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Arsenic trioxide induces apoptosis in B-cell chronic lymphocytic leukemic cells through down-regulation of survivin via the p53-dependent signaling pathway

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Abstract

Arsenic trioxide (As_2O_3) can induce apoptosis in many tumors. However, the associated mechanisms are not clearly understood. We found that As_2O_3 significantly inhibited the proliferation of WSU-CLL cells and induced apoptosis in dose- and time-dependent manners. WSU-CLL cells treated with 2 μM As_2O_3 showed survivin down-regulation and p53 up-regulation. Survivin siRNA combined with As_2O_3 further inhibited the proliferation of WSU-CLL cells. p53 inhibition by siRNA prevented the down-regulation of survivin by As_2O_3 and prevented the As_2O_3-induced cytotoxicity of WSU-CLL cells. These results suggest that As_2O_3 may be of therapeutic value for chronic lymphocytic leukemia.

Keywords

Arsenic trioxide; WSU-CLL; Apoptosis; Survivin; P53 and CLL

1. Introduction

B-cell chronic lymphocytic leukemia (CLL) is characterized by the extensive accumulation of monoclonal, relatively mature CD5+ B cells in lymphoid organs, bone marrow, and

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Conflict of interest
All authors declare no conflict of interest.

Contributions. Xiao-Hui Zhang and Ru Feng share first authorship. X.L. Zheng and X.H. Zhang designed the study, reviewed the data analysis, reviewed the manuscript; R. Feng helped with the design of the study and writing the manuscript; X.H. Zhang and R. Feng performed the laboratory testing and analysis of the experimental work and final submission of the manuscript. M.L., Q.J., H.H.Z., and Y.Z.Q. contributed to the acquisition of data of F.C.S. and P.C.R. and performed the statistical analysis. J.L.B. and X.L.Z. co-ordinated the research.

peripheral blood. CLL cells accumulate because of defective apoptosis, which extends survival [1].

Arsenic trioxide (As_2O_3) has been shown to be useful in the treatment of patients with acute promyelocytic leukemia (APL). Preclinical studies have demonstrated that As_2O_3 can induce apoptosis and inhibit tumor cell growth in a wide variety of tumors [2–5]. As_2O_3 is also being used to treat other hematologic malignancies, such as myelodysplastic syndrome [6]. Few studies have addressed the effect of As_2O_3 on B-CLL cells. It was recently reported that As_2O_3 preferentially induced apoptosis in B-CLL cases with unfavorable prognoses [7–11], and in analyses of a small number of B-CLL samples, As_2O_3 was shown to induce B-CLL apoptosis [8]. These previous studies suggest that As_2O_3 could be a possible therapeutic agent for B-CLL. The mechanism by which As_2O_3 induces B-CLL apoptosis has not been established.

Previous studies have shown that survivin expression is closely related to apoptosis resistance [12,13]. Survivin is a member of the inhibitor of apoptosis protein (IAP) family [14]. It has been shown that survivin is overexpressed in CLL patients, particularly those with poor prognostic factors [15]. A previous study demonstrated that survivin inhibits apoptosis, enhances proliferation, and promotes angiogenesis [16], and it is regarded as a promising target for the treatment of cancer.

The induction of apoptosis by As_2O_3 in tumors such as acute promyelocytic leukemia, Burkitt's lymphoma and human glioma is thought to occur through the down-regulation of survivin [2,17,18]. Li et al. [2] found that As_2O_3 inhibited the proliferation of Burkitt's lymphoma cell lines, and after treatment with different concentrations of As_2O_3, survivin gene expression decreased. Ghaffari et al. [17] demonstrated that As_2O_3 might inhibit cell growth and proliferation and induce apoptosis in NB4 cells through the transcriptional repression of survivin.

As_2O_3 has previously been shown to induce apoptosis in many malignancies, such as multiple myeloma, neuroblastoma and human pre-monocytic leukemia, depending on the p53 status in the cells [19–21]. p53 plays a central role in the activation of apoptosis via the mitochondrial apoptosis pathway [20]. Previous studies have shown that p53 is one of the most important components in the apoptosis pathway, which is affected by As_2O_3, and that it may be a target of As_2O_3 [22,23]. Kircelli et al. [19] proposed that As_2O_3 could induce apoptosis in multiple myeloma in a p53-dependent manner.

Cell growth and cell death are usually determined by a balance between the activities of oncogenes and tumor-suppressor genes. Survivin over-expression and loss of p53 function occur in many cancers [24–27]. Shao et al. [28] suggested a correlation between the levels of p53 and survivin proteins expressed in prostate tumors. Their data suggest that p53 may be directly altered conformationally in the presence of survivin. Nabilsi et al. [29] proved that the balanced regulation of the p53-survivin signaling pathway is necessary for cell survival in mammalian cells. They suggested that p53, a negative regulator of survivin, could decrease the expression of survivin protein through transcriptional regulation.

Given these results, we hypothesized that As_2O_3 can induce cytotoxic effects in WSU-CLL cells, a CLL cell line that is refractory to fludarabine and combination therapies, including cyclophosphamide, vincristine and prednisone (CVP) and vincristine, adriamycin and dexamethasone (VAD) [30–32]. The mechanism may be modulated through the induction of apoptosis due to the p53-dependent down-regulation of survivin. Therefore, in the present study, we evaluated whether As_2O_3 could inhibit the proliferation of WSU-CLL cells. Furthermore, we examined whether this inhibition was due to decreased survivin expression through the p53 signaling pathway.

