotoxicity of HeLa cells also involves inhibition of cell cycle progression and increases DNA content at sub-G1 level. Figure 2(a) shows that sulforaphene treatment causes significant increase in sub-G1 cell population in a time dependent manner. A significant reduction in cell population at the G0/G1 stage was observed with increase in treatment time. Sub-G1 cell population of control cells was found to be 5.1 ± 1.07 % whereas 22.5 ± 2.09 %, 36.5 ± 2.57 %, 45.1 ± 2.64 % and 59.0 ± 1.69 % were found after 3, 6, 12 and 24 h treatment, respectively. The histogram shows the percentages of cell count in different subpopulations. It also indicates that the number of apoptotic cells in sub-G1 region was significantly higher in all time intervals compared to the control group. Increase in the sub-G1 cells after sulforaphene treatment in all time intervals also indicates that HeLa cell proliferation was inhibited as and apoptosis was increased.

Sulforaphene-induced apoptosis involves modulation of Bax and Bcl2

Intracellular ROS generation is associated with, another important apoptotic process, Bax activation. So we analysed Bax expression in HeLa cells after sulforaphene treatment at different time intervals. As shown in Figure 3(a), sulforaphene treatment upregulates Bax expression and concomitantly down regulates Bcl2 expression in a time dependent manner. From the histogram of relative band intensities, sulforaphene treatment significantly increased Bax expression and decreased Bcl2 expression with time simultaneously.

