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Synergistic induction of apoptosis by sulindac and arsenic trioxide in human lung cancer A549 cells via reactive oxygen species-dependent down-regulation of survivin

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ABSTRACT

Survivin, a member of the inhibitor of apoptosis protein (IAP) family, may be a good target for cancer therapy because it is expressed in a variety of human tumors but not in differentiated adult tissues. In the present study, we show that a combination of sulindac and arsenic trioxide (ATO) induces more extensive apoptosis than either drug alone in A549 human non-small cell lung carcinoma (NSCLC) cells. Treatment with sulindac/ATO reduced the expression of survivin and promoted major apoptotic signaling events, namely, collapse of the mitochondrial membrane potential, release of cytochrome *c*, and activation of caspases. Combined sulindac/ATO treatment did not significantly affect the levels of other members of the IAP family (XIAP, cIAP1 and cIAP2), indicating that the effects were specific to survivin. In addition, sulindac/ATO treatment induced the production of reactive oxygen species and the antioxidant *N*-acetyl-L-cysteine blocked the down-regulation of survivin and induction of apoptotic signaling by the combination of sulindac and ATO. Combined sulindac/ATO treatment also activated p53 expression, and inhibition of p53 expression by small interfering RNA (siRNA) prevented sulindac/ATO-induced down-regulation of survivin, suggesting that survivin expression is negatively regulated by p53. Overexpression of survivin reduced sulindac/ATO-induced apoptosis in A549 cells and reduction of survivin levels by siRNA sensitized the cells to sulindac/ATO-induced cell death. These results demonstrate that, in A549 human NSCLC cells, sulindac/ATO-induced apoptosis is mediated by the reactive oxygen species-dependent down-regulation of survivin.

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1. Introduction

Cancer chemotherapy commonly requires the combination of multiple agents. Combination therapy, however, can have increased toxicity and may therefore require dose reduction, worsening the outcome of treatment. De novo and acquired resistance to chemotherapeutic agents and toxicity of drugs to normal cells are the major causes of treatment failure in most solid tumors [1]. To overcome such problems, additional combination chemotherapies that are more selectively toxic to tumor cells are needed.

Cancer of the lung is the leading cause of cancer-related death, and its incidence continues to rise. Strategies for the treatment of lung cancer include radiation therapy, chemotherapy and combinations of the two. Despite recent advances in the treatment of lung cancer, the response and remission rates for non-small cell lung cancer (NSCLC) remain relatively low [2]. Efforts are now being focused on finding novel combinations of anticancer agents with non-overlapping mechanisms of action to obtain enhanced anticancer efficacy and reduced adverse side-effects. For example, selective cyclooxygenase (COX)-2 inhibitors and cytotoxic drugs have synergistic antitumor effects in models of lung cancer [3].

Survivin is a member of the inhibitor of apoptosis protein (IAP) family. It plays an important role not only in inhibiting apoptosis but also in regulating mitosis [4,5]. Moreover, it is highly expressed in almost all types of human cancer but cannot be detected in most adult tissues [6]. High levels of survivin expression are associated with cancer progression, drug resistance, poor prognosis and short survival [7,8]. On the other hand, inhibition of survivin expression or interference with survivin function reduces cancer cell growth, induces cancer cell death, and sensitizes cancer cells to radiation or chemotherapy [9,10]. Therefore, survivin is regarded as a promising target for the treatment of cancer.

Sulindac, a non-steroidal anti-inflammatory drug (NSAID), is well known for its anti-inflammatory activity, which is due to its ability to inhibit the COX enzymes [11]. Recent studies have revealed a link between COX-2 expression and carcinogenesis, suggesting that inhibiting COX-2 can prevent cancer growth or progression [12]. In fact, in patients with adenomatous polyposis coli, NSAIDs cause the regression of colonic adenoma, thereby reducing the risk of colon cancer [13–15]. Based on these findings, the beneficial effects of NSAIDs have also been tested on other tumors where COX-2 is constitutively expressed, including lung, esophageal, prostate, pancreatic and gastric cancers [12,15–19]. These studies have shown that the antitumor activity of sulindac and other NSAIDs is due to the inhibition of COX-2.

Interestingly, however, sulindac sulfone, a metabolite of sulindac that lacks the ability to inhibit COX-2, reduces the incidence of tumors in animal models of breast and colon cancer [17,20]. Subsequent studies have shown that concentrations (90–240 μM) of sulindac and its metabolites induce apoptosis of various cultured tumor cell lines, including a lung cancer cell line, via COX-independent pathways [16,17,20,21]. Sulindac sulfone has been shown to exert its antiproliferative and antineoplastic effects by inhibiting cyclic guanosine 3′,5′ monophosphate phosphodiesterase, activating protein kinase G and promoting the phosphorylation of selective substrate, such as β-catenin [22]. Based on these results, it is evident that sulindac and its metabolites have potent antitumor activity against a broad spectrum of human cancer cells due to the inhibition of a variety of signaling pathways.