2. Materials and methods

2.1. Cell culture and reagents

WSU-CLL is a human CLL cell line, and the cells were generously provided by Dr. Bo Huang (Department of Immunology, Institute of Basic Medical Sciences, Chinese Academy of Medical Science, China). The cells were cultured in 5% CO_2 at 37 °C in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Life Technologies, Inc.), 2 mM L-glutamine, 100 units/ml penicillin G, and 100 mg/ml streptomycin (Life Technologies, Inc.). The characteristics of this cell line have been described in detail elsewhere [30–32].

A stock solution of arsenic trioxide (As_2O_3) was kindly supplied by Harbin Yida Pharmaceutical Company (Harbin, China). It was diluted in RPMI-1640 medium to a concentration range of 1–8 μM. Antibodies against survivin, myc and p53 were purchased from BD Biosciences Pharmingen.

2.2. Cell viability assay

The in vitro effect of As_2O_3 on WSU-CLL cell growth was determined by counting the viable cells with trypan blue staining. Briefly, cells were seeded in 96-well plates at a density of 5×10^4 cells/ml and pre-incubated overnight. After varying lengths of exposure to various concentrations of As_2O_3, each cell suspension (0.02 ml) was mixed well with 0.02 ml trypan blue solution. After 1 or 2 min, each solution was placed on a hemocytometer, and the blue-stained cells were counted as non-intact.

2.3. Assessment of apoptosis

WSU-CLL cell apoptosis was assessed by observing the translocation of phosphatidyl serine to the cell surface, as detected with an Annexin V-FITC/PI apoptosis detection kit (BD Biosciences Pharmingen, USA). The cells were collected, washed with cold PBS and suspended in binding buffer. The cells were stained with 5 μl annexin V-FITC and 10 μl PI and analyzed with a FACScan flow cytometer.

2.4. Western blotting analysis

After various treatments, the cells were lysed by adding lysis buffer (1 M Tris–HCl, pH 6.8, 2 M NaCl, 0.1 M EDTA, 0.05 M EGTA, 0.5% NP-40 and 10 mM PMSF). Then, samples were vortexed briefly and incubated in lysis buffer for 30 min on ice and centrifuged at 15,000 × *g* for 15 min. The protein concentration was determined using the Bradford

method. Samples of 40 μg of total protein were subjected to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose membrane (Roche). The membranes were incubated with primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies, and the immunoblots were visualized by enhanced chemiluminescence procedures according to the manufacturer's recommendations. β-actin was used as the protein loading control. Each immunoblot was repeated at least three times.

2.5. RT-PCR analysis

Total RNA was isolated from WSU-CLL cells treated with or without As_2O_3 using TRIzol reagent. RNA was quantified using a UV spectrometer, and cDNA was synthesized with 2 μg of total RNA (Fermentas Inc.). PCR was performed in a final volume of 20 μl containing 10 μl SYBR-Green master mix (including TaqE), 0.5 μl of each forward and reverse primer (10 pmol), 2 μl cDNA samples and 7 μl nuclease-free water. The thermal cycling conditions were as follows: 95 °C for 5 min; 40 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and 90 °C for 5 s; and an annealing/extension step at 72 °C for 5 min.

The specific PCR primers were as follows: [33]

survivin: 5′-GGACCACCGCATCTCTAC-3′ and 5′-CAGCCTTCCAGCTCCTTG-3′;

β-actin: 5′-GGATTCCTATGTGGGCGACAG-3′ and 5′-CGCTCGGTGAGGATCTTCATG-3′.

The primers were synthesized by Shanghai GenePharma Co. (Shanghai, China).

2.6. Small interfering RNA design

We used http://www.jura.wi.mit.edu/bioc/siRNA to construct a series of expression plasmids containing small interfering RNA (siRNA) specific to survivin, p53 or negative control siRNA expression elements.

The following primers were used:

survivin siRNA: 5′-AGCAUUCGUCCGGUUGCGCdTdT-3′ and 5′-GCGCAACCGGACGAAUGCUTT-3′;

p53 siRNA: 5′-GCAUGAACCGGAGGCCCAUdTdT-3′ and 5′-AUGGGCCUCCGGUUCAUGCTT-3′

negative control siRNA: 5′-UUCUCCGAACGUGUCACGUdTdT-3′ and 5′-ACGUGACACGUUCGGAGAATT-3′.

After these siRNAs were designed, they were compared with the sequence in the human expressed sequence tag (EST) database to confirm that no other genes were targeted, and they were then synthesized by Shanghai GenePharma Co. (China).

2.7. Transient transfection and reporter assay

The survivin luciferase reporter and Myc-tagged survivin were a kind gift from Dr. Bo Huang (Department of Immunology, Institute of Basic Medical Sciences, Chinese Academy of Medical Science, China). The cells were incubated in fresh media without antibiotics for 24 h before transfection. On the following day, the cells were transfected with plasmid DNA for 20 h.

For the survivin reporter assay, the cells were transfected with a survivin-Luc reporter and pCMV-β-gal, and reporter transcription was measured with a luciferase assay. The relative luciferase activity was calculated by normalizing the total luciferase activity by the β-galactosidase activity. The results were presented as the fold increase in activity relative to control.

