ORIGINAL

# Resistance to topoisomerase II inhibitors in human glioma cell lines overexpressing multidrug resistant associated protein (MRP) 2

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**Abstract : For understanding of the resistance to topoisomerase Ⅱ inhibitors, 50 sublines were isolated as single clones from parental glioma cell lines by exposure to VP-16 or m-AMSA. The quantitative aspects of topoisomerase Ⅱα, multidrug resistant gene (MDR)-1, breast cancer resistance protein (BCRP), and multidrug resistant associated protein (MRP) 1-5 were studied by Northern blotting in 50 resistant cell lines. By understanding the function of MRP2, we picked up three drug resistant sublines (T98G-m1, T98G-m2, and gli36-VP1) that overexpressed MRP2, but did not overexpress MDR-1 or MRP1-5 except 2. Moreover, in the results of northern blot analysis of mRNA for topoisomerase Ⅱα identical results are observed in parental cell lines and their resistant cell lines, suggesting that alterations in topoisomerase Ⅱ do not account for the resistance in these cells. To determine whether the cellular sensitivity to anticancer agents was closely associated with the cellular levels of MRP2, we established cell lines with the same levels of MRP2 as their parental cells by introducing the MRP2 antisense expression plasmid into resistant cells. Etoposide (VP-16) accumulation and efflux studies were carried out in the parental cell lines and their drug resistant cell lines. Decreases in the $H^3$-VP-16 accumulation and increases in the efflux were observed in these drug resistant cell lines. In the cytotoxicity assay, these drug resistant cell lines were resistant to multiple topoisomerase Ⅱ inhibitors with little cross resistance to vincristine, and display efflux of VP-16. We found that the resistant cells transfected with MRP2 antisense cDNA displayed increased cellular levels of VP-16 and enhanced sensitivities to topoisomerase Ⅱ inhibitors. In this study on the T98G-m1, T98G-m2, and gli36-VP1 cell lines, we showed a high correlation between MRP2 mRNA and VP-16 efflux, suggesting that MRP2 could be a new transporter for topoisomerase Ⅱ inhibitors. J. Med. Invest. 52 : 41-48, February, 2005**

***Keywords*** **:** *topoisomerase Ⅱ, mutation, drug resistance, multidrug resistance, chemotherapy, MRP*

## INTRODUCTION

The epipodophyllotoxins, Etoposide (VP-16) and Teniposide, are useful antineoplastic agents with activity in both hematologic malignancies and solid tumors (1). The anticancer activity of these agents is thought to result at least in part from stabilization of the cleavable complex, an intermediate in which topoisomerase Ⅱ is covalently bound to DNA in a step that precedes DNA religation (2). This mechanism of action is shared by other antitumor agents including the anthracyclines, the aminoacridines, and the ellipticines(3).

The efficacy of topoisomerase Ⅱ inhibitors (epipodophyllotoxins, aminoacridine, and mitoxantrone) is limited by the occurrence of drug resistance in the tumor cell population. Cellular insensitivity to drugs that stabilize the cleavable complex is frequently expressed

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as multidrug resistance (MDR)(4). In some cell lines, overexpression of MDR-1/P-glycoprotein or the multidrug resistance associated protein (MRP)1, has been demonstrated and implicated as the mechanism of resistance (5, 6). These proteins are known to confer multidrug resistant phenotypes to the cancer cells. Although the primary structures of P-glycoprotein and MRP share only 15% amino acid homology (7), both P-glycoprotein and MRP are involved in drug resistance to a similar profile of chemotherapeutic agents that include anthracyclines, vinka alkaloid, and epipodophyllotoxins (8). Another transporter, termed breast cancer-resistance protein (BCRP), also known as mitoxantrone-resistance protein and placenta ATP binding cassette (ABC) protein, was described in breast and colon carcinoma cell lines selected for higher-level resistance to the antineoplastic drug, mitoxantrone.

Typically, these cells have reduced drug accumulation, secondary to increased drug efflux. Recently, the cDNA of a new ABC superfamily, MRP2 has been isolated (9). The size of MRP2 mRNA was estimated to be about 6.5 KB(10), similar to that of MRP1 mRNA but larger than the human MDR-1 mRNA(4.5 KB).

Using a single clone selection process, we successfully isolated and characterized three human glioma cell lines isolated for their resistance to amsacrine (mAMSA) or VP-16, T98G-m1, T98G-m2, and gli36-VP1, which overexpress MRP2 but do not express MRP1-5 except 2 and MDR-1. We herein discuss a possible correlation between MRP2 and the cellular VP-16 accumulation.