Recent in vitro and in vivo studies have shown that anticancer drugs, such as cisplatin, paclitaxel or docetaxel, in combination with sulindac metabolites, synergistically inhibit the growth of lung cancer cells [23]. Also, previous studies have shown that sulindac and its metabolites enhance the potency of other chemotherapeutic agents [24,25].

Arsenic trioxide (ATO) has multiple mechanisms of action, and it has different effects on differentiation and apoptosis, depending on the dose [26]. At low, clinically achievable concentrations (1–2 μM), ATO has potent activity against acute promyelocytic leukemia but little toxicity [27,28]. Recently, preclinical studies have demonstrated that ATO can induce apoptosis and inhibit tumor cell growth in a wide variety of solid tumors [29]. The combination of ATO with other drugs that activate additional apoptotic signals or inhibit survival signals may provide a rational molecular basis for novel chemotherapeutic strategies. Therefore, in the present study, we tested the combined effects of sulindac and ATO on the induction of apoptosis in a lung carcinoma cell line. We found that sulindac and ATO synergistically enhance the death of A549 cells. Furthermore, we show that this is due to decreased expression of survivin. Although sulindac is considered a chemopreventative agent, our findings suggest that it can be used to enhance the effect of the anticancer agent ATO by promoting the production of reactive oxygen species (ROS).

2. Materials and methods

2.1. Cell culture and reagents

A549 human NSCLC cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in recommended growth media (Invitrogen, Carlsbad, CA, USA). ATO was purchased from Sigma–Aldrich (St. Louis, MO, USA) and sulindac and N-acetyl-L-cysteine (NAC) were from Calbiochem (San Diego, CA, USA). Anti-cytochrome c antibody was purchased from BD Biosciences Pharmingen (San Diego, CA, USA), anti-XIAP antibody was from Cell Signaling Technology (Beverly, MA, USA), and antibodies against survivin, myc, cIAP-1, cIAP2, caspase 3 and caspase 9 were from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Measurement of cell viability

Cell viability was determined by measuring the mitochondrial conversion of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) to a colored product. A549 cells were treated with drugs for 48 h, after which MTT reagent was added. After 1 h at 37 °C, the cells were solubilized in isopropanol containing 0.04N HCl. The amount of converted MTT was determined by measuring the absorbance at 570 nm.

2.3. Evaluation of cell death

Cells were stained with the annexin V-FITC and propidium iodide (PI) according to the manufacturer's instructions (BD Biosciences Pharmingen). Briefly, cells were collected, washed with cold PBS and suspended in binding buffer (10 mM HEPES/NaOH [pH 7.4], 140 mM NaCl and 2.5 mM $CaCl_2$). The cells were stained with 5 μl annexin V-FITC and 10 μl PI and then analyzed with a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA).

2.4. Detection of ROS

ROS were detected using the cell-permeable indicator 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H_2DCFDA). Briefly, A549 cells were treated with drugs and then were loaded 20 μM H_2DCFDA for 30 min of treatment. Prepared cells were collected, washed with cold PBS and analyzed with a FACScan flow cytometer.

2.5. Assay of mitochondrial membrane potential (MMP)

The MMP was measured with the voltage-sensitive lipophilic cationic fluorescence probe 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1). Briefly, cells were collected, washed with cold PBS and then incubated with JC-1 for 15 min. The cells were washed with PBS and analyzed with a FACScan flow cytometer.

2.6. Measurement of caspase activation

Active caspases were detected using the CaspaTag™ Caspase 3/7 and Caspase 9 In Situ Assay kit (Chemicon, Temecula, CA, USA) according to the manufacturer's instructions. This kit employs carboxyfluorescein-labeled fluoromethyl ketone peptide inhibitors of caspases 3/7 (FAM-DEVD-FMK) and 9 (FAM-LEHD-FMK), which are cell-permeable and non-cytotoxic fluorochrome inhibitors of that covalently bind to a reactive cysteine residue on the large submit of the active caspase heterodimer, inhibiting enzymatic activity and producing green fluorescence. Thus, the green fluorescent signal directly corresponds to the amount of active caspases present in the cell at the time the reagent was added. The stained cells were analyzed with a FACScan flow cytometer.