For siRNA transfection, the cells were incubated in fresh media without antibiotics in 96- and 6-well plates for 24 h. Then, they were transfected with 0, 25, 50, 100, 200, or 400 nmol/L (final concentration) of siRNA per well for 20 h using lipofectamine 2000 transfection reagent (Invitrogen, USA), according to the manufacturer's instructions. Western blots were used to detect the efficiency of siRNA transfection. The results of preliminary experiments indicated that 100 nmol/L siRNA could successfully inhibit target gene expression (data not shown). We therefore used 100 nmol/L as the siRNA concentration in this study.

Cells were treated with either As_2O_3 (2 μM) or solvent control for an additional 24 h after transfection. Treated cells were collected and used for measurement. Cell viability was measured using trypan blue exclusion as described above.

2.8. Statistical analysis

The data are expressed as the mean ± standard deviation (SD). All experiments were performed in triplicate. Statistical analyses were performed using SPSS software (SPSS, Version 16.0, Chicago, IL, USA). Differences among treatment groups were analyzed by one-way analysis of variance (ANOVA) with Dunnett's post hoc analysis. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Effect of As_2O_3 on WSU-CLL cell viability

Previous studies have shown that As_2O_3 exerts cytotoxic effects on cancer cells [2,3,17]. To evaluate the inhibitory effects of As_2O_3 on the growth of the WSU-CLL cell line, WSU-CLL cells were incubated with 0–8 μM As_2O_3 for 24, 48, 72 and 96 h. The inhibition rates and 50% inhibitory concentration (IC_{50}) were evaluated by trypan blue dye exclusion. WSU-CLL cell growth was inhibited by As_2O_3 in time- and dose-dependent manners. The IC_{50} after 72 h of As_2O_3 treatment in CLL cells was 1.96 μM.

As shown in Fig. 1, As_2O_3 was cytotoxic to WSU-CLL cells at concentrations at or above 2 μM. The therapeutic range of As_2O_3 in treating APL is 1–2 μM [17]. On the basis of our

observations as well as others [11,17], we examined the effect of 2 μM As_2O_3 in subsequent experiments.

3.2. Apoptosis induction by As_2O_3 in WSU-CLL cells

To explore whether the cytotoxic effect of As_2O_3 in WSU-CLL cells was the result of apoptosis induction, Annexin V-FITC/PI staining and flow cytometry were performed. WSU-CLL cells were treated with different concentrations of As_2O_3 for 0, 24, 48, 72 and 96 h. There was a dose- and time-dependent increase in the number of apoptotic cells, as shown in Fig. 2. The apoptosis rates of WSU-CLL cells in the presence of 2 μM As_2O_3 at 0, 24, 48, 72 and 96 h were 2.0%, 18.9%, 42.3%, 58.3% and 67.2%, respectively.

3.3. Down-regulation of survivin by As_2O_3

Survivin was recently reported to modulate the balance between cell death and viability in cancer [33]. It is regarded as one of the most important anti-apoptosis proteins in malignant proliferation. It has been shown that survivin is overexpressed in WSU-CLL cells [30]. We hypothesized that the cytotoxic effects of As_2O_3 on WSU-CLL cells may be due, in part, to survivin down-regulation. We therefore examined the effects of As_2O_3 on survivin expression in WSU-CLL cells. Western blotting of whole-cell extracts revealed that As_2O_3 strongly decreased the level of survivin protein (Fig. 3A and B). Our results demonstrated that As_2O_3 induced a time-dependent down-regulation of survivin protein in WSU-CLL cells.

To investigate whether As_2O_3 exposure triggered a decrease in survivin protein levels at the transcriptional level, we evaluated the impact of As_2O_3 on survivin mRNA levels. As shown in Fig. 3C and D, RT-PCR revealed that 2 μM As_2O_3 caused a large reduction in the survivin mRNA level.

3.4. Involvement of survivin expression in As_2O_3-induced cytotoxicity

To determine whether survivin down-regulation was responsible for As_2O_3-induced cytotoxicity, we examined the effects of As_2O_3 in cells transiently transfected with a plasmid encoding myc-survivin or pc-DNA. As shown in Fig. 4A, cells pre-treated with myc-survivin showed a high level of survivin protein. The cell viability of WSU-CLL cells treated with As_2O_3 was increased in the survivin group compared with the vector group (Fig. 4B). These observations suggest that the overexpression of survivin in CLL cells may help to overcome As_2O_3-induced cytotoxicity.

We next investigated the role of survivin in As_2O_3-induced cell death using survivin siRNA. Western blotting demonstrated that survivin siRNA successfully inhibited survivin expression in WSU-CLL cells (Fig. 5A). As shown in Fig. 5B, increased cytotoxicity was observed in cells transfected with survivin siRNA compared with cells treated with control siRNA. Moreover, treatment with survivin siRNA and As_2O_3 resulted in a greater extent of cell death than control siRNA.

These results demonstrate that survivin can counteract the cytotoxicity induced by As_2O_3 in WSU-CLL cells. The induction of cell death by As_2O_3 is due, at least in part, to the down-regulation of survivin.