## MATERIALS AND METHODS

*Cell Lines and Cell Cultures*

The cell lines described in the present report were initially isolated as single clones. Four parental glioma cell lines were used : T98G, Gli36, U87, and SNB19. The selections were performed at different drug concentrations for each cell line (T98G:300nM VP-16 or 30 nM mAMSA ; Gli 36 : 300 nM VP-16 or 50 nM mAMSA ; U87 : 500nM VP-16 or 50nM mAMSA ; and SNB 19 : 500nM VP-16 or 50nM mAMSA). These concentrations were those that were toxic to most cells (IC 99), and resulted in the survival of only a few cells that grew as isolated clones. 50Clones were picked as single colonies and subsequently, a population of cells from each concentration of the drugs was maintained in the same concentration of which was used in the selection. After a single clone selection process, we have characterized 50 human glioma cell lines by the Northern analysis with probes of Topoisomerase IIα, MRP1-5, BCRP, and MDR-1. By understanding the function of MRP2, we picked up three drug resistant sublines (T98G-m1, T98G-m2, and gli36-VP1) for resistance to mAMSA or VP-16 that overexpress MRP 2, but do not overexpress MDR-1, BCRP, and MRP 1-5 except 2.

*Transfection with the MRP2 Antisense Expression Vector*

Exponentially growing the resistant cell lines and the parental cell lines ($5\times10^6$) were washed and placed in serum-free medium. A mixture of 50μg of Lipofectin, 10μg of the MRP2 antisense expression vector (11), and 1 μg of pSV2-neo DNA was added for 12h, after which fresh medium was added. The cells were incubated in the selection medium (i.e. containing 400μg/ml G418) for 3-4 weeks. Three stable transfectants (T98G-m1-R, T98G-m 2-R, and Gli 36-VP 1-R), which also showed reduced cellular MRP2 levels, were selected among the G418-resistant clones.

*Cytotoxicity Assays*

Cytotoxicity assays were performed as previously described (10,12). Briefly, 300 to 1000 cells/well plated in 96-well dishes were incubated overnight after which time drug was added and the cells were incubated for an additional 5 days. At the end of the incubation period, cells were fixed with trichloroacetic acid, and stained with 0.4% sulforhodamine B dissolved in acetic acid. Unbound dye was removed by washing four times with 1% acetic acid, and protein bound dye was extracted with 10mM unbuffered Tris base. Optical density was determined on a 96-well microtiter plate reader by measuring the OD at 570nm. The cytotoxic effects of the drugs were expressed quantitatively as the dose required to kill 50% of the cells ($IC_{50}$). Each experimental group consisted of six independent wells.

*Cloning of topoisomerase IIα, MRP1-5, MDR-1, and BCRP cDNA by Reverse Transcription-PCR*

Synthetic oligonucleotides corresponding to the published cDNA sequence of human MRP1-5 were used to isolate by RT-PCR specific products for direct cloning into pGEM-3z vectors (Promega, Madison, WI). The identity of the cDNA clones was confirmed by direct sequence analysis prior to their use as probes for northern analysis. The sequences of the oligonucleotides used in the RT-PCR were as follows : topoisomerase IIα : -915 'TGTGGAGAAGCGGCTTGGTC3' and 449 5 'TAGTTACTAGAAGTTAGGAGCT3' ;MRP1:733 5 'CGGAAACCATCCACGACCCTAATCC3' and 1027 5 'ACCTCCTCATTCGCATCCACCTTGG 3' ; MRP2 : 41365 'CTGCCTCTTCAGAATCTTAG' and 4376 5

'CCCAAGTTGCAGGCTGGCC' ; MRP 3 : 5'GATAC GCTCGCCACAGTCC3' and 5 'CAGTTGGCCGTG ATGTggCTG 3' ; MRP 4 : 5 'CCATTGAAGATCTT CCTGG 3' and 5'GGTGTTCAATCTGTGTGC 3' ; MRP 5 : 5 'GGATAACTTCTCAGTGGG 3' and 5'G GAATGGCAATGCTCTAAAG 3' ; MDR-1 : 3525'C AGTGTTTGCCATAGTATTTTCAAGGATTG 3'an d 7415'CCCTTTAACACTAGAAGCATCAC 3' ; BCRP : 19795'TGCCCAGGACTCAATGCAACAG' and 25405'GACTGAAGGGCTACTAACC 3'.