2.7. RT-PCR analysis

RT-PCR was analyzed as described previously [30]. Two micrograms of total RNA isolated using TRI REAGENT (Molecular Research Center, Cincinnati, OH, USA) was transcribed into cDNA using M-MLV reverse transcriptase (Invitrogen). The specific primers for PCR were as follows: survivin, 5′-GGACCACCGCATCTCTAC-3′ and 5′-CAGCCTTCCAGCTCCTTG -3′; β-actin, 5′-GGATTCCTATGTGGGCGACAG-3′ and 5′-CGCTCGGTGAGGATCTTCATG-3′.

2.8. Transient transfection and reporter assay

The survivin-luciferase reporter and Myc-tagged survivin were a kind gift from Dr. Jin Q. Cheng (Department of Pathology, University of South Florida College of Medicine, Tampa, FL, USA). Survivin, p53 and silencer negative control siRNAs were purchased from Ambion (Austin, TX, USA). Transfections with plasmids and siRNAs were performed using Lipofectamine plus™ reagent and Lipofectamine™ 2000, respectively, according to the manufacturer's instructions (Invitrogen). For the survivin reporter assay, cells were transfected with survivin-Luc reporter along with pCMV-β-gal, and reporter transcription was measured by luciferase assay according to the manufacturer's instructions (Promega, Madison, WI, USA). The relative luciferase activity was calculated by normalizing the total luciferase activity by the β-galactosidase activity. Results are presented as the fold increase in activity relative to control.

2.9. Western blot analysis

Cells were collected, washed with ice-cold PBS and lysed in lysis buffer (50 mM Tris–HCl [pH 7.5], 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor cocktail (Roche, Mannheim, Germany). Insoluble components were removed from the lysate by centrifugation at 12,000 × *g* for 15 min, and protein concentrations were measured by the Bradford method. Samples (20–40 μg of protein) were separated by SDS-PAGE (10–14% acrylamide) and transferred to nitrocellulose membranes. The membranes were incubated with primary antibodies, followed by horseradish peroxidase-conjugated secondary antibodies, and immunoreactive bands were visualized with ECL reagents (Amersham Pharmacia, Uppsala, Sweden).

3. Results

3.1. Combined effect of sulindac and ATO on the death of A549 cells

Because previous studies have shown that sulindac and ATO exert cytotoxic effects on cancer cells [16,29], we conducted preliminary experiments to determine the minimum cytotoxic dose of sulindac and ATO in A549 NSCLC cells. As shown in Fig. 1A and B, MTT assays revealed that sulindac and ATO are cytotoxic at concentrations at or above 200 and 2 μM, respectively. It has been reported that the therapeutic range of ATO in treating APL is 1–2 μM [27,28]. And, the 200 μM of sulindac concentration has also been reported as the IC_{50} for various cell lines and lower than a dose (240 μM) to induce apoptosis in HT-29 colon or MCF-7 breast carcinoma cells [17,20]. On the basis of our observations in Fig. 1A and B as well as others [17,20,27,28], we examined the combined effect of 200 μM sulindac and 2 μM ATO in subsequent experiments.

We found that the combination of sulindac and ATO resulted in a synergistic, time-dependent induction of cell death as assessed by annexin V/PI staining (Fig. 1C and D). The combination of sulindac and ATO also caused a similar synergistic effect on the depolarization of the MMP (Fig. 1E) and caspase activation (Fig. 1F and G). These results showed that combination of sulindac and ATO results in a much greater extent of apoptosis than treatment with either drug alone.