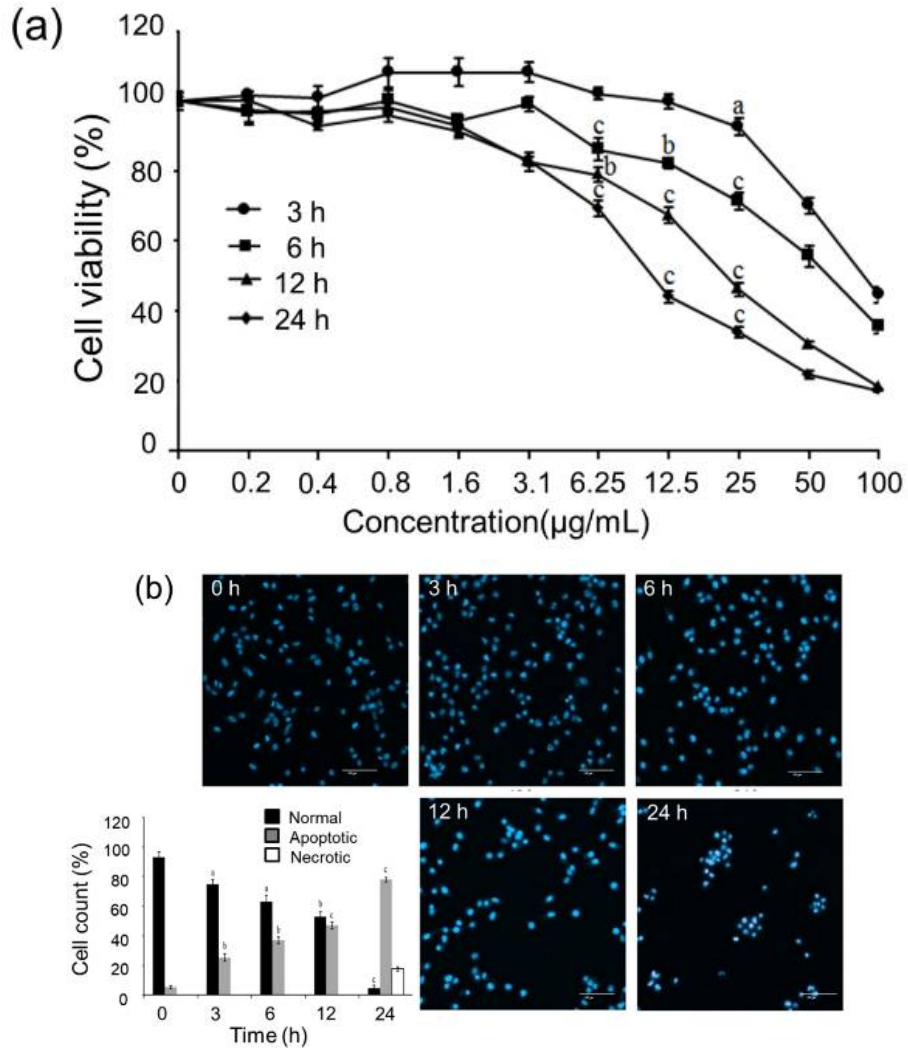

Figure 1: (a) The cytotoxicity of treated cells was studied by MTT assay. (b) Hoechst and PI double staining after sulforaphene treatment to study the apoptotic and necrotic cells a = $p<0.05$, b = $p<0.01$ and c = $p<0.001$ were considered as statistically significant

Sulforaphene-induced apoptosis involves intracellular ROS generation

Reactive oxygen species (ROS) induce cancer cell apoptosis by activation of capase cascade and therefore the generation of ROS in treated cells was tested to confirm whether ROS is involved in sulforaphene induced apoptosis. Flow cytometric analysis, in our experiment, figure 2(b) indicates that sulforaphene treatment stimulates intracellular ROS generation leading to increase in cell fluorescence at different time intervals. Approximately 45.43 ± 2.67 %, 63.04 ± 3.14 %, 83.65 ± 1.51 % and 77.92 ± 4.10 % cells showed an increase in fluorescence after 3, 6, 12, 24 h, respectively. The percentage of fluorescent cells, which indicates intracellular ROS generation, were significantly increased with sulforaphene treatment compared to the control untreated cells in all time intervals.

Sulforaphene-induced apoptosis involves mitochondrial depolarization and cytochrome c, AIF higher expression

Intracellular ROS generation and Bax/ Bcl2 modulation strongly suggest mitochondrial damage and mitochondrial membrane depolarization by sulforaphene treatment. Therefore we investigated changes in mitochondrial membrane potential ($\Delta\psi_m$) by confocal microscopy and flow cytometry. Confocal images in figure 3(b) of our experiment, indicates reduction in relative green fluorescence in treated cells compared to the control cells. $\Delta\psi_m$ noticeably reduced after 3 and 6 h sulforaphene treatment, however, after 12 and 24 h, $\Delta\psi_m$ was also found to decrease than respective control. The fluorescence intensity correlate with the time dependent differential changes in $\Delta\psi_m$.

