Transcription Factor c-Jun Activation Represses mdr-1 Gene Expression

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ABSTRACT

Expression of mdr-1 is complex and highly regulated. Several lines of evidence indirectly suggest that transcription factor c-Jun may negatively regulate human mdr-1 gene expression. We recently found that salvicine, a novel topoisomerase II inhibitor, is cytotoxic for multidrug resistance (MDR) tumor cells and down-regulates mdr-1 expression in MDR K562/A02 cells. Salvicine also stimulates a significant increase in the level of c-jun mRNA in HL60 cells. This study investigated the relationship between c-Jun activation and down-regulation of mdr-1 expression by salvicine in K562/A02 cells. Reverse-transcription PCR and Western blotting analyses revealed that salvicine suppressed mdr-1 expression in MDR cells and promoted c-jun expression in both MDR and parental K562 cells. Moreover, levels of c-jun expression were enhanced by salvicine before reduction of mdr-1 expression in K562/A02 cells. Furthermore, c-jun antisense oligodeoxynucleotides prevented salvicine-stimulated enhancement of c-Jun protein and reduction of mdr-1 gene expression, but did not affect the increase in c-jun mRNA levels. Salvicine promoted phosphorylation of c-Jun-N-terminal kinase and c-Jun protein in MDR K562/A02 and parental K562 cells. Electrophoretic mobility shift assay analysis showed that salvicine enhanced DNA binding activity of transcription factor activator protein 1. Additionally, c-jun antisense oligodeoxynucleotides also inhibited salvicine-induced apoptosis and cytotoxicity in MDR and parental K562 cells. A possible pathway emerges from these results: salvicine stimulates c-Jun-N-terminal kinase phosphorylation and activation, resulting in c-Jun phosphorylation and activation. Activated c-Jun promotes expression of c-Jun itself, represses mdr-1 transcription, and triggers pro-apoptotic signals, resulting in low mdr-1 expression and cell death. The present results demonstrate that transcription factor c-Jun plays a principal role in down-regulation of mdr-1 expression and induction of apoptosis in salvicine-treated human MDR K562/A02 cells, providing new insights into the complicated mechanisms regulating mdr-1 expression. The findings also suggest that c-Jun might be a potential drug target for circumventing tumor MDR.

INTRODUCTION

MDR1 is a major problem in treatment of human cancers with conventional chemotherapeutic drugs. One of the most important mechanisms by which tumor cells resist cytotoxic effects of anticancer agents is overexpression of the mdr-1 gene and its product P-gp (1). P-gp protein plays roles in inhibition of both drug accumulation and caspase activation in MDR tumor (2). This implies that reduction of mdr-1/P-gp expression may circumvent tumor MDR. Salvicine is a diterpenoid quinone compound synthesized by structural modification of a natural product isolated from the Chinese medicinal plant Salvia Prionitis Lance (Labiatae; See Ref. 3; Fig. 1). The compound has significant in vitro and in vivo activity against malignant tumor cells and xenografts, particularly in some human solid tumor models (4). Salvicine is a novel Topo II inhibitor that greatly promotes Topo II-DNA binding and inhibits pre- and post-strand Topo II-mediated DNA religation without interfering with forward cleavage steps (5, 6). One of the major features of salvicine is its activity against MDR tumor cells. It effectively kills MDR cell sublines with IC50 values of 1.55 μM for K562/A02 cells, 4.50 μM for KB/VCR cells, and 1.40 μM for MCF-7/ADM cells, close to those for their corresponding parental cell lines: 0.87 μM for K562 cells, 2.26 μM for KB cell, and 2.61 μM for MCF-7 cells. The cytotoxic activity of salvicine is much more potent than that of several classic anticancer drugs, with the average resistance factor for salvicine being 1.42, compared with 344.35, 233.19 and 71.22 for vincristine, doxorubicin, and etoposide, respectively. Salvicine induces similar levels of apoptosis in MDR K562/A02 and parental K562 cells, accompanied by an increased ratio of bax to bcl-2 mRNA (7). A clinical trial involving salvicine is currently underway in China.