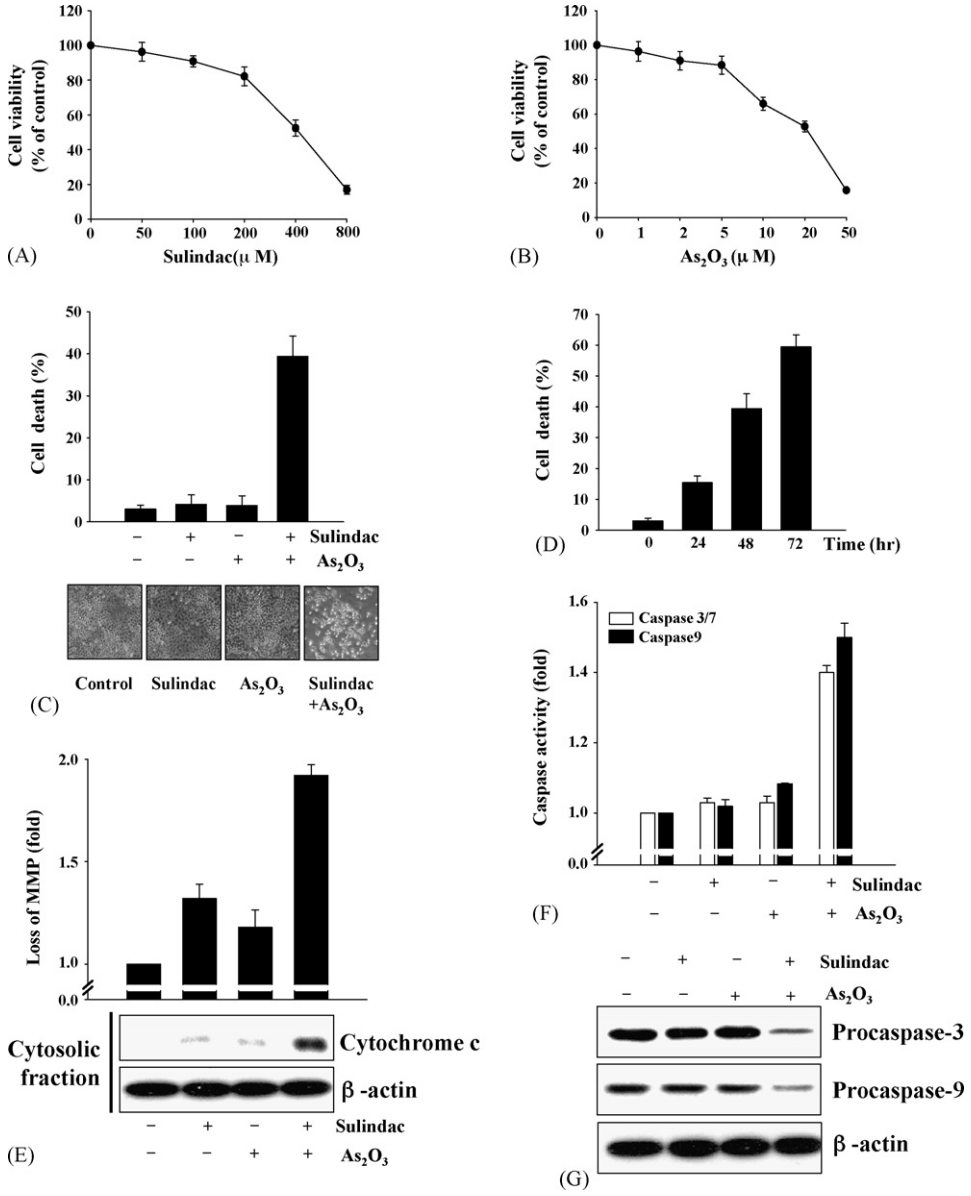

Fig. 1 – Cytotoxicity of the sulindac/ATO combination. (A and B) Individual effects of sulindac and ATO on cell viability. A549 cells were treated with the indicated concentrations of sulindac or ATO for 48 h. Cell viability was measured by MTT assay. The viability of control cells was set at 100% and the survival relative to the control is presented. (C and D) The combination of sulindac and ATO induces cell death. A549 cells were treated with or without 2 μM ATO and with or without 200 μM sulindac for 48 h (C) or treated with 200 μM sulindac and 2 μM ATO for the indicated times. Cell morphology was photographed under a microscope (C). Cell death was evaluated by flow cytometry after annexin V and PI staining (C and D). (E) The combination of sulindac and ATO induces mitochondrial dysfunction. A549 cells were treated with drugs as described in panel (C). The MMP was analyzed by flow cytometry using JC-1. The levels of cytochrome c protein in the cytosolic fraction were analyzed by Western blotting. (F and G) The combination of sulindac and ATO induces caspase activation. A549 cells were treated with drugs as described in panel (C). Active caspases were detected with the CaspaTag reagent (F) and the levels of caspases 3 and 9 protein were determined by Western blot analysis (G). In panels (E and G), β-actin protein levels were measured as loading controls.

3.2. *Role of ROS in the combined effect of sulindac and ATO*

To explore the molecular mechanism for the synergistic effect of sulindac and ATO, we first investigated whether these drugs induce the generation of ROS using the fluorescent indicator carboxy-H_2DCFDA. Both sulindac and ATO induced the generation of ROS. And, the enhancement of ROS generation by the sulindac/ATO combination was abrogated by the free radical scavenger NAC (Fig. 2A).

