Investigation of the mechanism involved in the As_2O_3-regulated decrease in MDR1 expression in leukemia cells

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Abstract. Arsenic trioxide (As_2O_3) inhibits the expression of P-glycoprotein (P-gp) in leukemia cells; however, the mechanism behind this inhibition is unclear. The present study aimed to explore the effect of As_2O_3 on the expression and regulation of P-gp in leukemia cells, and elucidate the mechanism of the reversal of drug resistance. In the present study, electrophoretic mobility shift assay results indicated that p65 binds to the NF-κB binding site of MDR1, specifically in K562/D cells. Expression of p65 and phosphorylated IκB was reduced, while the expression of IκB was increased in K562/D cells treated with As_2O_3. The activity of luciferase increased up to 9-fold with 40 ng/ml TNF-α, and it was suppressed by ~25% following treatment with 1 μM As_2O_3. These findings suggest that As_2O_3 reverses the P-gp-induced drug resistance of leukemia cells through the NF-κB pathway. As_2O_3 may inhibit the activity of phosphorylase to inhibit IκB phosphorylation, thereby inhibiting NF-κB activity and MDR1 gene expression, leading to reversal of drug resistance.

Introduction

Our previous study and other authors demonstrated that As_2O_3 inhibits the expression of P-glycoprotein (P-gp) in leukemia cells (unpublished data) (1); however, the mechanism behind this inhibition is unclear. Induction of apoptosis is considered to be the mechanism by which As_2O_3 acts as a chemotherapeutic agent. Based on this finding, Mathas *et al* (2) found that As_2O_3 inhibits the activity of nuclear factor-κB (NF-κB) and induces apoptosis in Hodgkin/Reed-Sternberg cell lines. NF-κB is a widely distributed and functional eukaryotic cell transcription factor that can be activated by many cell factors and chemotherapeutic agents (2). In addition, a large body of evidence has demonstrated that NF-κB is related to tumor drug resistance (3-5).

Previous research suggests that, in addition to inducing the expression of antiapoptotic genes, NF-κB also induces the expression of P-gp. Bentires-Alj *et al* (7) and Kuo *et al* (8) demonstrated that the promoter of multi-drug resistance gene 1 (MDR1, expressing P-gp) contained an NF-κB binding sequence that activates transcription of an MDR1 promoter-driven reporter gene. These findings support the notion that As_2O_3 has an effect on the reversal of drug resistance by inhibition of NF-κB, consequently inducing apoptosis and expression of MDR1.

Currently, As_2O_3 is mainly used to treat promyelocytic leukemia patients and cases for which chemotherapeutic agents are ineffective; however, As_2O_3 is not currently used as a drug-resistance modulator. According to analyses based on the clinical effects and proposed mechanism of As_2O_3, we hypothesized that it not only possesses apoptosis-inducing capabilities, but also the ability to reverse drug resistance. Exploitation of these capabilities could lead to increasing effectiveness of chemotherapeutics for treatment-resistant patients.

Therefore, we aimed to study the impact of As_2O_3 on the expression and regulation of P-gp in leukemia cells in order to explore the mechanism of the reversal of drug resistance. The findings of the present study provide an important theoretical foundation for the improvement of chemotherapy. Moreover, study of the function of the reversal of drug resistance by As_2O_3 may improve its therapeutic value and expedite further studies concerning the mechanism of tumor drug resistance.

Materials and methods

Cell culture. K562/D, K562/S and HEK293T cells were maintained in RPMI-1640 with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere with 5% CO_2 at 37˚C. The study was performed in accordance with the ethical standards of the 1975 Declaration of Helsinki, as revised in 2000, and was approved by the appropriate institutional review boards.

In the present study, we used the K562/D and 293T cell lines in the process of analysis of the inhibition of the MDR1

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gene promoter. K562/D cells have a high level of P-gp expression, and the function of its external input pump leads to low efficiency of cell transfection; therefore, we choose human embryonic kidney 293T cells instead of K562/D cells for the transfection experiments.

Identification of the NF-κB response element of the MDR1 promoter by EMSA. Nuclear extracts were prepared from K562/D cells. The wild-type probe of the MDR1 promoter was generated by annealing two complementary oligonucleotides (5'-GCACTGCAGGGGCTTTCCTGTGCGC-3' and 5'-GCGCACAGGAAAGCCCCTGCAGTGC-3'). The core sequence of the NF-κB binding site is underlined and the 3' recessive ends were filled by repair synthesis with dATP, dTTP, dGTP [α-^{32}P]-dCTP and the Klenow fragment of DNA polymerase I. The mutated probe of the MDR1 promoter was generated using the same procedure, except that several oligonucleotides were mutated (5'-GCACTGCACTCGCTTTCC TGTGCGC-3' and 5'-GCGCACAGGAAAGCGAGTGCAG TGC-3'). Nuclear extracts of the protein were preincubated in 20 μl of binding buffer and 4% glycerol with or without unlabeled excess competitor. For supershift assays, 1 μg of rabbit IgG (Sigma) was added to the preincubation mixture. After preincubation on ice for 30 min, the DNA probe labeled with (α-^{32}P) dCTP was added, and the samples were incubated at room temperature for 30 min. The reaction mixtures were resolved on 4% polyacrylamide gels. Antibodies used in the supershift assays were rabbit polyclonal antibodies against NF-κB p65 from Santa Cruz Biotechnology.