*Northern Blotting*

Total RNA (20μg/lane) separated on a 6% formaldehyde gel and transferred to Hybond $N^+$ with 10X SSC. The membranes were hybridized at 42°Covernight with the radiolabeled probe in Hybrisol I (Oncor, Gaithersburg, MD)(13). We used the RNA of ZR-VP 13 cells that overexpressed all drug resistant genes (MDR-1, MRP1-5, topoisomerase IIα, and BCRP) as positive control(14).

*Immunoblotting*

Immunoblotting for the MRP2 protein was performed using detergent-solubilized membrane proteins resolved on a 6% SDS-polyacrylamide. Immunoblotting was performed with a 1:2000 dilution of a monoclonal MRP antibody (cosmobio, Tokyo, Japan). After three washes in TBS-T, the membranes were incubated with HRP-conjugated anti-mouse secondary antibody at a dilution of 1 : 1000 for 1 hr. Five washes were then performed and immunolabeled protein was detected by chemiluminescence (15).

*Preparation of crude nuclear extracts*

Crude nuclear extracts were prepared from $1\times10^8$ cells in early log phase culture as previously described (15). Briefly, cells washed 3 times with ice-cold PBS, were harvested with a rubber scraper, and collected by centrifugation. Cell pellets were then lysed in10 nM Tris-HCl (pH 7.5), 1.5 mM $MgCl_2$, 10 nM NaCl, and 1% Nonidet P-40. Nuclei were isolated by centrifugation at600 X g for10 min, suspended in 2ml of 50 mM Tris-HCl (pH7.5), 25mM KCl, 2mM $CaCl_2$, 5mM $MgCl_2$ buffer, and layered over a 0.6 ml cushion of the suspension buffer containing 0.6M sucrose. Following centrifugation at 2000 X g for10 min, the nuclear pellets were resuspended in 0.2ml of 50mM Tris-HCl (pH7.5), 25mM KCl, 5mM $MgCl_2$ ; and20μl of 0.2 M EDTA (pH8.0) was added followed by 2 volumes of 80mM Tris-HCl (pH7.5), 1mM dithiothreitol, 2mM EDTA, 0.53 M NaCl, and 20% glycerol ; the final concentration of NaCl was 0.35M. Extraction then proceeded on ice for 60 min. Protein concentration of the extracts was determined by the method of Bradford.

*DNA topoisomerase II activity assay*

The standard reaction contained 50mM Tris-HCl (pH 7.5), 85mM KCl, 10mM $MgCl_2$, 5mM dithiothreitol, 0.5mM EDTA, 0.03mg/ml bovine serum albumin, and 1mM adenosine triphosphate. The decatenation reaction of catenation DNA was carried out with serial dilutions of nuclear extract and 0.1μg of kinetoplast DNA (TopoGEN, Columbus, Ohio) in a final volume of 20 μl at 30°C for 30 min. The reaction was terminated by adding 2μl of 1.2mg/ml proteinase K in 22 mM EDTA followed by incubation for15 min. at 37°C. Then, 5ml of 0.05% bromophenol blue in 50% glycerol was added to the reaction mixture. Samples were separated by electrophoresis through a 1% agarose gel. After staining with ethidium bromide, gels were photographed under UV illumination (15).

*Cellular Accumulation and Efflux Assay for[$^3$H]-VP-16*

$3.5\times10^5$ cells were plated in each well of a six-well plate and incubated for 24hr at 37°C. Growth medium was then replaced with serum-free MEM, and the cells were incubated with 2.6μM[$^3$H]-VP16 for various times up to 45 min. at 37°C. At the completion of the incubation period, cells were quickly washed three times with ice-cold PBS, lysed with 2ml of 0.05% SDS, and mixed with 3 ml of Scintisol. For efflux studies, cells plated as for accumulation assays were incubated with 2.6 M[$^3$H]-VP-16 for 45 mins then washed free of extracellular VP-16 by rinsing with ice-cold PBS. Cells were incubated in VP-16-free medium at 37°C. Then over a 30 min period, the levels of intracellular radioactivity were measured(15).

*Statistical analysis*

All cytotoxicity assays (MRP2 expressing cells versus parental cells) were analyzed by two-way analysis of variance (ANOVA)(n=6), with differences between individual means determined by Bonferroni's post-tests. In accumulation and efflux tests, each point represents the means±SD of independent six experiments (differences were considered statically significant ($P<0.05$) in MRP2 expressing resistant cells or their MRP 2 antisense transfectants versus parental cells).