We next investigated whether this elevated production of ROS is crucial for the combined effect of sulindac and ATO on apoptotic cell death. For these assays, the cells were incubated

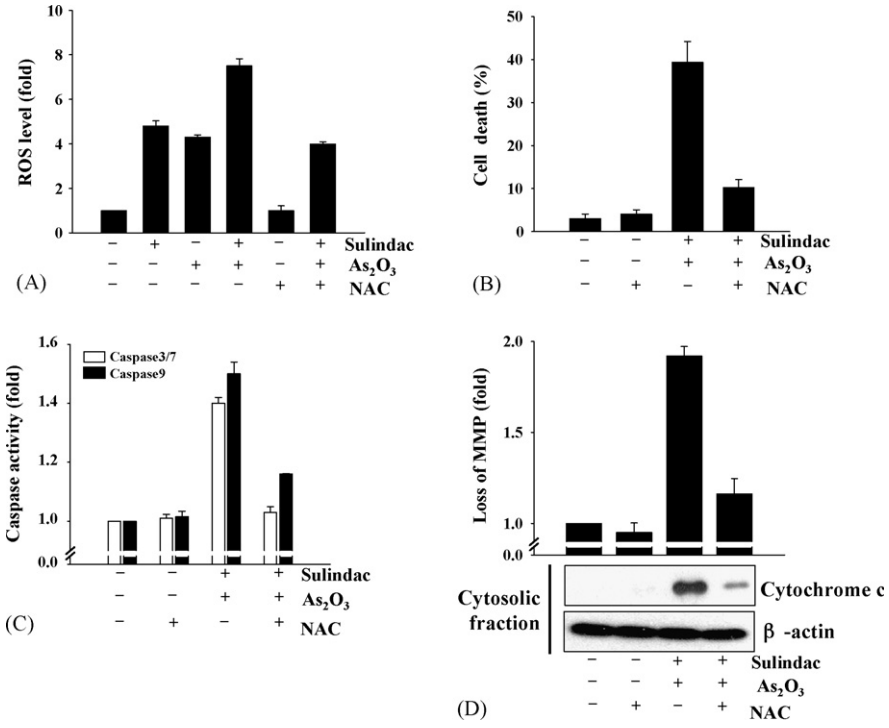

Fig. 2 – Role of ROS of the sulindac/ATO combination. (A) Sulindac and ATO induce the production of ROS. A549 cells were pretreated with 10 mM NAC for 30 min, followed by a 48 h treatment with or without 200 μM sulindac and with or without 2 μM ATO. ROS were detected with H_2DCFDA. (B–D) NAC blocks sulindac/ATO-induced cell death (B), caspase activation (C) and mitochondrial dysfunction (D). A549 cells were pretreated with 10 mM NAC for 30 min, followed by 200 μM sulindac and 2 μM ATO for 48 h. Cell death, caspase activation and MMP were analyzed as described in Fig. 1.

with NAC prior to the addition of the drugs. We found that NAC markedly inhibited the induction of cell death and the activation of caspases by the combination of sulindac and ATO (Fig. 2B and C). Our observations in Fig. 2A and B were consistent with data reported by Minami et al. [31] that sulindac and its metabolites greatly elevated carboxy-H_2DCFDA-detectable ROS levels and apoptosis in human colon DLD-1 cells.

In agreement with this result, NAC also suppressed the ability of the sulindac/ATO combination to depolarize the MMP and cause the release of cytochrome *c* to the cytosol from mitochondria (Fig. 2D). Together, these findings indicate that ROS generation plays a primary role in the ability of the sulindac/ATO combination to induce mitochondrial injury and apoptosis in A549 lung carcinoma cells.

3.3. *Down-regulation of survivin by the combination of sulindac and ATO*

Survivin, a member of the IAP family, was recently reported to modulate the balance between cell death and viability in cancer [6]. We therefore examined the effects of sulindac and ATO on the expression of survivin by A549. Western blotting of whole-cell extracts revealed that the combination of sulindac and ATO strongly decreases the level of survivin protein (Fig. 3A and B) but does not significantly affect the levels of other IAP family members, cIAP1/2 and XIAP (Fig. 3A).

We next evaluated whether the decrease in survivin levels is mediated at the level of transcription. As shown in Fig. 3C, RT-PCR revealed that the combination of survivin and ATO caused a much greater reduction in the level survivin mRNA than either drug alone. To confirm these results, we also examined the activity of the survivin promoter using a luciferase reporter gene containing the survivin promoter. As shown in Fig. 3D, there was a marked reduction of luciferase activity in cells treated with the sulindac/ATO combination. These results indicate that the combination of sulindac and ATO represses *survivin* promoter-mediated transcription in A549 cells.

3.4. *Role of ROS in the down-regulation of survivin expression by the sulindac/ATO combination*

To explore the relationship between ROS generation and the down-regulation of survivin expression, we measured the effect of NAC on the expression of survivin protein and survivin promoter activity. As shown in Fig. 4A and B, down-regulation of survivin protein and survivin promoter activity by the combination of sulindac and ATO were greatly abrogated by NAC. These results indicate that the down-regulation of survivin by the combination of sulindac and ATO is mediated by the production of ROS.