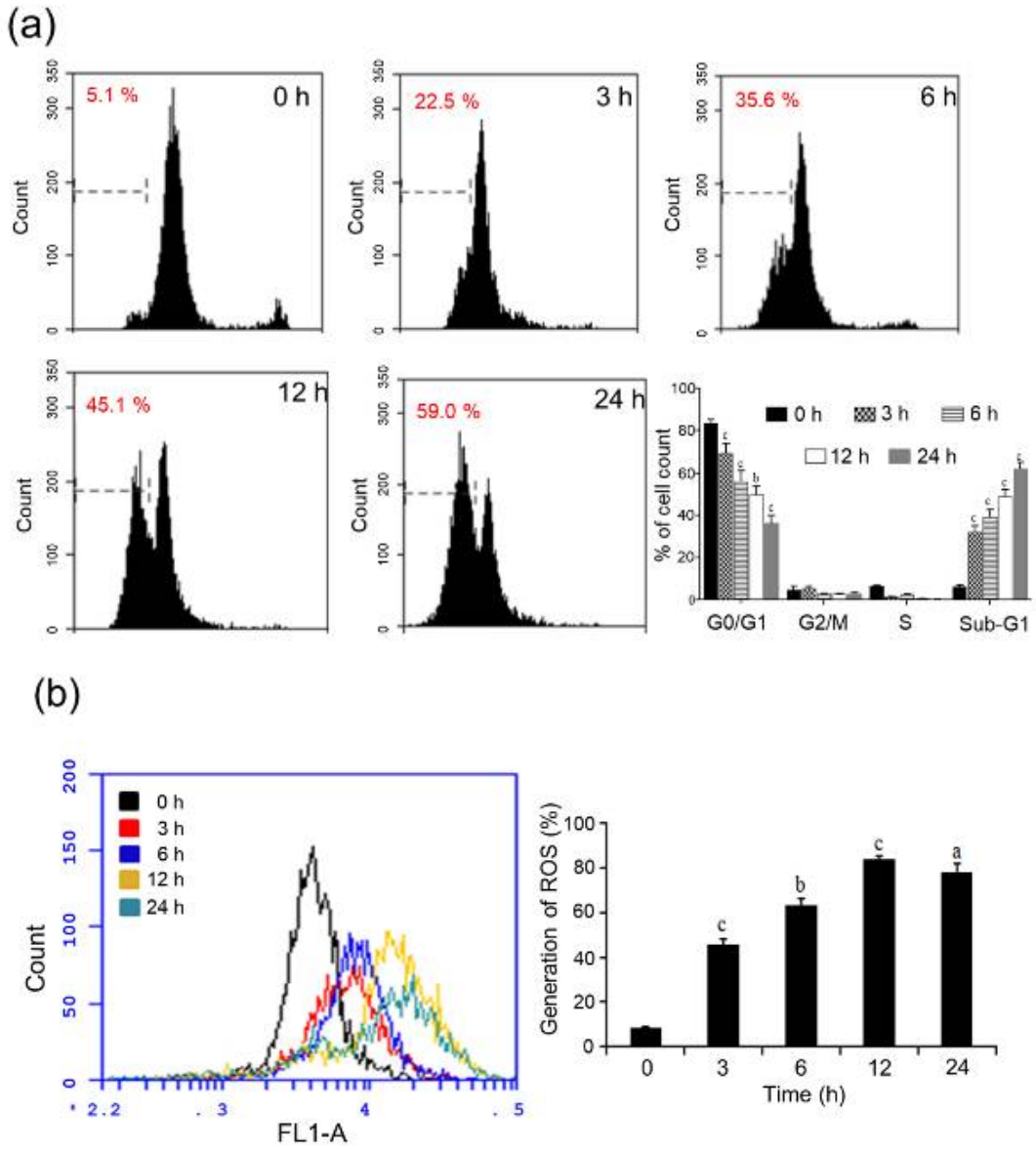

Figure 2: (a) Flow cytometric analysis of cell cycle after sulforaphene treatment at different time intervals. (b) Intracellular ROS increases with time after sulforaphene treatment. a = $p<0.05$, b = $p<0.01$ and c = $p<0.001$ were considered as statistically significant

These time dependent decrease in $\Delta\psi_m$ were significant compared to their respective control. Sulforaphene treatment induced time dependent mitochondrial membrane depolarization was also confirmed by flow cytometry. Histograms in figure 3(c) shows 29.6, 36.4, 34.9 and 57.6 % cells reduced fluorescence intensity after sulforaphene treatment for 3, 6, 12 and 24 h, respectively. This time dependent mitochondrial depolarization promotes the release of cytochrome c and AIF which in turn induce mitochondria mediated apoptosis. In this experiment, sulforaphene treatment was found to increase the expressions of cytosolic cytochrome c and AIF. In figure 3(d) higher expression of cytochrome c was observed at 6 and 12 h whereas expression of AIF was higher at 3 and 6 h. Therefore the time-dependent decrease in $\Delta\psi_m$ and cytochrome c and AIF expression confirmed that sulforaphene treatment activates mitochondrial apoptotic pathway in HeLa cell.

Sulforaphene-induced apoptosis involves modulation of caspases and cleavage of PARP

To evaluate the pathway of apoptosis after sulforaphene treatment the expressions of caspase 9, caspase 8, pro-caspase 3 and PARP were analyzed by western blot. From figure 4(a) caspase 9 expression was elevated with time and highest expression was found at 6 h after treatment.