Western blot analysis. Cells were lysed in RIPA buffer containing a complete protease inhibitor cocktail, followed by centrifugation at 12,000 x g at 4˚C. The protein concentration was determined from the supernatant by a bicinchoninic acid assay (Beyotime Institute of Biotechnology, Haimen, China), and the results were assessed using an ELISA plate reader. Protein extracts were diluted using 5X SDS loading buffer, boiled and resolved on SDS-PAGE. After electrophoresis, proteins were transferred to a PVDF membrane by semidry blotting. Detection was performed using the anti-IκB antibody (1:1,000); and the anti-actin antibody (1:100) was used as a loading control. The anti-rabbit and anti-mouse IgG coupled with horseradish peroxidase were used at a dilution of 1:3,000. Blots were developed with the Western Lightning Chemiluminescence Reagent Plus (Pierce, Thermo Fisher Scientific) and chemiluminescence detection film.

Generation of the MDR1 promoter reporter, and the IκB and IκB mutant vector constructs. The MDR1 genomic sequence, 986 nucleotides of the MDR1 TSS, was PCR-amplified using the MDR1 forward primer 5'-GCACTGCAGGGGCTTTCC TGTG-3' and reverse primer 5'-CTGCAGAAAAATTTCTC CTAGCC-3'. The transcriptional site was defined as +1, and human genomic DNA was used as the template. Next, 1094 bp nucleotides of IκB were amplified using primers (5'-GTCCG CGCCATGTTCCAG-3' and 5'-TGGGCTAGGCAGTGTGC AGT-3') and the cDNA from K562/D cells as the template. The PCR product was cloned into the pMD18-T vector. The MDR1 reporter vector was constructed into the PGL3-Basic vector with *Hin*dIII and *Sac*I restriction sites. Using the IκB sequence as the template, the IκB expression vector was constructed in the pcDNA3.1 vector containing *Hin*dIII and *Eco*RI restriction sites. We constructed the IκB mutant vector according to the operating instructions of the Takara MutanBEST kit using the primers CCTCGTCTTTCATGTAGTCCAGGCCGATGTCGTGGCGGTC and GACCGCCACGACATCGGCCT GGACTACATGAAAGACGAGG (underlined are mutated sites that changed the ser 32/36 to ile 32/tyr 36). All of the constructed vectors were confirmed by sequencing.

Luciferase assay. K562D cells treated with 1 μM As_2O_3 and 293T cells treated with or without 40 ng/ml TNF-α stimulation were seeded at $2x10^5$ cells/well in a 24-well plate. After 24 h, the cells were transfected with the plasmid including IκB, the IκB mutant and p65 siRNA using Lipofectamine 2000 (Invitrogen) according to the protocol recommended by the manufacturer. Two days after transfection, the cells were lysed, and the luciferase activities were assayed using the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA) and measured with a Lumat LB 9507 luminometer (Bethold Technologies, Bad Wildbad, Germany). The luciferase levels were calculated by the ratio of firefly luciferase to *Renilla* luciferase; the results were averaged from at least three separate transfection assays in all of the experiments.

Results

Identification of the NF-κB protein binding site on the MDR1 promoter. We demonstrated that response elements exist in the MDR1 promoter by EMSA (Fig. 1). The wild-type NF-κB-labeled probe formed a complex with the nuclear extract, and the addition of a cold self-competitor resulted in weakened binding. However, a mutant competitor could not abolish the binding. Supershift assays revealed that the binding was blocked by the p65 antibody. These results indicated that p65 could bind with the NF-κB binding site of MDR1 specifically in K562/D cells.

Influence of As_2O_3 on p65, IκB and phosphorylated IκB in K562/D cells. The results of the western blot analysis demonstrated that As_2O_3 reduced the expression of p65 and phosphorylated IκB but increased the expression of IκB. We then extracted the nuclear protein and total protein from untreated K562/D cells and those treated with As_2O_3 to identify whether the MDR1 gene was inhibited by As_2O_3 via the NF-κB pathway.