Salvicine down-regulates mdr-1 and P-gp expression in MDR K562/A02 cells (7). Salvicine also stimulated significant increases in c-jun mRNA levels in HL60 cells (8). This is intriguing because the transcription factor c-Jun may play a role in regulation of mdr-1 expression (9). Moreover, there are several lines of evidence indirectly suggesting that c-Jun activation is negatively correlated with human mdr-1 gene expression (10–13). The present study explored the relationship between activation of the transcription factor c-Jun and down-regulation of mdr-1 expression by salvicine in MDR K562/A02 cells.

MATERIALS AND METHODS

Materials. Salvicine was kindly provided by Prof. Jin-Sheng Zhang (Phytochemistry Department of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences). It was dissolved at a concentration of 0.1 μM in 100% DMSO as a stock solution, stored at −20°C, and thawed and diluted with complete medium before each experiment. The final DMSO concentration did not exceed 0.1%. Primary antibodies sc-1694, sc-571, sc-1616, sc-822, and sc-6254 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and JSB-1 was purchased from Alexis Biochemicals (San Diego, CA).

Cell Culture. The human leukemia K562 parental cell line was obtained from the American Type Culture Collection (Rockville, MD). The doxorubicin-selected MDR K562/A02 (14, 15) subline was obtained from the Institute of Hematology, Chinese Academy of Medical Sciences (Tianjin, People’s Republic of China). The MDR cells displayed 310.0-, 146.8-, and 76.1-fold resistance to doxorubicin, etoposide, and vincristine compared with their corresponding parental cells, respectively (7). Cells were grown in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated FCS, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen, Grand Island, NY) at 37°C in a 5% CO2 humidified atmosphere. MDR K562/A02 cells were routinely maintained in medium containing 200 μg/L doxorubicin (14) and incubated in drug-free medium for at least one week before use. Cell viability was determined by trypan blue exclusion.

Oligodeoxynucleotides and Antisense Preparation. c-jun AODs (5′-CGT TTC CAT CTT TGC AGT-3′) and SODs (5′-ACT GCA AAG ATG GAA ACG-3′; phosphorothioate-modified; see Ref. 16) corresponding to the first 18 bases following the AUG sequence of c-jun mRNA were synthesized...
Fig. 1. Chemical structure of salvicine.

by the Shanghai Research Center of Biotechnology, Chinese Academy of Sciences.

Semiquantitative RT-PCR. After treatment of cells (5 × 10^5/ml) with salvicine, total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. RNA yield and purity were assessed by spectrophotometric analysis. Total RNA (1 µg) from each sample was subjected to reverse transcription with random hexamer, deoxynucleoside triphosphates, and Moloney murine leukemia virus reverse transcriptase in a total reaction volume of 20 µl. PCR was performed on cDNA with the use of Taq DNA polymerase, deoxynucleoside triphosphates, and the corresponding primers. The following PCR primers synthesized by the Shanghai Research Center of Biotechnology, Chinese Academy of Sciences, were used: 5'-CCA TGG AGA AGG CTG GGG-3' (sense) and 5'-CAA TGG CAT GTA TGA GGA TGA-3' (antisense) for the GAPDH gene (17); 5'-CCC ATT GTA GCA ATA GGA GGA-3' (sense) and 5'-GTT CAA ACT TCT GCT CCT GA-3' (antisense) for the mdr-1 gene (11); and 5'-AAC GAC CTT CTA TGA CGA ACC CAA CCC TTC CCT GTC TCA-3' (sense) and 5'-GGC ACC CCC TCC TGC CCA GTG GAT GAT-3' (antisense) for the c-jun gene (8). An aliquot of each reaction mixture was analyzed by electrophoresis on a 1.8% agarose gel, and amplified DNA was visualized by ethidium bromide staining. For quantitation of cDNA, densitometric analysis of a digital image of the agarose gel was performed using a GDS8000 Gel Documentation System (UVP Inc., Upland, CA).