## RESULTS

*Cytotoxicity assays*

Table1 depicts the cross-resistance profiles of the

**Table 1.** Cross-resistance profiles of the three drug resistant sublines

| | VP-16 | mAMSA | Mitoxantrone | Adriamycin | Vincristine |
|---|---|---|---|---|---|
| a) $IC_{50}$ Gli 36 | 48.7±4.0 | 2.4±0.4 | 0.11±0.09 | 0.42±0.12 | 4.2±0.95 |
| Gli36-VP1 | 6234±118 | 48.0±20.2 | 0.528±0.44 | 1.7±0.6 | 3.9±0.36 |
| b) relative resistance | 128c) | 20c) | 4.8c) | 4.0c) | 0.93 |
| Gli36-VP1-R | 54.7±7.3 | 3.5±1.1 | 0.18±0.11 | 0.63±0.18 | 4.5±1.32 |
| b) relative resistance | 1.1 | 1.5 | 1.6 | 1.5 | 1.1 |
| a) $IC_{50}$ T98G | 5.1±1.9 | 0.6±0.44 | 0.023±0.02 | 2.0±0.5 | 0.7±0.1 |
| T98G-m1 | 398±226 | 10.8±3.8 | 0.13±0.05 | 6.6±5.7 | 1.3±0.08 |
| b) relative resistance | 78c) | 18c) | 5.7c) | 3.3c) | 1.9 |
| T98G-m1-R | 6.3±1.5 | 0.8±0.32 | 0.031±0.05 | 2.5±0.3 | 1.1±0.3 |
| b) relative resistance | 1.2 | 1.3 | 1.3 | 1.3 | 1.6 |
| T98G-m2 | 833±139 | 17±1.3 | 0.12±0.02 | 9.0±2.3 | 0.7±0.1 |
| b) relative resistance | 163c) | 28c) | 5.2c) | 4.5c) | 1.0 |
| T98G-m2-R | 7.9±2.1 | 0.7±0.86 | 0.026±0.10 | 3.4±1.2 | 1.2±0.4 |
| b) relative resistance | 1.5 | 1.2 | 1.1 | 1.7 | 1.7 |

Each experimental group consisted of six independent wells
a) $IC_{50}$ : The cytotoxic effects of the drugs were expressed quantitatively as the dose required to kill 50% of the cells (ng/ml)
b) relative resistance ; ratio of $IC_{50}$ of the individual cell lines
c) Significant different ($p<0.05$) from their parental cell lines

three drug resistant sublines (T98G-m1, T98G-m2, and Gli36-VP1)and their transfectants (T98G-m1-R, T98G-m2-R, and Gli36-VP1-R) to various chemotherapeutic agents. Cross-resistance to four topoisomerase II poisons($p<0.05$) (VP-16, mAMSA, mitoxantrone, adriamycin) was observed in these drug resistant cells with little cross resistance to the microtubule active agent, vincristine. The transfectants displayed enhanced sensitivities to topoisomerase II inhibitors, VP-16, mAMSA, mitoxantrone, and adriamycin.

*Expression of mRNA of drug resistance genes*

We examined the expressions of topoisomerase IIα, MRP 1-5, BCRP, and MDR-1, and the results are shown in Fig.1. The Northern blot analysis showed that these resistant cells overexpressed MRP2, but did not overexpress MDR-1 or MRP1-5 except 2. Moreover, in the results of the Northern blot analysis of mRNA for topoisomerase IIα identical results were observed in the parental cell lines and their resistant cell lines.

*Topoisomerase II activity*

When the topoisomerase II activity was measured in decatenation assays using crude nuclear extracts, similar levels of activity were observed in the crude nuclear extracts of resistant cell lines (Gli36-VP1, T98G-m 1, and T98G-m 2 cells) and their parental cells, suggesting that alterations in topoisomerase II did not account for the resistance in these cells (Fig.2).