3.5. The reduction of survivin expression by As_2O_3 treatment is dependent on p53 accumulation

Previous reports have suggested that p53 down-regulates the expression of survivin in some cell models and cancer cell lines [29,34]. To examine the effect of As_2O_3 on p53 expression and determine the relationship between survivin and p53, we first monitored the p53 protein levels of WSU-CLL cells treated with As_2O_3. We found that p53 expression was increased 24 h after exposure to As_2O_3 and that it was sustained for at least 72 h (Fig. 6).

Previous studies have suggested that survivin overexpression inhibits the p53-dependent apoptosis pathway [34]. Therefore, survivin inhibition may allow for the re-activation of this p53-mediated apoptosis program.

We next examined whether p53 up-regulation by As_2O_3 was responsible for survivin down-regulation using an siRNA targeting p53. Western blot analysis revealed that the siRNAs were highly effective at decreasing the p53 protein level (Fig. 7A). As shown in Fig. 7B, survivin protein expression increased as p53 protein decreased. These results indicate that survivin down-regulation by As_2O_3 is mediated by p53 activation.

To further investigate the role of p53 in As_2O_3-induced WSU-CLL cell cytotoxicity, we used trypan blue exclusion to examine cell viability in the presence of As_2O_3 and/or p53 siRNA. As shown in Fig. 7C, As_2O_3 significantly decreased cell viability. Furthermore, when cultured with p53 siRNA, As_2O_3-induced cytotoxicity was partially rescued. Our data suggest that the p53-survivin pathway is involved in the effects of As_2O_3-induced apoptosis in WSU-CLL cells.

4. Discussion

Studies have demonstrated that As_2O_3 slows cancer cell growth, including cells from leukemias and solid tumors [10,35]. Although As_2O_3 influences numerous signal transduction pathways, cell cycle progression, and programmed cell death, the detailed mechanisms of how programmed cell death is induced by As_2O_3 are complex and not entirely delineated [36]. The current study found that As_2O_3 reduced WSU-CLL cell viability in dose- and time-dependent manners. Our findings support the results of previous studies showing similar effects on various types of human cancer cell lines [17,20,29,33]. Pharmacokinetic analyses of APL patients successfully treated with As_2O_3 have shown that the peak plasma concentration is 5.5–7.3 μM and that the steady state is believed to be between 1 and 2 μM [37]. In our study, the IC_{50} value of As_2O_3 in WSU-CLL cells after 72 h of treatment was 1.96 μM, which is similar to those reported previously [8]. Taken together, the results of our study suggest that As_2O_3 may be clinically useful in patients with malignant chronic lymphoblastic leukemia.

The mechanisms of As_2O_3-induced cell growth inhibition have been extensively investigated. Apoptosis appears to be the primary phenomenon resulting in significant cell death [2,7,20]. In our experiments using dual staining with Annexin V and PI, we clearly demonstrated that WSU-CLL cells treated with As_2O_3 underwent apoptosis.

The balance between apoptosis and survival signals plays an important role in the pathogenesis of a variety of cancers [14,18]. Survivin is a unique member of the inhibitor of apoptosis protein (IAP) family. It is expressed in most human tumors, but it is barely detectable in terminally differentiated normal cells/tissues [14]. The differential expression of survivin in cancer versus normal tissues suggests that it is a promising therapeutic target. Disruption of the survivin induction pathway resulted in an increase in apoptosis and the inhibition of tumor growth.

Survivin has been shown to inhibit apoptosis, promote tumor-associated angiogenesis, and serve as a resistance determinant to various anti-cancer therapies [38]. Survivin expression inhibits cell death induced by various apoptotic stimuli in vitro and in vivo [28,38]. In the current study, we demonstrated that As_2O_3 is a potent down-regulator of survivin expression at both the protein and mRNA levels and a potent inducer of apoptosis in WSU-CLL cells. Thus, As_2O_3 may serve as a novel chemotherapeutic method for the treatment of CLL.

Overall, previous studies have reported two major findings. First and foremost is that a decrease in the survivin level plays an important role in CLL. Second, these studies clearly suggest that survivin is an important molecular factor in CLL cell survival as well as in resistance to apoptosis and that a decrease in survivin expression leads to the apoptosis of CLL cells. Several studies have concluded that there is a strong association between increased survivin expression and the progression of human CLL [14,15].

In our study, we found that the down-regulation of survivin expression by siRNA sensitized cells to As_2O_3-induced apoptosis. This finding further supports the hypothesis that survivin modulates the sensitivity of WSU-CLL cells to apoptosis. Our result indicated that the effect of this treatment was specific.

In mammalian cells, p53, the “guardian of the genome,” is involved in the maintenance of genomic stability. As a downstream factor that is highly expressed in cancer and regulated by p53 [39], survivin is a dual mediator of resistance to apoptosis and cell cycle progression [40]. Thus, regulation of the p53-survivin signaling pathway is important for cell survival [29,41]. Because p53 binds to the survivin promoter and suppresses its transcription, we examined the role of p53 in the down-regulation of survivin by As_2O_3. We found that p53 is activated in WSU-CLL cells by As_2O_3. p53 inhibition by transfection with p53 siRNA prevented the down-regulation of survivin by As_2O_3. Further studies are needed to determine whether additional survivin-independent pathways participate in the induction of apoptosis by As_2O_3.