3.5. *Involvement of survivin expression in sulindac/ATO-induced cell death*

To determine whether the repression of survivin is responsible for sulindac/ATO-induced cell death, we examined the

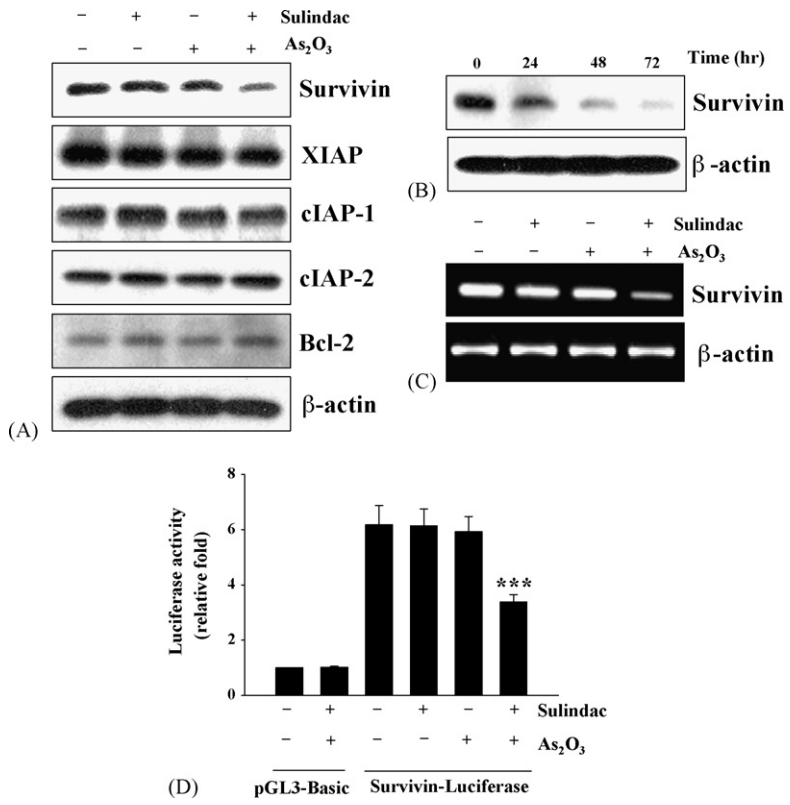

Fig. 3 – Down-regulation of survivin by the sulindac/ATO combination. (A–C) Combined treatment with sulindac and ATO inhibits transcription and translation of survivin. A549 cells were treated with or without 200 μM sulindac and with or without 2 μM ATO for 48 h (A and C) or with 200 μM sulindac and 2 μM ATO for the indicated times (B). Survivin RNA was measured by RT-PCR (C) and survivin protein was analyzed by Western blotting (A and B). (D) Combined treatment with sulindac and ATO reduces survivin transcriptional activity. A549 cells were transiently transfected with pGL3-Basic or survivin-luciferase along with pCMV-β-gal (encoding β-galactosidase). After 24 h, cells were treated with 200 μM sulindac and 2 μM ATO for 48 h. Luciferase activity was measured and normalized by the β-galactosidase activity. $^{***}P < 0.001$ vs. the survivin-luciferase-transfected group.

effects of the drugs in cells transiently transfected with a plasmid encoding Myc-survivin. Expression of Myc-survivin in transiently transfected cells, which normally showed approximately 50% of transfection efficiencies in our experimental settings (data not shown), was confirmed by Western blot analysis with an anti-Myc antibody (Fig. 5A, inset). As shown in Fig. 5A, the combination of sulindac and ATO resulted in decreased levels of apoptosis in A549 cells overexpressing myc-survivin than in control A549 cells. These observations imply that ectopic expression of survivin in A549 cells may help them to overcome sulindac/ATO-induced apoptosis in certain levels.

We next investigated the role of survivin in sulindac/ATO-induced apoptosis using siRNA. As shown in Fig. 5B, cells transfected with survivin siRNA showed an increased cell death compared with control siRNA treated cells. Moreover, combined treatment with survivin siRNA and sulindac/ATO resulted in a greater extent of cell death than combined treatment with control siRNA and sulindac/ATO. Western blot analysis confirmed that the survivin siRNA reduced the level of total survivin protein without affecting the level of XIAP or actin, indicating that its effects were specific and selective (Fig. 5B, inset). These results show that survivin can counteract apoptosis induced by sulindac and ATO in A549 cells, and they suggest that the induction of cell death by the combination of sulindac and ATO is due, at least of in part, to the down-regulation of survivin.

3.6. *Reduction of survivin expression by sulindac/ATO treatment is dependent on the accumulation of p53*

Previous reports suggest that p53 down-regulates the expression of survivin in some cell models and cancer cell lines [32,33]. To examine the effect of p53 on the expression of survivin, we first monitored the p53 protein levels in cells treated with sulindac and ATO. We found that the expression of p53 is induced 24 h after exposure to sulindac and ATO and that it is sustained for at least 72 h (Fig. 6A).