*Immunoblotting*

By immunoblotting with polyclonal antibody against the human MRP2, we demonstrated the presence of a Mr 200,000MRP2 protein in the resistant cell lines (densitometry quantitation : T98G-m1, 17times of the parental T98G ; T98G-m2, 125 times ; and Gli36-VP

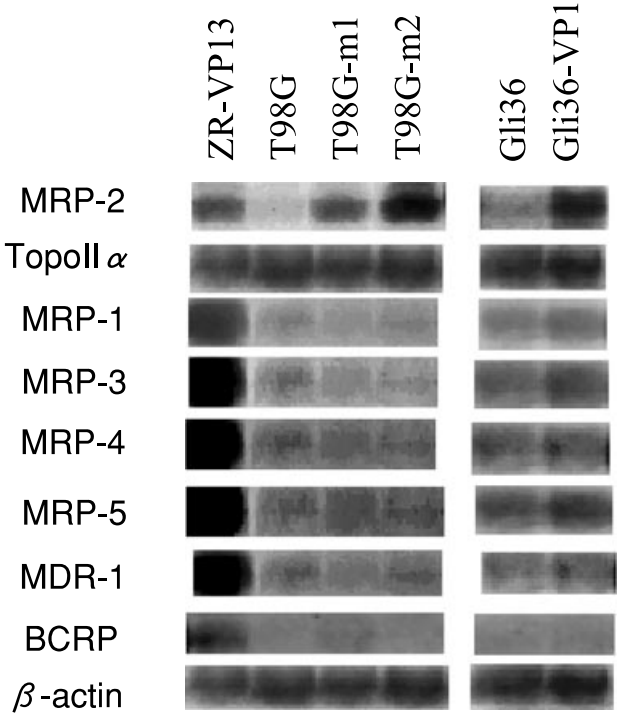


**Figure 1** Northern blot analysis of topoisomerase IIα(4.5 KB), MRP 1-5(4.5 KB, 4.4 KB, 4.7 KB, 4.3 KB, 6.5 KB), MDR-1 (4.5 KB), and BCRP (2.8 KB) expression in the drug resistant sublines and their parental cell lines. β-actin was used as internal control.

1, 78times of the parental Gli36)(Fig 3). To determine whether the cellular sensitivity to the anticancer agents was closely associated with the cellular levels of MRP2, we established cell lines with the same levels of MRP2 as their parental cells by introducing the MRP 2 antisense expression plasmid into the resistant cell lines. As compared with the parental cell lines, the resistant cell lines transfected with MRP2 antisense cDNA had decreased to the same levels of MRP2 as their parental cells (densitometry quantitation : T98G-m1-R, 1.1times of the parental T98G; T98G-m2-R, 1.4times; and Gli 36-VP 1-R, 1.5 times of the parental Gli 36)(Fig 3).

*VP-16 accumulation and efflux*

VP-16 accumulation and efflux studies were carried out in the parental cell lines and their drug resistant sublines (Fig.4). Figure 2A and 2C of demonstrate the $^{3}$H-VP-16 accumulation in the parental cells and the drug resistant sublines. VP-16 accumulation over 45 min is displayed as p mol of VP-16/$10^{7}$ cells. $^{3}$H-VP-16 accumu-