In conclusion, this study demonstrated that As_2O_3 induced apoptosis in the WSU-CLL cell line, which was associated with the up-regulation of p53 and down-regulation of survivin. Our data indicate that As_2O_3 plays an important role in apoptosis induction in WSU-CLL cells. These results demonstrate that As_2O_3 may be a novel therapeutic strategy for treating

CLL. Future studies are needed to verify these results in primary patient samples and identify patients who are suitable for this therapeutic strategy.

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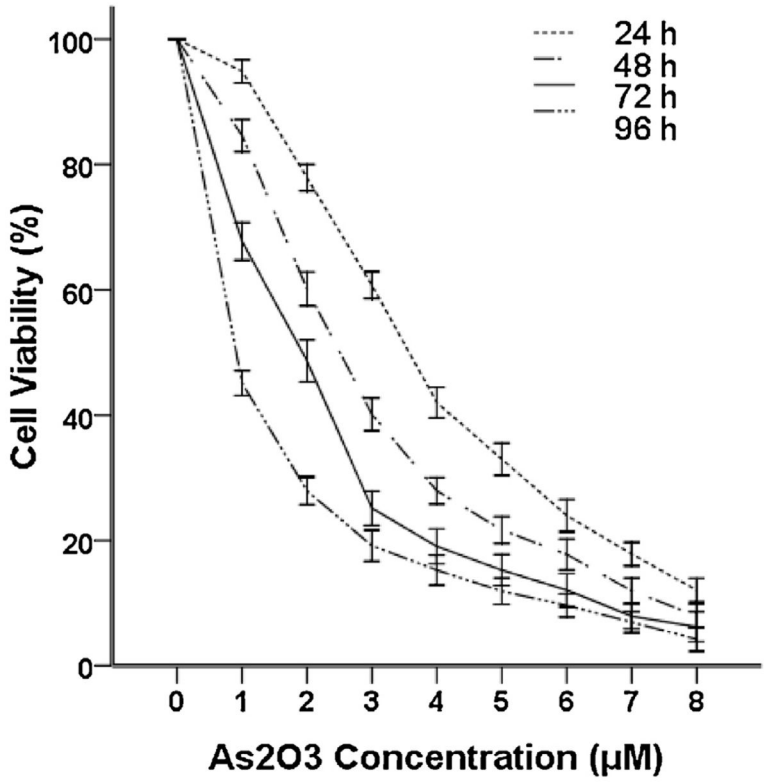

Fig. 1.

Cytotoxicity of As_2O_3 to WSU-CLL cells. WSU-CLL cells were cultured without or with different concentrations of As_2O_3 for 24, 48, 72 and 96 h. Cell viability was determined based on trypan blue exclusion. Control cell viability was set at 100%, and the survival relative to the control is presented. Each point represents the mean ± SD of three experiments with nine replicates per dose.

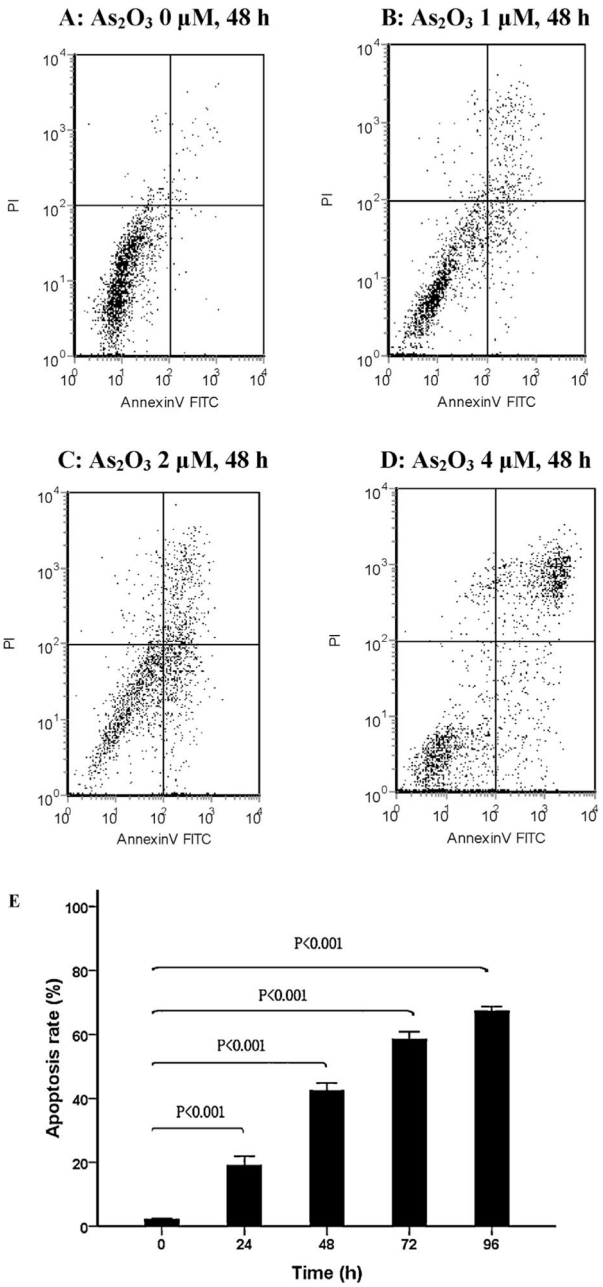

Fig. 2.