We next examined whether the up-regulation of p53 by sulindac and ATO is responsible for the down-regulation of survivin using a siRNA targeting p53. Western blot analysis revealed that the siRNAs were highly effective at decreasing the level of p53 protein. As shown in Fig. 6B, the p53 siRNA prevented sulindac/ATO from reducing the level of survivin protein. These results indicate that down-regulation of survivin by the sulindac/ATO combination is mediated by the activation of p53.

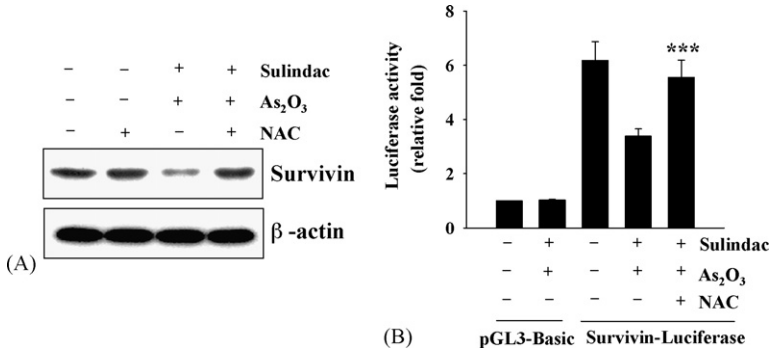

Fig. 4 – Role of ROS in the down-regulation of survivin protein and promoter activity by the sulindac/ATO combination. Survivin protein levels were determined by Western blot analysis (A). A549 cells were pretreated with 10 mM NAC for 30 min, followed by 200 μM sulindac and 2 μM ATO for 48 h. Survivin promoter activity was measured by luciferase activity (B). A549 cells were transiently transfected with pGL3-Basic or survivin-luciferase along with pCMV-β-gal (encoding β-galactosidase). After 24 h, the cells were pretreated with 10 mM NAC for 30 min, followed by 200 μM sulindac and 2 μM ATO for 48 h. Luciferase assay was measured and normalized by the β-galactosidase activity. $^{***}P < 0.001$ vs. the survivin-luciferase-transfected and survivin/ATO-treated groups.

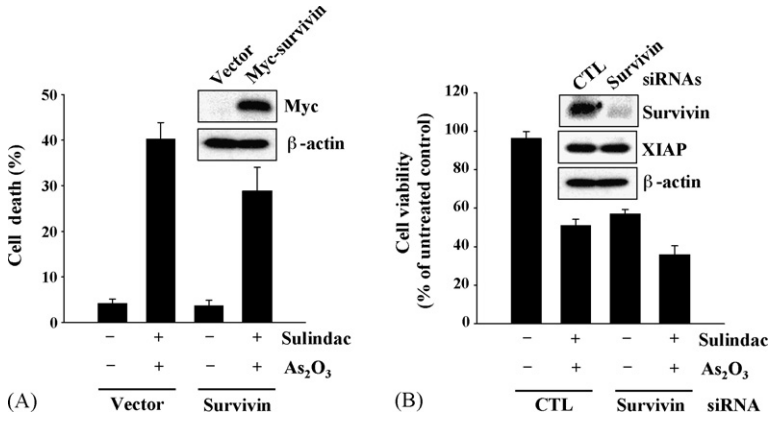

Fig. 5 – Involvement of survivin in sulindac/ATO-induced cell death. (A) Overexpression of survivin reduces sulindac/ATO-induced cell death. A549 cells were transfected with Myc-survivin or pcDNA (vector) for 20 h and then treated with 200 μM sulindac and 2 μM ATO for 48 h. Cell death was evaluated by flow cytometry after annexin V and PI staining. (B) Inhibition of survivin by siRNA sensitizes A549 cells to sulindac/ATO-induced cell death. A549 cells were transfected with survivin or silencer negative control (CTL) siRNAs for 20 h and then treated with 200 μM sulindac and 2 μM ATO for 48 h. Cell viability was determined by MTT assay. The viability of control cells was set at 100%, and the viability relative to the control is shown.