Western Blotting Analysis. K562/A02 cells (5 × 10^5/ml) were exposed to different concentrations of salvicine at 37°C for the indicated times. Harvested cell pellets were suspended in suspension buffer [0.1 M NaCl, 0.01 M Tris-HCl (pH 7.6), 1.0 mM EDTA (pH 8.0), 1.0 µg/ml aprotinin, 100 µg/ml phenylmethylsulfonylfluoride] and then lysed in lysis buffer [100 mM Tris-HCl (pH 6.8), 200 mM DTT, 4% SDS, 0.2% bromphenol blue, 20% glycerol]. Equal amounts of cell lysate were separated on 7.5% Tris-glycine-SDS polyacrylamide gels and proteins electroblotted onto nitrocellulose membranes. Proteins were visualized using the Gelshift kit (Geneka Biotechnology, China) according to the manufacturer’s instructions. The following AP1 double-strand oligonucleotides used in this experiment were: 5'-CCG TTG ATG CAG GAA-3' for wild-type and 5'-CCG TTG ATG AAC ACC CAG CCG GAA-3' for mutant-type, respectively. The gel shift reaction was conducted as experimental procedure C1 in the protocol. DNA-protein complexes were electrophoresed on 5% polyacrylamide (38:2) gels in 0.25× Tris-boric acid electrophoresis buffer. Gels were dried on 3 µm Whatman paper and visualized by autoradiography.

MTT Assay. After pretreatment with 50 µg/ml c-jun AODs or SODs for 1 h, both MDR and parental K562 cells (5 × 10^5/ml) were exposed to 20 µM salvicine for 24 h in 96-well plates. At the end of incubation, cytotoxicity was measured by adding 20 µl 5 mg/ml MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma Chemical Co.; see Refs. 19, 20] to each well and the plates incubated at 37°C for 4 h. "Triplex" solution (10% SDS, 5% isobutanol, 12 mM HCl) was then added and the plates were incubated at 37°C for 12–20 h. Media and DMSO control wells, in which salvicine was absent, were included in all experiments. Absorbance values at A570 nm were determined using a VERSAmax tunable microplate reader (Molecular Devices). The growth inhibition rate was calculated by the equation: growth inhibition rate = [1 - (A570 treated/A570 control)] × 100%.

RESULTS

Salvicine Increases c-jun mRNA Levels before Reduction of mdr-1 mRNA Levels in MDR K562/A02 Cells. We investigated the effects of salvicine on levels of c-jun and mdr-1 mRNA in MDR

![](image125x682 to 216x749)

C-JUN REPRESSION MDR-1 EXPRESSION

![Fig. 2. Effect of salvicine on expression of c-jun and mdr-1 mRNA in MDR K562/A02 cells. RT-PCR was performed to detect c-jun and mdr-1 mRNA in MDR K562/A02 cells (5 × 10^5/ml) treated with 20 µM salvicine for 0.5–16 h (A) or 5–40 µM salvicine for 2 or 16 h (B). C–E, semiquantitation of c-jun and mdr-1 cDNA in both MDR and parental K562 cells at A and B by densitometric analysis of a digital image, respectively. Relative expression percentage of genes (Y axis) represented the intensities of c-jun or mdr-1 expression relative to those of GAPDH expression (taken as 100%). Data expressed as mean ± SD, n = 3. Con, control group.

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Salvicine Raises c-Jun, and Lowers P-gp, Protein Expression in MDR K562/A02 Cells. We sought to confirm the above-mentioned mRNA findings by analyzing protein expression using Western blotting. After treatment of both MDR and parental cells with 20 μM salvicine, c-Jun protein levels began to rise as early as 0.5 h (Fig. 3A). In contrast to the changes at the mRNA level, c-Jun protein reached a plateau after 12 h and remained high to 24 h. A decline in P-gp protein levels was observed, consistent with that of mdr-1 mRNA levels in salvicine-treated K562/A02 cells. A similar phenomenon appeared in experiments exploring concentration-response relationship (Fig. 3B). Levels of c-Jun increased concentration-dependently in both cell lines treated with salvicine for 24 h, but levels of P-gp decreased progressively at concentrations higher than 10 μM in MDR cells. As was the case for mRNA levels, enhancement of c-Jun protein levels occurred earlier than the decline in P-gp protein levels in MDR cells.

The above-mentioned mRNA and protein results indicate the existence of a possible cause-and-effect relationship between activation of c-Jun and down-regulation of mdr-1 expression in salvicine-treated K562/A02 cells.