Apoptosis of WSU-CLL cells after As_2O_3 treatment. (A–D) Apoptosis of WSU-CLL cells after treatment with different concentrations of As_2O_3 for 48 h. (A) 0 μM, (B) 1 μM, (C) 2 μM, (D) 4 μM and (E) WSU-CLL cells were treated with 2 μM As_2O_3 for 0, 24, 48, 72 and 96 h. Cell apoptosis was evaluated by flow cytometry after Annexin V and PI staining. The data are presented as the mean ± SD of three independent experiments.

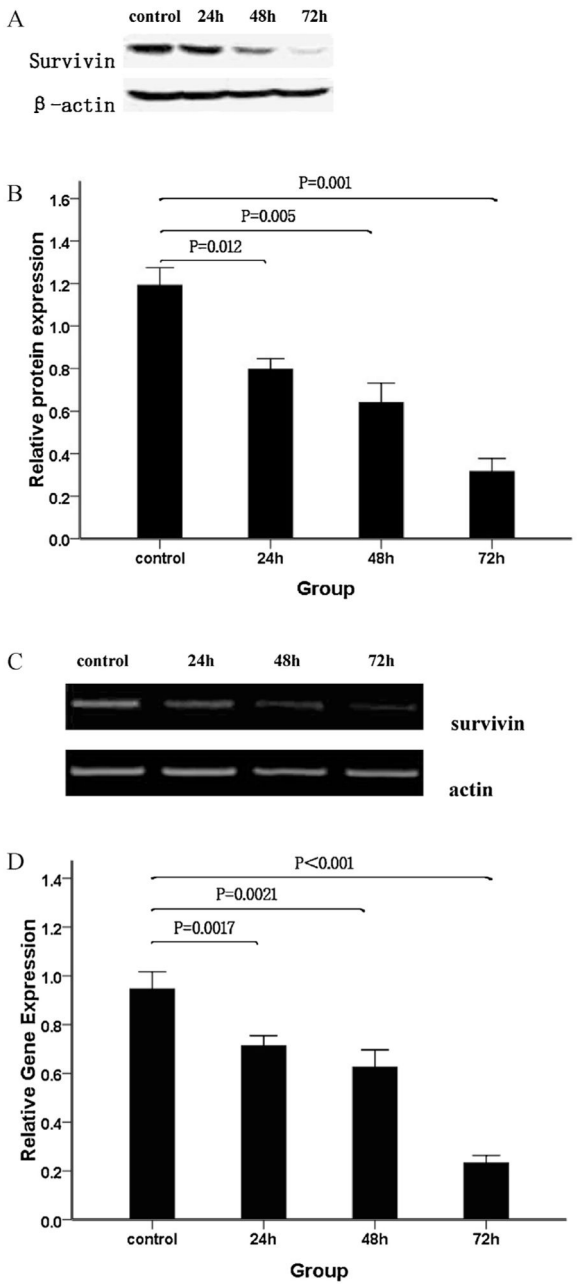

Fig. 3.

Analyses of survivin protein and mRNA levels. WSU-CLL cells were exposed to 2.0 μM As_2O_3 for 0, 24, 48 and 72 h. Survivin was analyzed by Western blotting. (A) Representative immunoblots of the expression level of survivin in cells treated with 2.0 μM As_2O_3 for 0, 24, 48 and 72 h. (B) β-actin expression was used as a loading control, and the relative survivin protein levels (means ± SD, $n = 3$) were determined. Survivin mRNA levels were measured by RT-PCR. (C) RT-PCR images of survivin in WSU-CLL cells treated with As_2O_3 for different lengths of time. (D) Quantification of the survivin mRNA level in WSU-CLL cells

treated with As_2O_3 for different lengths of time in three separate experiments. As_2O_3 reduced survivin transcriptional activity.

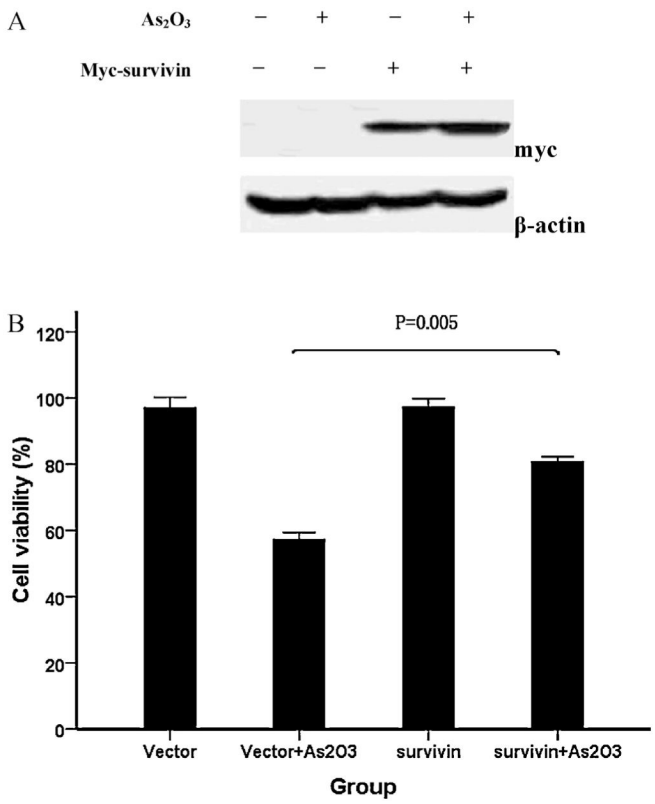

Fig. 4.