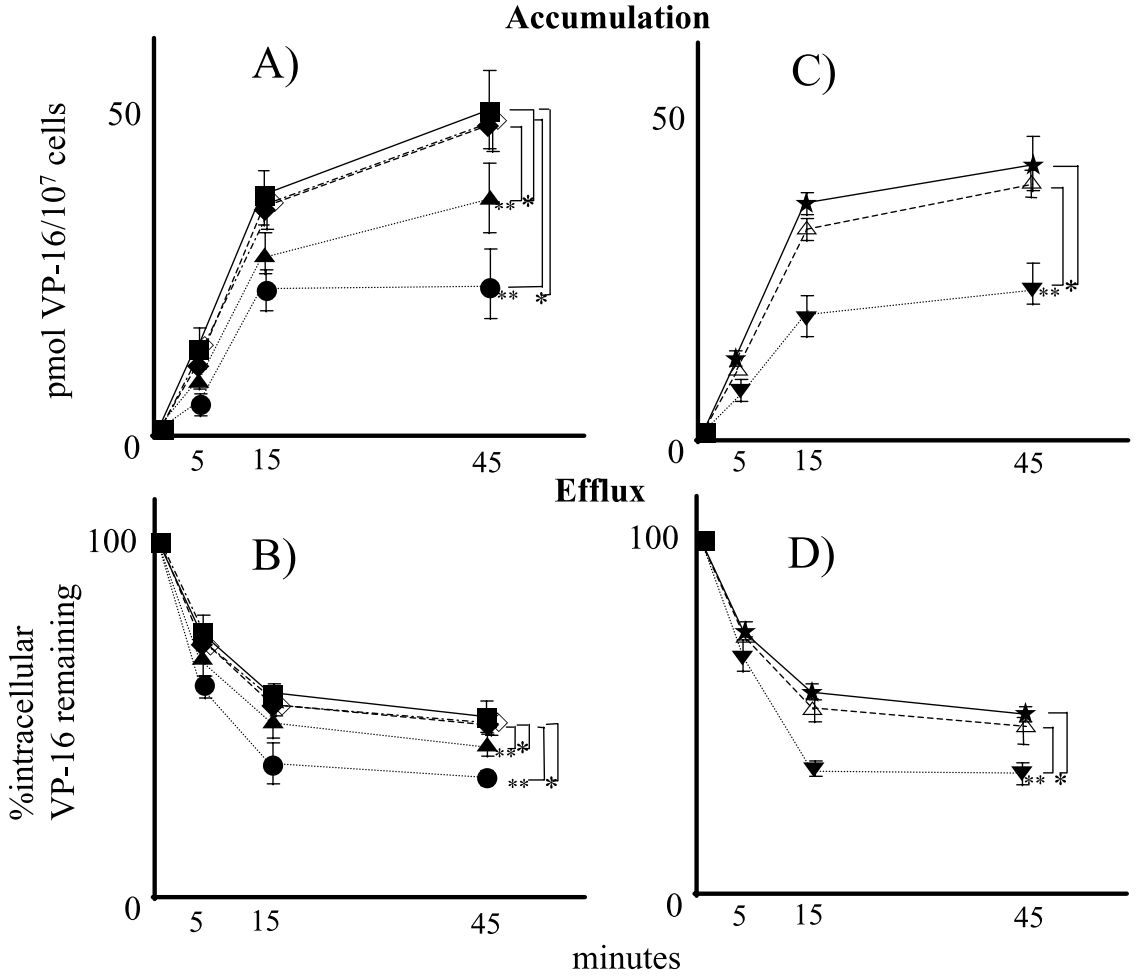


Figure 2 VP-16 accumulation and efflux studies were carried out in the parental cell lines and their drug resistant sublines. A) and C) demonstrate the $^{3}$H-VP-16 accumulation in the parental cells and the drug resistant sublines. Accumulation over 45 min period is displayed as p mol of VP-16/$10^{7}$ cells. $^{3}$H-VP-16 accumulations for the resistant sublines relative to the parental cells were T98G-m1, 76% (T98G:42.0±9.0, T98G-m1:31.9±4.3, $p<0.05$); T98G-m2, 48%(T98G:42.0±9.0, T98G-m2:20.2±3.8, $p<0.05$) and Gli36-VP1, 58%(Gli36:41.1±3.7, Gli36-VP1:20.2±3.8,$p<0.05$). Increased accumulation was observed in the three sublines transfected with the MRP 2 antisense cDNA, with partial restoration of levels to that of the parental cells (T98G-m1-R, 96% of the parental T98G; T98G-m2-R, 98%; and Gli36-VP1-R, 94% of the parental Gli36). B) and D) demonstrates the $^{3}$H-VP-16 present in the drug resistant cells, compared to the efflux present in parental cells. Relative to the amount of $^{3}$H-VP-16 present following loading, the proportion of $^{3}$H-VP-16 remaining after 45 min. of efflux in the T98G-m1, T98G-m2, and gli36-VP1 were 41%, 33%, and 34%, compared to 49%, and 48% in their parental cells respectively. As compared with the resistant cells, cellular efflux levels of VP-16 were increased in the transfectants and their parental cells (T98G-m1-R, 96% of the parental T98G; T98G-m2-R, 96%; and Gli 36-VP 1-R, 93% of the parental Gli 36). Each experimental group consisted of six independent wells.
*significantly different ($p<0.05$) between the resistant cell lines and their parental cell lines (incubation time at 45 min).
**significantly different ($p<0.05$) between the resistant cell lines and their own transfected MRP2 antisense cDNA (incubation time at 45 min)
A), B) : ■ ; T98G, ▲ ; T98G-m1, ● ; T98G-m2, ◆ ; T98G-m1-R, ◇ ; T98G-m2-R
C), D) : ★ ; Gli36, ▼ ; Gli36-VP1, △ ; Gli36-VP1-R