Salvicine Promotes Phosphorylation of JNK Kinase and c-Jun Protein in MDR K562/A02 and Parental K562 Cells. Activation of c-Jun protein, performed by JNK kinase, which also must be phosphorylated to be active, requires phosphorylation on serines 63 and 73, (22, 23). We investigated whether salvicine-induced c-Jun protein was in its active, phosphorylated form (p-c-Jun) and whether this process involved phosphorylated JNK (p-JNK).

**Materials and Methods**

Materials and methods are well described in the article, including the use of appropriate reagents and protocols for protein and mRNA analysis.

**Results**

**Fig. 3. Effect of salvicine on c-Jun, p-c-Jun, JNK, p-JNK, and P-gp protein levels in MDR K562/A02 cells.** After pretreatment with 50 μg/ml c-Jun AODs or c-Jun SOCs for 1 h, both MDR and parental K562 cells (5 × 10^6/ml) were exposed to 20 μM salvicine for 2 h or 16 h (A) or 24 h (B). A, the results of RT-PCR analysis. B, the results of Western blot analysis. C, semiquantitation of c-Jun cDNA and mdr-1 mRNA levels shown in A by densitometric analysis of a digital image. Relative expression percentage of genes (Y axis) represented the intensities of c-Jun or mdr-1 expression relative to those of GAPDH expression (taken as 100%). Data are expressed as mean ± SD. Experiments were repeated 3 times. Con, control group.

**Fig. 4. Effect of c-Jun AODs on expression of c-Jun and mdr-1 genes in MDR K562/A02 cells.** After pretreatment with 50 μg/ml c-Jun AODs or c-Jun SOCs for 1 h, both MDR and parental K562 cells (5 × 10^6/ml) were exposed to 20 μM salvicine for 2 h or 16 h (A) or 24 h (B). A, the results of RT-PCR analysis. B, the results of Western blot analysis. C, semiquantitation of c-Jun cDNA and mdr-1 mRNA levels shown in A by densitometric analysis of a digital image. Relative expression percentage of genes (Y axis) represented the intensities of c-Jun or mdr-1 expression relative to those of GAPDH expression (taken as 100%). Data are expressed as mean ± SD. Experiments were repeated 3 times. Con, control group.
MDR or parental cells with salvicine led to increased levels of p-JNK and p-c-Jun, the altered amounts of which were reflected incubation time (Fig. 3A) and salvicine concentration (Fig. 3B). Basically, these changes paralleled those in the levels of c-Jun. In contrast, the total amount of JNK did not change (Fig. 3). The results indicate that salvicine stimulated phosphorylation of c-Jun at serines 63 and 73 by activating JNK kinase in a time- and concentration-dependent manner.

c-jun AODs Rescue Reduction of mdr-1 Gene Expression after Activation of c-Jun by Salvicine. AODs, with their high specificity, versatility, and cost-effect ratio, are invaluable reagents for specific regulation of gene expression. They are able to sequence-specifically promote mRNA degradation and/or block translation to protein (20, 21). To confirm whether c-Jun activation is a prerequisite for reduction of mdr-1 mRNA levels in salvicine-treated K562/A02 cells, we synthesized a sequence of c-jun AODs, which has previously been shown to block heat shock- and ceramide-induced apoptosis (14, 24). Pretreatment of cells with 50 μg/ml c-jun AODs for 1 h did not change the response pattern of c-jun mRNA to salvicine, but altered the levels of c-Jun protein, mdr-1 mRNA, and P-gp protein (Fig. 4). In both c-jun AODs-treated and -untreated groups, c-jun mRNA levels still rose at 2 h and declined approximately to control values at 16 h in salvicine-treated K562/A02 and K562 cells. Contrarily, c-jun AODs completely reversed the reduction of mdr-1 mRNA by salvicine in MDR cells (Fig. 4A). At the protein level, c-jun AODs prevented up-regulation of c-Jun and p-c-Jun and the down-regulation of P-gp by salvicine (Fig. 4B). In contrast, the internal controls, GAPDH mRNA and β-actin protein expression, did not change significantly (Fig. 4, A and B). These data further demonstrate that enhancement of c-jun expression might lead to down-regulation of mdr-1 expression in salvicine-treated MDR cells. The results also reveal that c-jun AODs mainly affect translation, not stability, of c-jun mRNA. As expected, c-jun SODs did not alter the effects of salvicine (Fig. 4).