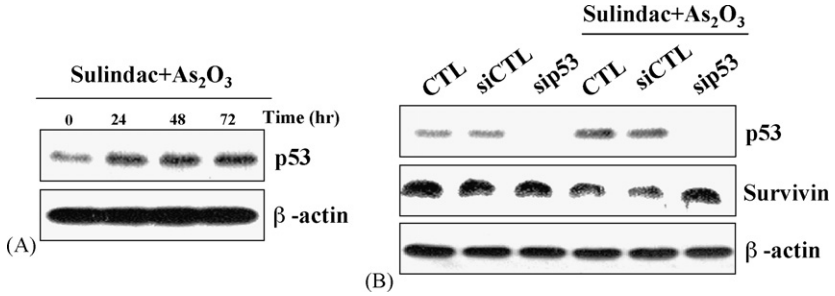

Fig. 6 – Reduction of survivin expression by sulindac/ATO treatment is dependent on the accumulation of p53. (A) Combined treatment with sulindac and ATO induces the expression of p53 protein. A549 cells were treated with 200 μM sulindac and 2 μM ATO for the indicated times. (B) Inhibition of p53 by siRNA prevents the down-regulation of survivin by the combination of sulindac and ATO. A549 cells were transfected with p53 or silencer negative control (CTL) siRNAs for 20 h and then treated with 200 μM sulindac and 2 μM ATO for 48 h. The levels of survivin and p53 protein were determined by Western blot analysis.

4. Discussion

The balance between apoptosis and survival signals plays an important role in the pathogenesis of a variety of cancers [34,35]. Survivin has been demonstrated to inhibit apoptosis and to promote mitotic progression in cancer cells [4,5]. Thus, it is expected that inhibition of survivin will be an important strategy for the treatment of cancer [36]; however, anticancer drugs, such as adriamycin and taxol, increase the expression of survivin in cancer cells [36], where it may serve as a radio- and chemo-resistance factor [9,10,36]. The key finding of the present study was that a combination of sulindac and ATO is exceptionally strong at down-regulating survivin expression at both the protein and mRNA level as well as depolarizing the MMP, activating caspases and inducing apoptosis in A549 human NSCLC cells. Interestingly, the combination of sulindac and ATO did not significantly affect the levels of other IAPs (XIAP, cIAP1 and cIAP2), indicating that the effect of this treatment was specific. Also, the finding that down-regulation of survivin expression by siRNA sensitizes the cells to sulindac/ATO-induced apoptosis further supports the idea that survivin modulates the sensitivity of A549 cells to apoptosis.

Overall, these findings have two major implications. First and foremost is that a decrease in survivin levels plays an important role in human NSCLC. In agreement with this, several studies have concluded that there is a strong association between increased survivin levels and progression of human lung cancer [37–39]. Second, these studies clearly suggest that survivin is an important molecular factor in lung cancer cell survival as well as in resistance to apoptosis and that a decrease in survivin expression leads to the apoptosis of lung cancer cells. Although it is possible that in most normal cells and terminally differentiated adult tissues which express survivin in undetectable levels [6,40,41] other yet unidentified molecules or signalling pathways may be involved in sulindac/ATO-induced apoptosis, the current results are highly significant because they show that a combination of sulindac and ATO causes the complete down-regulation of survivin in conjunction with a strong induction of apoptosis in A549 cells.

Cellular ROS are essential to cell survival, but the effect of ROS on cells is complex. Experimentally, a low concentration of H_2O_2 causes a modest increase in the proliferation of many tumor cell lines, whereas a higher level results in slowed growth, cell cycle arrest, and apoptosis or even necrosis [42]. This implies that the effects of ROS depend on their levels. This can explain how ATO, which was initially recognized as an environmental carcinogen, can act as an anticancer agent that induces apoptosis in tumor cells [29]. Our results suggest that when sulindac is combined with ATO, the elevation of ROS may exceed a certain threshold that finally overcomes anti-apoptotic forces, shifting the cell survival/death balance towards cell death. Specifically, our current results demonstrated that the combination of sulindac and ATO down-regulates the expression of survivin by promoting the generation of ROS. Thus, the combination of sulindac and ATO may serve as a novel chemotherapy for the treatment of lung cancer.

Our investigation of how sulindac/ATO down-regulates survivin in A549 cells revealed that these drugs reduce the level of survivin mRNA and the activity of the survivin promoter. This suggests that survivin is transcriptionally down-regulated by sulindac and ATO. Because p53 binds to the survivin promoter and suppresses its transcription [32], we examined the role of p53 in the down-regulation of survivin by the combination of sulindac and ATO. We found that p53 is activated in A549 cells by the combination of sulindac and ATO. Inhibition of p53 by transfection with a p53 siRNA prevented the down-regulation of survivin by the combination of sulindac and ATO. These results suggest that p53 mediates the down-regulation of survivin by the combination of sulindac and ATO. Further studies are needed to determine whether additional survivin-independent pathways participate in the induction of apoptosis by the combination of sulindac and ATO. Further studies are also needed to establish whether the sulindac/ATO combination is effective in other human lung cancer cells or cancer cell lines originating from other tissues.

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