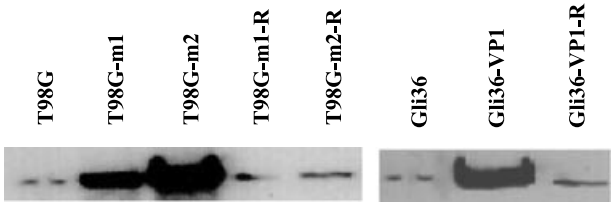


**Figure 3** Immunoblotting analysis for MRP2 in the drug resistant cell lines and their parental cell lines. By immunoblotting with the polyclonal antibody against human MRP2, we demonstrated the presence of a Mr200,000 MRP2 protein in the resistant cell lines. To determine whether the cellular sensitivities to anticancer agents were closely correlated with the cellular levels of the MRP2, we established the cell lines with reduced levels of the MRP2 by introducing the MRP2 antisense expression plasmid into the resistant cell lines. As compared with the parental cell lines, the resistant cell lines transfected with MRP2 antisense cDNA had the same levels of MRP 2 as their parental cells.

lations for the resistant sublines relative to the parental cells were T98G-m1, 76%(T98G : 42.0±9.0, T98G-m1 : 31.9±4.3, $p<0.05$); T98G-m2, 48%(T98G:42.0±9.0, T98G-m2:20.2±3.8, $p<0.05$) and gli36-VP1, 58%(Gli 36 :41.1±3.7, Gli36-VP1:20.2±3.8, $p<0.05$). Increased accumulation was observed in the three sublines transfected with MRP2 antisense cDNA, with partial restoration of the levels to that of the parental cells (T98 G-m1-R, 96% of the parental T98G;T98G-m2-R, 98%; and Gli36-VP-R, 94% of the parental Gli36). Figure 2B and 2D demonstrate the $^3$H-VP-16 present in the drug resistant cells, as compared to the efflux present in the parental cells. Relative to the amount of $^3$H-VP-16 present following loading, the proportion of $^3$H-VP-16 remaining at 45 min. of efflux in the T98G-m1, T98 G-m 2, and Gli 36-VP 1 were 41±6.1%, 33±0.91%, and 34±4.6%, as compared to 49±3.1% (T98G) and 48±1.2% (Gli 36) in their parental cells respectively. We also determined whether the reduced cellular levels of MRP affected the cellular accumulation of drugs. As compared with the resistant cells, the cellular efflux levels of VP-16 increased in the transfectants and their parental cells (T98G-m 1-R, 96% of the parental T 98 G; T 98 G-m 2-R, 96%; and Gli 36-VP-R, 93% of the parental Gli 36).

## DISCUSSION

In the early studies, it was recognized that resistance to the structurally unrelated natural products and decreased drug accumulation were the hallmarks of the phenotype that we now associate with overexpression of MDR-1(16). Approximately 20 years later, a second ATP-dependent transporter, MRP1 was linked with MDR. MRP1-overexpressing cells are primarily resistant to VP-16and doxorubicin (5,17). In addition to MRP 1, other genes of the MRP family (MRP2-5) were isolated. Of these MRP family genes, MRP1 and 3 were expressed in the human glioma cell lines and clinical glioma specimens. However, unlike MRP1, expressions of the MRP 2 and 3 have not been detected in the normal brain tissue(11, 20-21). For understanding the function of MRP2 in this study, we isolated 50 sublines by exposures to VP-16 or mAMSA and picked up three drug resistant sublines (T98G-m1, T98G-m2, and gli 36-VP1) that overexpressed MRP2, but did not overexpress MDR-1, BCRP, and MRP 1-5 except 2. The results of 50 sublines described in the present study (data not