Salvicine Enhances the DNA Binding Activity of AP1. There is an AP1 DNA binding element in the promoter region of the human mdr-1 gene, which can be bound by the AP1 transcription factor containing c-Jun (7). Because induction of c-jun expression does not always mean activation of AP1 (23), we studied the effect of salvicine on AP1 DNA binding activity using EMSA. The nuclear extract derived from phorbol 12-myristate 13-acetate (TPA)-treated human T lymphocyte leukemia Jurkat cells was used as a positive control. We observed that nuclear extracts from K562/A02 cells treated with 20 and 40 μM salvicine for 24 h contained a binding activity to the AP1 DNA binding consensus sequence, whereas those from untreated K562/A02 cells did not (Fig. 5). The results were consistent with increased levels of c-Jun and p-c-Jun and decreased levels of mdr-1 mRNA and P-gp protein. Interestingly, it appears that the components of AP1 in salvicine-treated K562/A02 cells might be different from those in phorbol 12-myristate 13-acetate-treated Jurkat cells because bandshift 1 lagged significantly behind bandshift 2 (Fig. 5).

To determine the specificity of the binding activity, EMSA binding reactions were also performed in the presence of up to 100-fold molar excess of unlabeled probe. Results showed that the labeled probe was competed away from the shifted complexes, whereas a 100-fold molar excess of a mutant oligonucleotide did not compete with the labeled probe (Fig. 5). Taken together, the data demonstrate that salvicine-stimulated AP1 complexes recognize and bind the AP1 DNA binding consensus sequence. These results provide further evidence supporting the hypothesis that activation of transcription factor c-Jun down-regulates mdr-1 gene expression in salvicine-treated MDR K562/A02 cells.

c-jun AODs Inhibit Salvicine-induced Apoptosis and Cytotoxicity in MDR K562/A02 and Parental K562 Cells. We examined the effects of c-jun AODs on salvicine-induced apoptosis in MDR K562/A02 and parental K562 cells to further confirm the role of transcription factor c-Jun in regulation of MDR. After 1 h preincubation with 50 μg/ml c-jun SODs or AODs, cells were treated with 20 μM salvicine for 24 h. DNA agarose gel electrophoresis showed that salvicine-induced apoptosis was clearly inhibited by c-jun AODs, but not by SODs (Fig. 6). Consistent with this, pretreatment with c-jun AODs also dramatically decreased the salvicine cytotoxicity against both cell lines (Fig. 6). Pretreatment with c-jun AODs caused the growth inhibitory rate elicited by salvicine to decrease from 64.64 to 9.53% in MDR K562/A02 cells and from 67.48% to 23.82% in parental cells. It is worth noting that the degree of growth inhibition of resistant cells was significantly greater than that of parental cells. These results demonstrate that alteration of c-jun expression is a critical feature of salvicine-stimulated apoptosis and cytotoxicity.

DISCUSSION

Most strategies developed to reverse the MDR phenotype involve the use of resistance modulators. These have in common the ability to...
reverse the phenotype through inhibition of MDR transporter function (1). A more efficient strategy to circumvent MDR would be to down-regulate expression of genes coding for the transporters. This requires a profound understanding of the molecular mechanisms and signal-transduction pathways involved in regulation of MDR-related genes. The regulation of mdr-1 gene expression is highly complex. Such complexity is embodied in multiple transcription-regulatory elements contained in the 5′ and 3′ flanking sequences of the mdr-1 gene, and numerous protein factors involved in transcription-regulatory processes in cell-type- and stimulus-dependent manners. The mechanisms regulating mdr-1 expression are not yet fully understood.