Involvement of survivin in As_2O_3-induced cytotoxicity. WSU-CLL cells were transfected with myc-survivin or pcDNA (vector) for 20 h and then incubated with or without 2 μM As_2O_3 for 48 h. (A) Survivin protein expression was analyzed by Western blotting. Survivin protein was overexpressed in WSU-CLL cells transfected with survivin. (B) Cell viability was determined by trypan blue exclusion. Survivin overexpression reduced As_2O_3-induced cytotoxicity. The viability of control cells was set at 100%, and the viability relative to the control is shown.

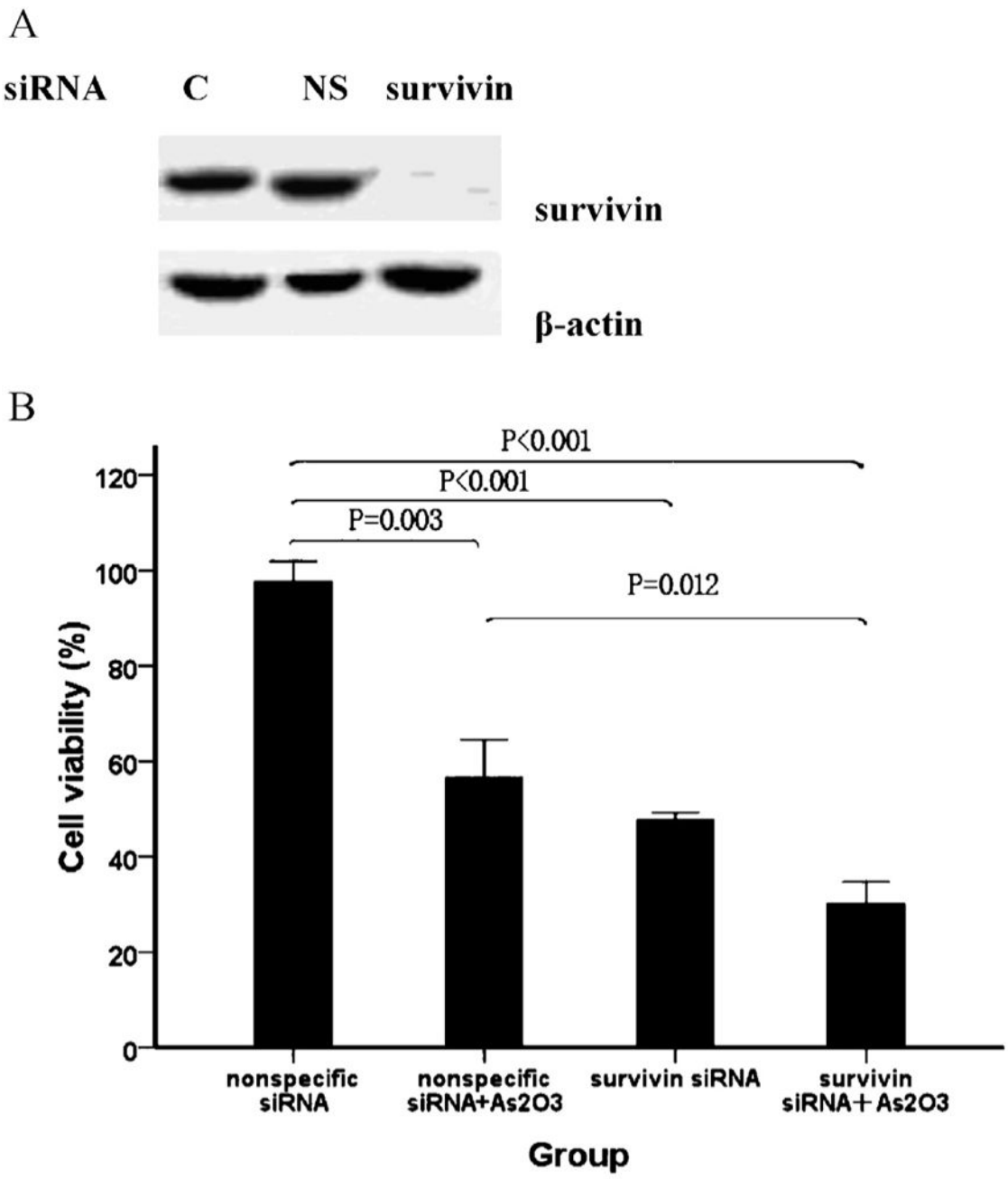

Fig. 5.

Survivin inhibition by siRNA sensitizes WSU-CLL cells to As_2O_3-induced cell death. WSU-CLL cells were transfected with survivin or nonspecific siRNA for 20 h and then treated with or without 2 μM As_2O_3 for 48 h. (A) Cells that were pre-treated with survivin siRNA (survivin) showed a significant decrease in survivin expression compared with the control group (C) and the nonspecific siRNA group (NS). (B) Cell viability was determined by trypan blue exclusion. Control cell viability was set at 100%, and the viability relative to the control is shown.