Western blot analysis was performed for p65 using nuclear proteins and IκB and phosphorylated IκB using total protein. The results demonstrated that the expression of p65 and phosphorylated IκB was reduced, while the expression of IκB was increased in K562/D cells treated with As_2O_3 (Fig. 2). In essence, phosphorylated IκB was reduced after treatment with As_2O_3, resulting in an increased IκB protein level and decreased NF-κB translocation to the cell nucleus.

Influence of TNF-α and As_2O_3 treatment on MDR1 promoter activity. Using the luciferase assay system, we found that negative regulation of the MDR1 promoter occurred after stimulation with TNF-α, while As_2O_3 inhibited this activity. Luciferase reporter vectors harboring the MDR1 promoter

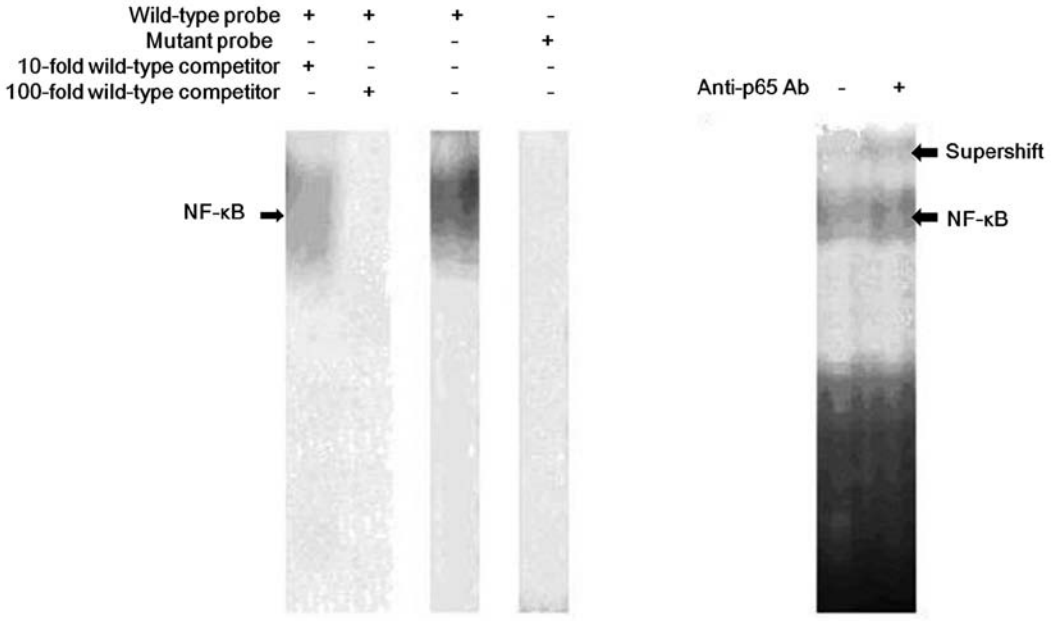

Figure 1. Identification of NF-κB in the MDR1 promoter by EMSA. The wild-type NF-κB-labeled probe formed a complex with certain proteins in the nuclear extract, and the addition of the 10- and 100-fold cold self-competitor resulted in weakened binding; however, a mutant competitor was not able to abolish the binding. Supershift assays demonstrated that the binding was blocked by the p65 antibody.