The data in the present study revealed that salvicine stimulated c-jun gene expression and inhibited mdr-1 gene expression in MDR K562/A02 cells. Moreover, elevation of c-Jun mRNA and protein levels occurred before reduction of mdr-1 mRNA and P-gp protein levels. Salvicine also enhanced levels of the active forms of JNK and c-Jun, and raised the DNA-binding activity of API. Using c-jun AODs, we confirmed that c-Jun activation is a prerequisite for reduction of mdr-1 mRNA and P-gp protein levels. The specific c-jun AODs disrupted enhancement of c-Jun and p-c-Jun by salvicine and simultaneously prevented the reduction of mdr-1 mRNA and P-gp protein levels. Most importantly, c-jun AODs inhibited apoptosis and cytotoxicity induced by salvicine in both MDR and parental K562 cells. Integrating these results, we suggest a possible pathway describing down-regulation of mdr-1 expression by salvicine (Fig. 7). Salvicine stimulates JNK phosphorylation, and activated JNK phosphorylates serines 63 and 73 of c-Jun, resulting in increased transcription activity. Phosphorylated c-Jun promotes expression of c-jun itself, thus increasing c-Jun levels (22). On the other hand, the transcription-factor complex containing c-Jun binds to the consensus API target element in the mdr-1 gene promoter and represses transcription, resulting in reduction of mdr-1 mRNA and P-gp expression in MDR K562/A02 cells. Although this pathway indicates how c-Jun activation represses mdr-1 expression, some molecular links remain to be clarified, such as how salvicine stimulates JNK protein phosphorylation and the connection between c-Jun activation and apoptosis.

Several previous studies suggest that c-Jun can inhibit expression of mdr-1 in human MDR cells. Firstly, MDR FM3A/M cells overexpressing P-gp have significantly lower basal and drug-stimulated JNK activity than parental FM3A/M cells, and are resistant to anticancer drugs. After JNK gene transfection, MDR FM3A/M cells recover the basal and drug-stimulated activities of JNK and the susceptibility to anticancer drugs (10). Secondly, reactive oxygen species down-regulate P-gp expression and activate JNK in multicellular prostate tumor spheroids (11). Thirdly, tumor necrosis factor α can suppress mdr-1 expression in MDR cells (25, 26) and can promote c-jun expression (27, 28). Finally, in mouse hepatoma cell lines, a canonical API binding sequence in the promoter of mdr3/mdr-1a negatively regulates gene expression (13). In this study, our data directly demonstrate for the first time that c-Jun activation down-regulates mdr-1 gene expression in a human MDR cell line.

It has been shown that JNK kinase and transcription factor c-Jun are required for apoptosis induced by various stimuli (29, 30). Using c-jun AODs, we come to the same conclusion in salvicine-treated MDR K562/A02 and parental K562 cells (Fig. 6). On the one hand, MDR tumor cells are generally resistant to apoptosis induction (31, 32). Functional P-gp inhibits activation of caspase-3 and -8 by some apoptotic stimuli, resulting in apoptosis resistance in MDR tumor cells (33, 34). We have shown that salvicine-induced MDR and parental K562 cell apoptosis is caspase-3-dependent (7). Our previous work (7) and the present study show that salvicine can induce apoptosis and cytotoxicity in both MDR and parental K562 cells. The activation of c-Jun and antagonism by c-jun AODs indicate that c-jun is principally responsible for the effects of salvicine and suggest that c-Jun activated by salvicine probably triggers a pro-apoptotic signal that leads to cell death in both MDR and parental cell lines. On the other hand, P-gp is expressed in MDR cells but not in parental cells (7, 14, 15), which challenges the notion that its down-regulation via c-Jun activation contributes to the effects of salvicine in MDR cells. However, inhibition of salvicine cytotoxicity by c-jun AODs is significantly greater in MDR cells than in parental cells (Fig. 6). An explanation may be that c-jun AODs, in addition to decreasing pro-apoptotic signals, restore P-gp inhibition of death in MDR K562/A02 cells. Therefore, we could reasonably infer that transcription factor c-Jun mediates the negative regulation of mdr-1 gene expression in salvicine-treated MDR K562/A02 cells. At the same time, our results also show the complex versatility of c-Jun.

In summary, we demonstrate that transcription factor c-Jun is a principal determinant in down-regulation of mdr-1 gene expression and induction of apoptosis by salvicine in human MDR K562/A02 cells, providing new insights into the complicated regulatory mechanisms of mdr-1 gene expression. The findings suggest c-Jun might be a potential drug target for circumventing tumor MDR. In addition, the present results provide a biochemical basis for possible clinical application of salvicine alone, or in combination with conventional antineoplastic agents in treating MDR tumors.

REFERENCES
12. Ogretmen, B., and Safa, A. Negative regulation of MDR-1 promoter activity in MCF-7, but not in multidrug resistant MCF-7/Adr, cells by cross-coupled NF-kappaB55.


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