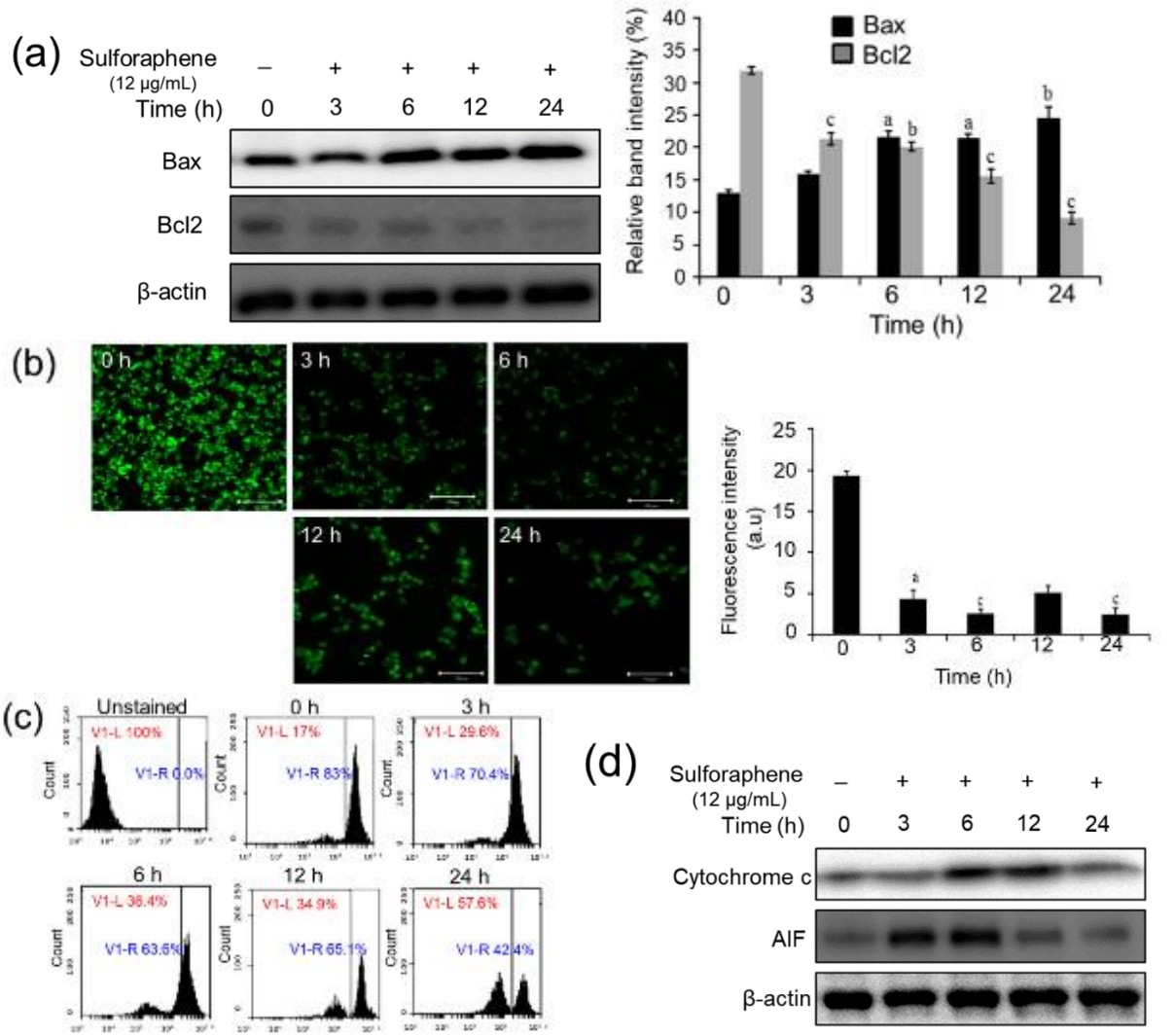

Figure 3: (a) Modulation of Bax and Bcl2 expression after sulforaphene treatment at different time intervals. (b) mitochondrial membrane depolarization with rhodamine 123 ($^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$. (c) Mitochondrial membrane potential of the cells. Cells in the right section of the histogram (V1-R) indicates normal cells with high fluorescence while the left side (V1-L) indicates cells with lower fluorescence, indicating depolarization of mitochondrial membrane potential. (d) Cytochrome c and AIF expression after sulforaphene treatment

In case of caspase 8, time dependent higher expression was observed and at 24 h cleaved form was observed. On the other hand time dependent modulation of caspase 3 was also observed after sulforaphene treatment. Expression of pro-caspase 3 in western blot analysis was observed to be decreased with time which indicates higher expression of active caspase 3. Hence we checked active caspase 3 by immunofluorescence staining. Results in figure 4 (b) clearly indicate time dependent increase of active caspase 3 after sulforaphene treatment. Highest active caspase 3 fluorescence intensity was observed for 12 h. After 24 h of sulforaphene treatment, maximum cells were found to be dead and the fluorescence intensity observed was less compared to the other treatment groups. Another downstream protein PARP was also found to be cleaved with time in sulforaphene treatment. As time increases, cleaved PARP expression was found to be increased.