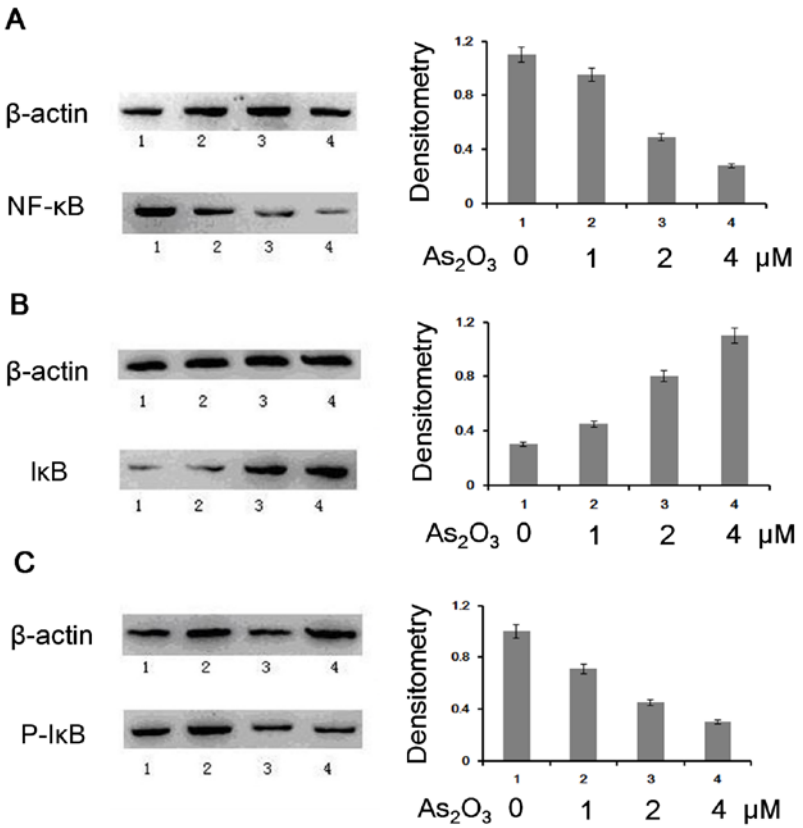

Figure 2. Expression of NF-κB, p65, IκB and phosphorylated IκB in As_2O_3-treated-K562/D cells. Left, western blot analysis of the K562D cells treated with different concentrations of As_2O_3 (1, control; 2, 1 μM As_2O_3; 3, 2 μM As_2O_3; 4, 4 μM As_2O_3). Right, analysis of the density by the ratio of NF-κB p65, IκB, phosphorylated IκB and β-actin. (A) Reduced NF-κB p65 expression, (B) increased IκB expression, (C) reduced phosphorylated IκB expression following treatment with As_2O_3.

(Fig. 3A and B) were transfected into 293T cells after treatment with different concentrations of TNF-α for 24 h. We found that without TNF-α stimulation, low luciferase activity was observed, and the activity of luciferase increased as the