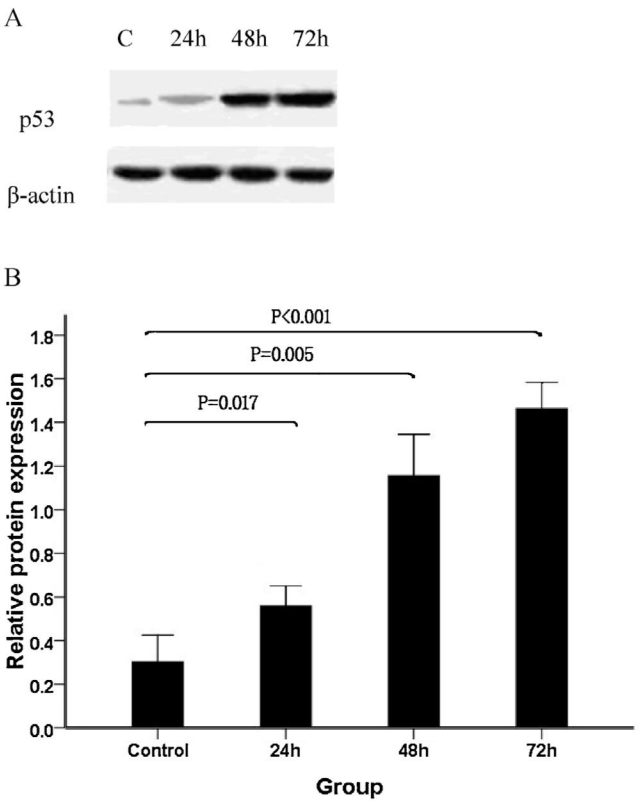

Fig. 6.

Effect of As_2O_3 on p53 protein expression in WSU-CLL cells. WSU-CLL cells were exposed to 2.0 μM As_2O_3 for 0, 24, 48, and 72 h. Western blot analyses were performed. (A) Representative immunoblots of the p53 expression level following treatment with 2 μM As_2O_3 for 0, 24, 48 and 72 h. (B) β-actin expression was used as a loading control. Relative p53 protein levels (means ± SD, $n = 3$) were determined.

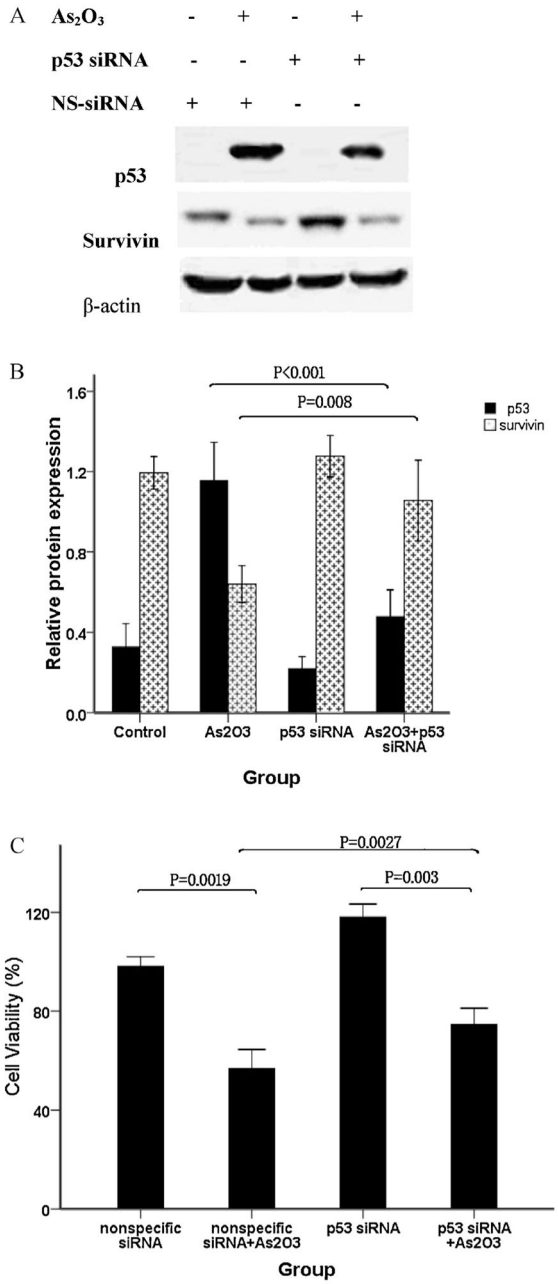

Fig. 7.

Knockdown of p53 with siRNA targeting p53 can prevent cell death due to As_2O_3. WSU-CLL cells were transfected with p53 siRNA or nonspecific siRNA for 20 h and then incubated with or without 2 μM As_2O_3 for 48 h. Western blot and cell viability analyses were performed. (A) Representative immunoblots of p53 and survivin. The p53 siRNAs were highly effective at decreasing the level of p53 protein and simultaneously increased survivin protein expression. (B) The relative protein expression of p53 and survivin (means ± SD, $n = 3$) was determined. p53 siRNA decreased p53 protein expression, which was up-regulated by As_2O_3. The decrease in the survivin protein level caused by As_2O_3 was

partially rescued by p53 siRNA. (C) Cell viability was determined by trypan blue exclusion. Control cell viability was set at 100%, and the viability relative to the control is shown. The knockdown of p53 using siRNA resulted in the partial rescue of cell viability.