Sulforaphene inhibits HeLa cell proliferation by activating PTEN and inactivating PI3Kinase

PI3Kinase has very important function in cell survival, cell differentiation and cell proliferation.. To determine whether sulforaphene can inhibit this cellular process via activation of PTEN and deactivation of PI3Kinase, we performed western blot analysis. Our experimental data in figure 4 (c) clearly demonstrated that PTEN expression increased with increasing time, whereas PI3kinase expression was found to be decreased. This time dependent increase of PTEN expression and decrease of PI3Kinase

was higher at 12 and 24 h after sulforaphene treatment. Relative band intensity histogram clearly indicates the time dependent significant modulation of PTEN and PI3Kinase.

DISCUSSION

Individual side effects of different cervical cancer modalities are the main disadvantage and have thrown this cancer as a challenge to the human being. Therefore introduction of some novel therapeutics without any adverse side effects is now highly recommended. Cruciferous vegetables like broccoli, cabbage, cauliflower, radish, mustard, etc. have already been reported for their anti-proliferative, anti-inflammatory, antioxidant and anticancer properties due to presence of various isothiocyanates [21].

The present study demonstrated that radish derived isothiocyanate sulforaphene treatment can reduce the viability of HeLa cells in both dose and time dependent manner. Sulforaphene induced time dependent apoptotic response was confirmed by the morphological changes with progressive detachment of cells containing condensed and fragmented chromatin along with reduction in cell viability. On the other hand, time dependent increase in sub-G1 cell population and concomitant decrease in G0/ G1 phase population after sulforaphene treatment suggest the efficacy of sulforaphene in arresting cell division and cell proliferation inhibition on human cervical cancer cells. Thus there was clear evidence that sulforaphene could bring about both cellular apoptosis and cell cycle progression inhibition in HeLa cells.