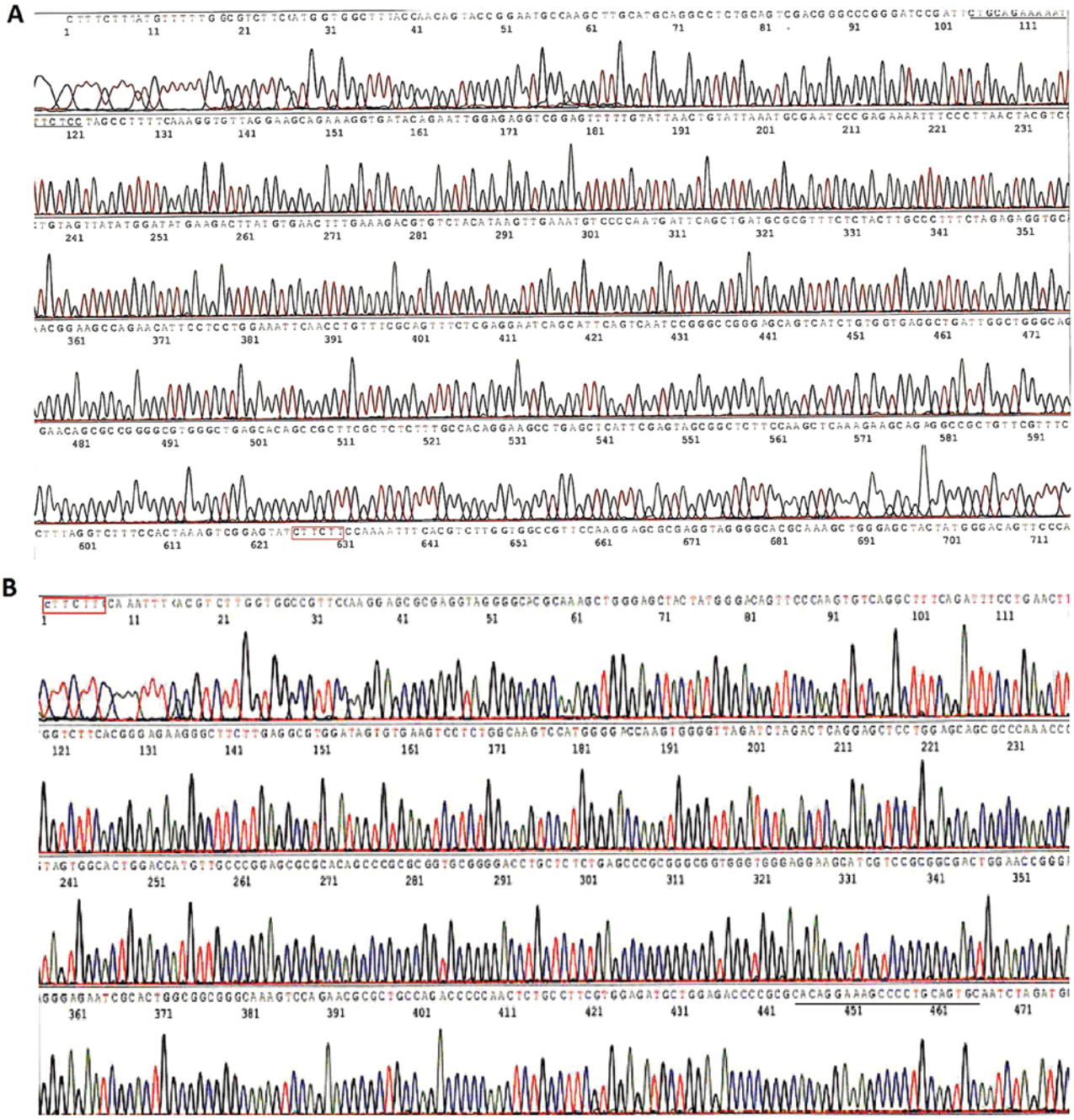

Figure 3. Sequencing of the MDR1 promoter vector. (A) Sequencing of the MDR1 promoter in the PGL3-Basic vector. The downstream primer is underlined, and the red frame (bottom row) indicates the overlay region of the sequencing. (B) Sequencing of the MDR1 promoter in the PGL3-Basic vector. The upstream primer is underlined, and the red frame (top row) indicates the overlay region of the sequencing.

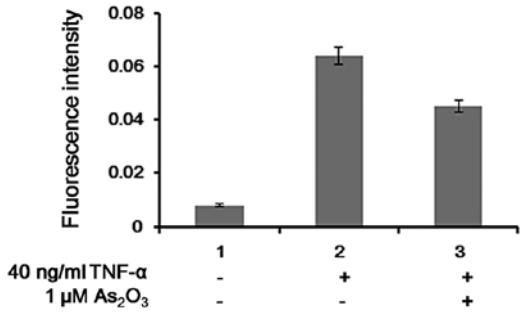

Figure 4. Analysis of fluorescence intensity. The activity of luciferase was increased up to 9-fold with 40 ng/ml TNF-α stimulation, and was suppressed by ~25% following treatment with 1 μM of As_2O_3.

concentration of TNF-α increased. The activity of luciferase was increased up to 9-fold with 40 ng/ml TNF-α, and it was suppressed by ~25% following treatment with 1 μM As_2O_3 (Fig. 4).

Influence of the NF-κB response element in the suppression of MDR1 promoter activity following As_2O_3 treatment. The activity of the MDR1 promoter induced by TNF-α was inhibited by As_2O_3. This inhibition was significantly reduced after the cells were transfected with p65 siRNA (Figs. 5 and 6). The results indicated that As_2O_3 partially inhibited the TNF-α induced-activity of the MDR1 promoter via NF-κB.

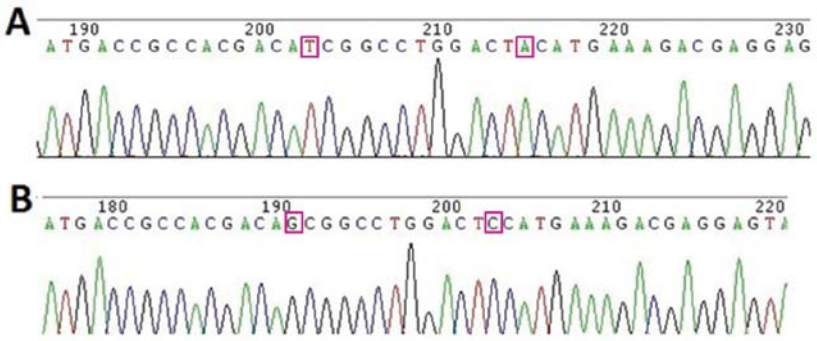

Figure 5. Sequencing of the IκB vector. (A) Wild-type IκB sequencing (pink frames indicate Ser32/36). (B) Mutant IκB sequencing (pink frames indicate the changes of Ser 32/36).

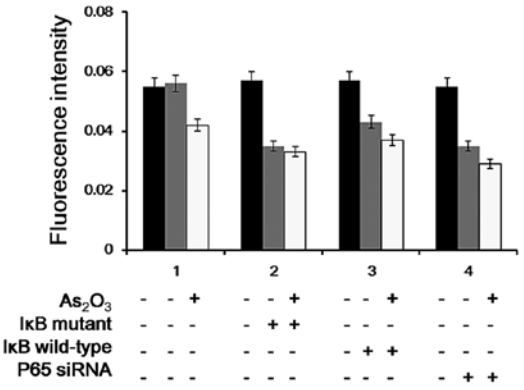

Figure 6. Influence of MDR1 promoter activities by factors in the NF-κB pathway. 1, control; 2, IκB mutant transfection group; 3, IκB transfection group; 4, p65 siRNA transfection group. Activities of the luciferase reporter gene were inhibited in the 293T cells following TNF-α stimulation, and the activities of luciferase were reduced in the IκB, IκB mutant and p65 siRNA transfection groups. Following treatment with As_2O_3, activities in the IκB mutant transfection group were decreased more than that in the IκB transfection group