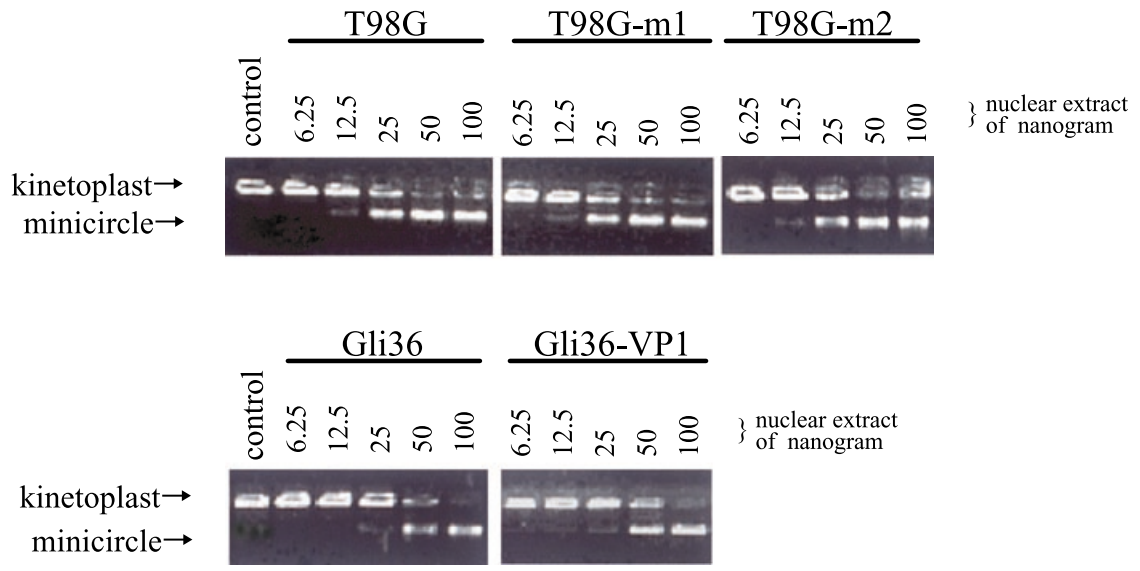


**Figure 4** Decatenation of kinetoplast DNA by nuclear extract. In the absence of nuclear extract, the catenated kinetoplast DNA remains in the gel. Comparable topoisomerase activity is demonstrated by comparable decatenation of kinetoplast DNA to the monomer minicircles, using increasing amounts of the nuclear extracts.

shown) support and extend previous observations. The initial change observed was a decrease in the expression and activity of topoisomerase IIα. Previous studies have documented reduced expression of topoisomerase II with drug selection, and this has been proposed as a mechanism of drug resistance(22-24). In our own experience, we have found decreased expression of topoisomerase IIα (less than 50% of parental cells) in 39 of 50 isolates, suggesting that reduced expression is a common adaptation. Reduction of topoisomerase levels effectively diminishes the main intracellular target of VP-16 and other topoisomerase poisons and can confer broad cross-resistance to these agents(25). Although in some cell lines, topoisomerase IIα expression was partially maintained with resistance to topoisomerase II poisons, as a results of increased expression of drug efflux pump [MRP1(33/50), MRP2 (28/50), MRP3(12/50), MRP4(0/50), MRP5(0/50), MDR 1(6/50), and BCRP(7/50)]. The three cell lines are resistant to topoisomerase II inhibitors (VP-16, mAMSA, mitoxantrone and Adriamycin) with little cross resistance to the microtubule active agent, vincristine. This resistance is not due to MDR-1, BCRP, and MRP 1-5 except 2 overexpression, as demonstrated by the Northern blot analysis and VP-16 accumulation and efflux studies. Especially in the three cell lines composed of T98G parental cells and their mAMSA-selected MRP 2-expressing sublines, we noticed a high correlation between MRP2 mRNA overexpression and cross-resistance to topoisomerase II inhibitors, suggesting that MRP2 could be a new transporter and a new drug resistant mechanism for topoisomerase II inhibitors. To determine whether the cellular sensitivity to anticancer agents closely correlated with the cellular levels of MRP2, we established cell lines with reduced levels of MRP2 by introducing the MRP2 antisense expression plasmid into the resistant cells and their parental cells. We found that the resistant cells transfected with MRP2 antisense cDNA displayed increased cellular levels of VP-16 and enhanced sensitivities to topoisomerase II inhibitors, VP-16, mAMSA, mitoxantrone, and Adriamycin (26).

We also found that resistant cell lines transfected with MRP2 antisense cDNA displayed an enhanced sensitivity to topoisomerase II inhibitors (VP-16, mAMSA, mitoxantrone, and adriamycin). It has also been shown that cells transfected with MRP1cDNA as well as drug-selected MRP1-overproducing cells acquired resistance to VP-16, adriamycin, and vincristine but not to camptothecin-11 and cisplatin. Thus, MRP2 appears to modulate the sensitivity to certain anticancer agents as MRP 1(27).

In contrast, a recent study has demonstrated that the expression of MRP2 is increased in the colorectal cancers as compared with non-cancerous tissues and also that the drug sensitivity to cisplatin closely correlated with the MRP mRNA levels in the colorectal cancers (28). Another study demonstrated that antisense MRP2 cDNA transfectants could increase the sensitivity to cisplatin. Like the MRP1, MRP2 protein appears to show strong affinities to glutathione and its conjugates. Further studies are necessary to determine whether MRP2 is directly involved in the sensitivity to VP-16, in addition to cisplatin, with or without glutathione or glucuronide (11).

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