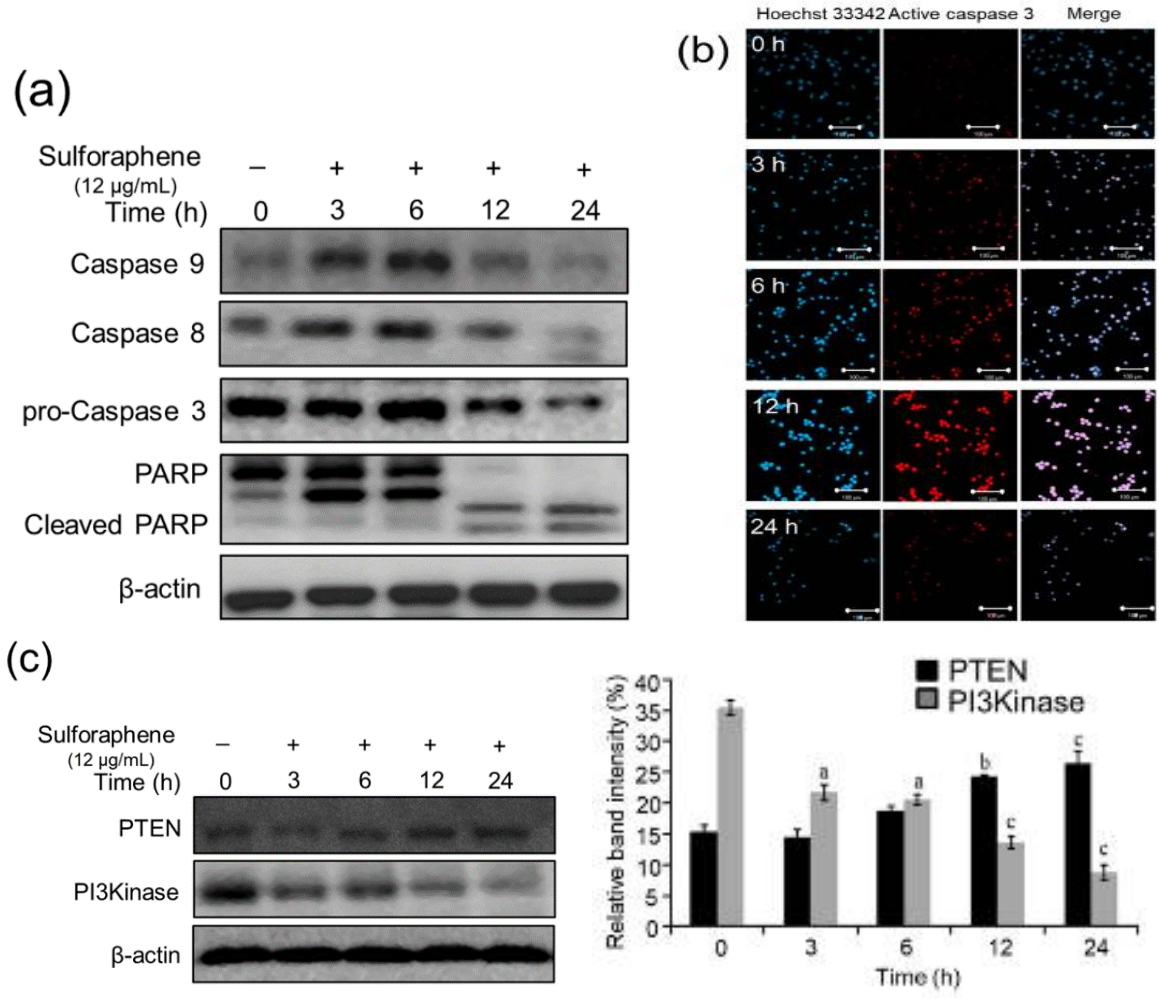

Figure 4: (a) Modulation of expressions of caspase 9, caspase 8, pro-caspase 3 and cleavage of PARP in active form. (b) Confocal microscopic analysis of active caspase 3 activity after sulforaphene treatment. (c) Alteration expressions of PTEN and PI3Kinase. a = $p<0.05$, b = $p<0.01$ and c = $p<0.001$ were considered as statistically significant

Reactive oxygen species (ROS) generation is responsible for the intracellular oxidative stress related mitochondrial membrane depolarization resulting in the activation of mitochondrial pathway of apoptosis [22]. In this present study, sulforaphene treatment increased intracellular