Discussion

Human P-gp is present not only in tumor cells, but also in normal tissues including the kidney, liver, adrenal glands and the pregnant uterus (9). The human MDR1 gene is located at 7p21-21.1; containing 28 exons (10). The upstream region of the MDR1 gene promoter lacks the TATA box commonly found in many protein-coding genes; however, it does contain a reverse CCAAT and GC-rich region that can bind with NF-Y and Sp family transcription factors, which recruit histone acetyltransferase to initiate histone acetylation, chromatin remodeling, thus, activating the MDR1 promoter. Moreover, the promoter contains binding sites for lymphoid enhancer factor/T cell transcription factor, heat shock factor, early growth response factor 1 and myocyte enhancer factor 1 (11). Research has demonstrated that cell stress such as DNA damage and various activation signals (including TNF-α and NF-κB) can activate the MDR1 gene and induce its expression (12-15). Bentires-Alj *et al* (7) found that the MDR1 promoter contained a binding sequence (GGGGCTTTCC) for NF-κB. In the present study, we verified this specific binding using EMSA, and demonstrated that TNF-α activates the transcription of the MDR1 promoter using a luciferase reporter system.

In the present study, we used the K562/D and 293T cell lines as our models. K562/D cells possess a high level of P-gp expression; therefore, the function of the external input pump of the cells leads to low efficiency of cell transfection. Thus, in the present study, we used 293T cells when undertaking the transfection experiments. After transfection of the reporter gene, the weak signal was enhanced by NF-κB expression induced by TNF-α, and this enhancement was significantly reduced again after treatment with As_2O_3. These results demonstrated that As_2O_3 inhibited activation of the MDR1 promoter induced by TNF-α.

In our previous research and in studies by other authors, we found that As_2O_3 directly affected the expression level of P-gp in K562/D cells (unpublished data) (1); As_2O_3 was observed to significantly inhibit the expression and function of P-gp by flow cytometry. These studies demonstrated that As_2O_3 could regulate the expression of MDR1. Further research on the regulatory mechanisms of MDR1 will facilitate the study of the reversal of drug resistance.

Expression of MDR1 is affected by many tumor-associated proteins, such as mutant ras isoforms, and c-raf can directly bind with the Sp-1 motif to increase expression of MDR1. SXR/PXR, steroid receptors, and a number of carcinogens can also directly bind to the MDR1 promoter and activate its expression. As previously demonstrated, mutant p53 specifically stimulated the MDR1 promoter and wild-type p53 exerted specific repression (16-18). These results imply that the MDR1 gene activated during tumor progression is associated with many factors. As this area of study has developed, researchers have recognized P-gp as a target for cancer therapy.

As_2O_3 regulates the activity of the MDR1 promoter via the NF-κB pathway. In order to further analyze the inhibitory effects of As_2O_3 on the activation of the MDR1 promoter, we examined IκB, phosphorylated IκB and the expression of NF-κB in K562/D cells treated with or without As_2O_3.

The NF-κB family consists of five correlative transcription factors (19,20): i) NF-κB1, including p50 and p105; ii) NF-κB2, including p52 and p100; iii) NF-κB3, also known as Rel-A or p65, generally considered to play an important role in the transcriptional activation of NF-κB (20); iv) Rel-B; and v) Rel (also known as c-Rel). In the cytoplasm, NF-κB is in an inactive form and binds to the inhibitor of IκBα. Under the stimulus of certain factors, it is often activated by a classical

pathway, in which two conserved serine residues (Ser32/36) of IκBα are phosphorylated by the IκB kinase (IKK) complex. Phosphorylation of IκB leads to rapid ubiquitination, followed by a conformational change, which is recognized and degraded by a catalytic ATP-dependent 26S protease. Thus, the suppression of NF-κB is reversed, and it is translocated into the nucleus where it exerts its function of transcriptional activation (22).

Our results demonstrated that phosphorylation of IκB and nuclear localization of NF-κB were decreased, while expression of IκB was increased in the As_2O_3-treated K562/D cells when compared with the control group. The activity increased with the increasing concentration of As_2O_3. We hypothesized that inhibition of phosphorylase activity caused by As_2O_3 resulted in the reduction of phosphorylation of IκB and degradation of IκB proteins. Thus, IκB proteins bound to NF-κB outside of the nucleus, and further reduced NF-κB translocation into the nucleus.

In the present study, we detected an influence on the promoter activity, resulting from the alteration in the binding of MDR1 and NF-κB. The results demonstrated that the MDR1 promoter-driven reporter gene, which contained NF-κB sites, had a high level of transcriptional activity following stimulation with TNF-α, and As_2O_3 inhibited the activity of transcription promoted by TNF-α. This inhibition was also enhanced by transfection with siRNA targeting p65 or an expression vector containing IκB. Hence, the NF-κB response element played an important role in the transcriptional activation of the MDR1 promoter. Moreover, the inhibition of activity was significantly reduced in the group transfected with the IκB mutant vector compared with the wild-type vector. These results further demonstrated that As_2O_3 inhibited the phosphorylation of IκB in the NF-κB pathway to regulate MDR1.

