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 II, 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 II, 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 H3-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.
Cell Lines and Cell Cultures

Cell Lines and Cell Cultures

Transfection with the MRP2 Antisense Expression Vector

Cytotoxicity Assays

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

Y. Matsumoto et al. MRP2 in topoisomerase IIα
Northern Blotting

Immunoblotting

Preparation of crude nuclear extracts

DNA topoisomerase II activity assay

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

Statistical analysis

Cytotoxicity assays
<table>
<thead>
<tr>
<th>MRP-2</th>
<th>Topoll α</th>
<th>MRP-1</th>
<th>MRP-3</th>
<th>MRP-4</th>
<th>MRP-5</th>
<th>MDR-1</th>
<th>BCRP</th>
<th>β-actin</th>
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**Expression of mRNA of drug resistance genes**

Expression of mRNA of drug resistance genes was determined by RT-PCR. The results showed that MRP-2 and Topoll α were highly expressed in ZR-VP13 and T98G cells, while MRP-1 was highly expressed in T98G-m1 and T98G-m2 cells. MRP-3 and MRP-4 were expressed at similar levels in all cell lines, and MRP-5 was not detected. MDR-1 and BCRP were expressed at low levels in ZR-VP13 and T98G cells, while they were highly expressed in T98G-m1 and T98G-m2 cells. β-actin was used as a control for RNA loading.

**Topoisomerase activity**

Topoisomerase activity was assessed by immunoblotting. The results showed that MRP-2 and Topoll α were highly expressed in ZR-VP13 and T98G cells, while MRP-1 was highly expressed in T98G-m1 and T98G-m2 cells. MRP-3 and MRP-4 were expressed at similar levels in all cell lines, and MRP-5 was not detected. MDR-1 and BCRP were expressed at low levels in ZR-VP13 and T98G cells, while they were highly expressed in T98G-m1 and T98G-m2 cells. β-actin was used as a control for protein loading.
VP-16 accumulation and efflux

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Accumulation

Efflux

minutes

% intracellular remaining

pnmol VP-16/10^7 cells