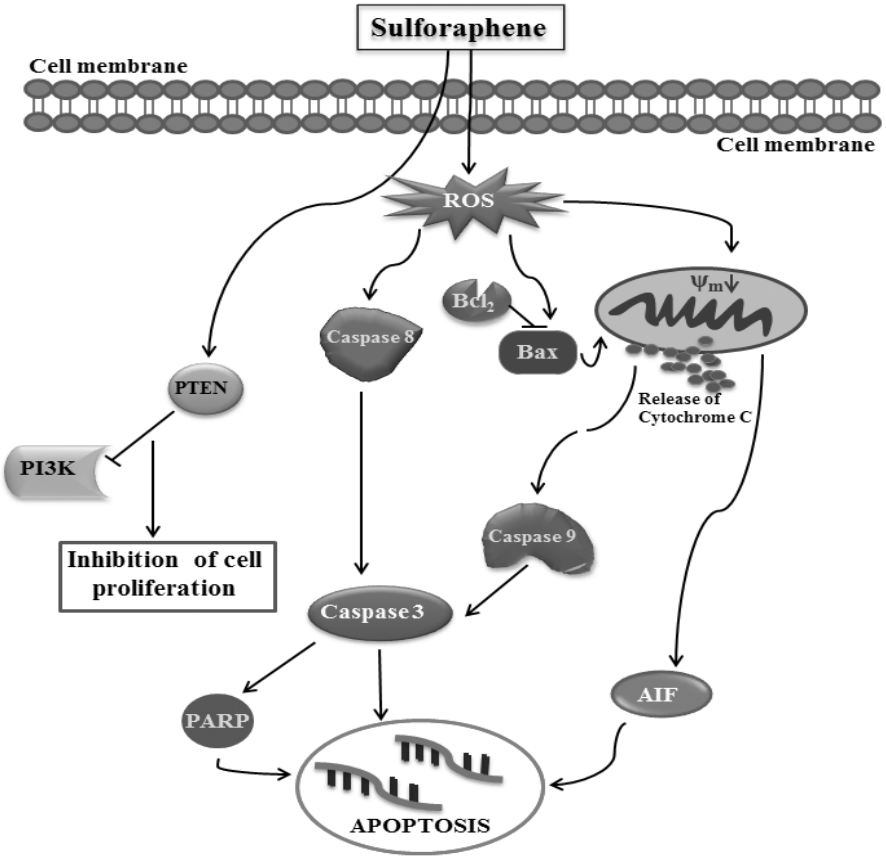

Figure 5: Schematic diagram represents intracellular ROS generation and modulation of pathway related signaling proteins after sulforaphene treatment in HeLa cells

ROS generation and subsequently enhanced pro-apoptotic Bax expression. Enhanced Bax, down regulates the anti-apoptotic protein Bcl2, which is the indication of mitochondrial membrane permeability change and activation of mitochondrial intrinsic pathway of programmed cell death [23]. Our data revealed that sulforaphene treatment decreased mitochondrial membrane potential in a time dependent manner and consequently increased the expressions of cytochrome c and AIF. This cytochrome c activates another mitochondrial pathway protein, caspase 9. On the other hand, sulforaphene treatment was also found to modulate the extrinsic pathway related protein, caspase 8. Activated caspase 8 and caspase 9 further activate one of the most important apoptosis regulating protein, caspase 3. Downstream protein PARP also found to be cleaved to its active form after sulforaphene treatment. Taken together, intracellular ROS plays a causative role in sulforaphene treated HeLa cells by the activation of mitochondrial pathway of programmed cell death. In the present study anti-apoptotic Bcl2 down-regulation and pro-apoptotic Bax up-regulation and further depletion of mitochondrial membrane potential indicates that mitochondria play a central role in sulforaphene induced apoptosis in HeLa cells. Higher expressions of cytochrome c and AIF also coincided with the mitochondrial membrane potential depletion and subsequent activation of caspase 9 and caspase 3. Moreover, caspase 8 was also involved in the sulforaphene mediated apoptosis. Therefore, higher expression of different caspases in our study could suggest that the apoptotic action of sulforaphene might be mediated through the activation of caspases.

The phosphatidylinositol 3-kinase (PI3Kinase) and phosphatase and tensin homolog (PTEN) are the two key signaling components which very frequently mutated in human cancers. Activated of PI3Kinase pathway regulates several cellular processes such as survival, proliferation, metabolism and growth resulting in tumorigenesis, migration and invasion [24]. On the other hand, PI3Kinase activity in the cancer cells is tightly regulated by the opposing activity of PTEN. Moreover, PTEN appears as a suppressor of cancer cell growth by cell cycle arrest and inducing apoptosis [25]. In the present study, the expression of PI3Kinase decreased and concomitantly the expression PTEN increases in a time dependent manner after sulforaphene treatment in HeLa cells. Based on

this result, it can be suggested that the dietary isothiocyanate, sulforaphene can inhibit cervical cancer cell proliferation and further functions by PI3Kinsase inhibition and PTEN activation. Figure 5 schematically demonstrate sulforaphene activity in the modulation of different pathway related proteins in HeLa cells.

Therefore, our present study is directed towards understanding the preliminary mechanisms in sulforaphene activity in HeLa cells. First, sulforaphene induced ROS appeared to play a crucial role in Bax/Bcl2 modulation as well as mitochondrial damage and cytochrome c and AIF higher expression. Second, sulforaphene treatment activates caspase 9-, 8-, 3- and cleaves PARP which leads HeLa cells to apoptosis. Third, sulforaphene down-regulates PI3Kinase and up-regulates PTEN resulting in inhibition of HeLa cell proliferation. However, more studies are required to overcome other detailed concerns including animal experiments but in the present study sulforaphene preliminarily qualifies as a better candidate and potent anticancer agent for therapeutic management of human cervical cancer.

DECLARATIONS

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Conflict of interest

The authors have no conflict of interest to declare with regard to this study.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Rhee Y performed the majority of experiments, analyzed the data, and wrote the manuscript; Mondal A. participated in experiments and Ahn J and Chung P designed and coordinated the research.

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