NF-κB has antiapoptotic functions. It can be activated by cytotoxic drugs, and once activated it can induce the expression of antiapoptotic genes (23-25), leading to the resistance to chemotherapy (15,26-28).

P-gp has been demonstrated to possess antiapoptotic functions in stem cells (29-31). As_2O_3 inhibits P-gp expression via the NF-κB pathway, and plays a dual role in the reversal of tumor resistance. Studies continue to demonstrate the special effects that As_2O_3 has on relapsed/refractory tumors. In the present study using As_2O_3-treated wild-type K562/S cells and drug-resistant K562/D cells, we found that the cell lethal concentration of the former was higher than that of the latter, which corroborated the collateral sensitivity theory of multidrug-resistant cells (32-34). The finding that resistant cells may be ultra-sensitive to unconventional drugs (such as As_2O_3) adds significant value to the study of cancer treatment.

In summary, the present study demonstrated that the +561 - +571 region of the MDR1 promoter is the response element of NF-κB. As_2O_3 regulated the binding of NF-κB through the phosphorylation of IκB, and consequently led to NF-κB-mediated transcriptional repression of the MDR1 promoter, which is one of the mechanisms of the reversal of the drug resistance by As_2O_3. Furthermore, the effect of As_2O_3 on the apoptosis of resistant cells may involve more complex mechanisms, which remain to be elucidated. Finally, As_2O_3 has been clinically used as an anticancer drug, and when its ability to reverse resistance is further developed and utilized, As_2O_3 may become an option for the first-line treatment of cancer.

In conclusion, we identified that As_2O_3 reversed the P-gp-induced drug-resistance of leukemia cells through the NF-κB pathway. A specific binding sequence for NF-κB was identified in the upstream region of the MDR1 gene, which NF-κB can use to affect MDR1 gene expression. Additionally, As_2O_3 was observed to inhibit NF-κB binding to this sequence.

In leukemia cells, As_2O_3 may inhibit the activity of phosphorylase to inhibit IκB phosphorylation, thereby inhibiting NF-κB activity and MDR1 gene expression, leading to the reversal of drug resistance.

References

1. Wang T, Ma LM, Zhang HP, Wang HW, Yang LH and Qiao ZH: The effect of arsenic trioxide (As_2O_3) combined with BSO on K562/ADM cells and its mechanisms. Zhonghua Xue Ye Xue Za Zhi 28: 438-443, 2007 (In Chinese).
2. Mathas S, Lietz A, Janz M, *et al*: Inhibition of NF-κB essentially contributes to arsenic-induced apoptosis. Blood 102: 1028-1034, 2003.
3. Thomas H and Coley HM: Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. Cancer Control 10: 159-165, 2003.
4. Lee HR, Cheong HJ, Kim SJ, Lee NS, Park HS and Won JH: Sulindac enhances arsenic trioxide-mediated apoptosis by inhibition of NF-κB in HCT116 colon cancer cells. Oncol Rep 20: 41-47, 2008.
5. Mathieu J and Besancon F: Arsenic trioxide represses NF-κB activation and increases apoptosis in ATRA-treated APL cells. Ann NY Acad Sci 1090: 203-208, 2006.
6. Park MJ, Lee JY, Kwak HJ, *et al*: Arsenic trioxide (As_2O_3) inhibits invasion of HT1080 human fibrosarcoma cells: role of nuclear factor-κB and reactive oxygen species. J Cell Biochem 95: 955-969, 2005.
7. Bentires-Alj M, Barbu V, Fillet M, *et al*: NF-κB transcription factor induces drug resistance through MDR1 expression in cancer cells. Oncogene 22: 90-97, 2003.
8. Kuo MT, Liu Z, Wei Y, *et al*: Induction of human *MDR1* gene expression by 2-acetylaminofluorene is mediated by effectors of the phosphoinositide 3-kinase pathway that activate NF-κB signaling. Oncogene 21: 1945-1954, 2002.
9. Tanigawara Y: Role of P-glycoprotein in drug disposition. Ther Drug Monit 22: 137-140, 2000.
10. Callen DF, Baker E, Simmers RN, Seshadri R and Roninson IB: Localization of the human multiple drug resistance gene, MDR1, to 7q21.1. Hum Genet 77: 142-144, 1987.
11. Scotto KW and Johnson RA: Transcription of the multidrug resistance gene MDR1: a therapeutic target. Mol Interv 1: 117-125, 2001.
12. Hirsch-Ernst KI, Ziemann C, Schmitz-Salue C, Foth H and Kahl GF: Modulation of P-glycoprotein and *mdr1b* mRNA expression by growth factors in primary rat hepatocyte culture. Biochem Biophys Res Commun 215: 179-185, 1995.
13. Hirsch-Ernst KI, Ziemann C, Foth H, Kozian D, Schmitz-Salue C and Kahl GF: Induction of mdr1b mRNA and P-glycoprotein expression by tumor necrosis factor alpha in primary rat hepatocyte cultures. J Cell Physiol 176: 506-515, 1998.
14. Fardel O, Lecureur V, Daval S, Corlu A and Guillouzo A: Up-regulation of P-glycoprotein expression in rat liver cells by acute doxorubicin treatment. Eur J Biochem 246: 186-192, 1997.
15. Cheng Q, Lee HH, Li Y, Parks TP and Cheng G: Upregulation of Bcl-x and Bfl-1 as a potential mechanism of chemoresistance, which can be overcome by NF-κB inhibition. Oncogene 19: 4936-4940, 2000.
16. Chin KV, Ueda K, Pastan I and Gottesman MM: Modulation of activity of the promoter of the human MDR1 gene by Ras and p53. Science 255: 459-462, 1992.
17. Synold TW, Dussault I and Forman BM: The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. Nat Med 7: 584-590, 2001.
18. Bush JA and Li G: Regulation of the *Mdr1* isoforms in a *p53*-deficient mouse model. Carcinogenesis 23: 1603-1607, 2002.

19. Sen R and Baltimore D: Inducibility of κ immunoglobulin enhancer-binding protein NF-κB by a posttranslational mechanism. Cell 47: 921-928, 1986.
20. Lin L, DeMartino GN and Greene WC: Cotranslational biogenesis of NF-κB p50 by the 26S proteasome. Cell 92: 819-828, 1998.
21. Schmitz ML and Baeuerle PA: The p65 subunit is responsible for the strong transcription activating potential of NF-kappa B. EMBO J 10: 3805-3817, 1991.
22. Quivy V and Van Lint C: Regulation at multiple levels of NF-κB-mediated transactivation by protein acetylation. Biochem Pharmacol 68: 1221-1229, 2004.
23. Wu M, Lee H, Bellas RE, *et al*: Inhibition of NF-kappaB/Rel induces apoptosis of murine B cells. EMBO J 15: 4682-4690, 1996.
24. Ryan KM, Ernst MK, Rice NR and Vousden KH: Role of NF-κB in p53-mediated programmed cell death. Nature 404: 892-897, 2000.
25. Pham LV, Tamayo AT, Yoshimura LC, *et al*: A CD40 signalosome anchored in lipid rafts leads to constitutive activation of NF-κB and autonomous cell growth in B cell lymphomas. Immunity 16: 37-50, 2002.
26. Ueda M, Kokura S, Imamoto E, *et al*: Blocking of NF-κB activation enhances the tumor necrosis factor α-induced apoptosis of a human gastric cancer cell line. Cancer Lett 193: 177-182, 2003.
27. Wang CY, Mayo MW, Korneluk RG, Goeddel DV and Baldwin AS Jr: NF-κB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 281: 1680-1683, 1998.
28. Thevenod F, Friedmann JM, Katsen AD and Hauser IA: Up-regulation of multidrug resistance P-glycoprotein via nuclear factor-κB activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis. J Biol Chem 275: 1887-1896, 2000.
29. Pallis M, Turzanski J, Higashi Y and Russell N: P-glycoprotein in acute myeloid leukaemia: therapeutic implications of its association with both a multidrug-resistant and an apoptosis-resistant phenotype. Leuk Lymphoma 43: 1221-1228, 2002.
30. Ruefli AA, Tainton KM, Darcy PK, Smyth MJ and Johnstone RW: P-glycoprotein inhibits caspase-8 activation but not formation of the death inducing signal complex (disc) following Fas ligation. Cell Death Differ 9: 1266-1272, 2002.
31. Turzanski J, Grundy M, Shang S, Russell N and Pallis M: P-glycoprotein is implicated in the inhibition of ceramide-induced apoptosis in TF-1 acute myeloid leukemia cells by modulation of the glucosylceramide synthase pathway. Exp Hematol 33: 62-72, 2005.
32. Schuurhuis GJ, Pinedo HM, Broxterman HJ, van Kalken CK, Kuiper CM and Lankelma J: Differential sensitivity of multidrug-resistant and -sensitive cells to resistance-modifying agents and the relation with reversal of anthracycline resistance. Int J Cancer 46: 330-336, 1990.
33. Ludwig JA, Szakacs G, Martin SE, *et al*: Selective toxicity of NSC73306 in MDR1-positive cells as a new strategy to circumvent multidrug resistance in cancer. Cancer Res 66: 4808-4815, 2006.
34. Hall MD, Handley MD and Gottesman MM: Is resistance useless? Multidrug resistance and collateral sensitivity. Trends Pharmacol Sci 30: 546-556, 2009.