Transfection of Glutathione S-Transferase (GST)-\(\pi\) Antisense Complementary DNA Increases the Sensitivity of a Colon Cancer Cell Line to Adriamycin, Cisplatin, Melphalan, and Etoposide

Noriyoshi Ban, Yasuo Takahashi, Tetsuji Takayama, Toshiro Kura, Tatsuro Katahira, Sumio Sakamaki, and Yoshiro Niitsu

Department of Internal Medicine (Section 4), Sapporo Medical University School of Medicine, South-1, West-16, Chuo-ku, Sapporo 060, Japan

ABSTRACT

The goal of this study was to demonstrate that glutathione S-transferase (GST)-\(\pi\) is directly involved in the intrinsic and acquired resistance of cancer cells to anticancer drugs. To this end, GST-\(\pi\) antisense cDNA was transfected into the cultured human colon cancer cell line M7609, which expresses an innately high level of GST-\(\pi\) and shows intrinsic drug resistance, and into an M7609 strain with acquired resistance to Adriamycin (ADR; i.e., M7609/ADR cells). The changes in the sensitivity of these transfecants to various anticancer drugs were investigated. The intracellular concentrations of GST-\(\pi\) in M7609/anti-1 cells and M7609/anti-2 cells, two clones that were established by transfection of GST-\(\pi\) antisense cDNA into M7609 cells, were decreased to approximately half of those detected in the parent cells (M7609) and in the control cells transfected with vector alone (M7609/pLJ). The sensitivities of the antisense transfecants in relation to ADR, cisplatin, melphalan, and etoposide were increased—3.3-fold, 2.3-fold, 2.2-fold, and 2.1-fold, respectively, compared with those of M7609 and M7609/pLJ. On the other hand, the sensitivities of the antisense transfecants to Taxol, vincristine, 5-fluorouracil, and mitomycin C were not significantly changed. Similarly, the transfection of antisense cDNA into M7609/ADR cells resulted in the reduction of intracellular GST-\(\pi\) concentration (by about half) and an increased sensitivity to ADR (4.4-fold), but no increase in 5-fluorouracil sensitivity. Thus, GST-\(\pi\) is considered to be a multidrug resistance factor that is responsible for both the intrinsic and acquired resistance of cancer cells to anticancer drugs such as ADR, cisplatin, melphalan, and etoposide.

INTRODUCTION

GST-\(\pi\) expression is increased in various human cancer tissues, including gastric cancer, colon cancer, lung cancer, oral cavity cancer, and uterine cancer; thus it is employed in cancer research as a tumor marker (1–5). There have also been numerous reports showing that the expression of GST-\(\pi\) is elevated in various cultured cell lines possessing resistance to anticancer drugs such as ADR (6), melphalan (7), CDDP (8), cyclophosphamide (9), and chlorambucil (10) as well as in vivo cancer tissues that have become resistant to therapy after administration of anticancer agents (11). We have recently established, from a human colon cancer cell line (M7609), an ADR-resistant cell line (M7609/ADR) that showed increased expression of GST-\(\pi\) but not of p-glycoprotein, multidrug-resistance-associated protein (MRP), and topoisomerase II. These observations suggest that the expression of GST-\(\pi\) is involved in the acquisition of resistance to anticancer drugs. Furthermore, elevated expression of GST-\(\pi\) has been demonstrated even in cancers that show resistance to anti-cancer agents before treatment, which suggests that GST-\(\pi\) is involved in intrinsic resistance as well (12). However, these studies have not shed light directly on the question of whether the expression of GST-\(\pi\) is in fact a cause of drug resistance or merely a reactive change that accompanies the development of resistance.

Accordingly, various investigators have attempted to obtain direct proof of the involvement of GST-\(\pi\) in anticancer drug resistance by the transfection of GST-\(\pi\) expression vectors to cultured cancer cells. For example, Moscow et al. (13) transfected a GST-\(\pi\) expression vector to NIH3T3 cells (a cultured human breast cancer cell line) and showed that the cells acquired resistance to etoposide and ethacrynic acid, but there was no change in the cells’ sensitivity to CDDP or melphalan. Another group, Miyazaki et al. (14), transfected a GST-\(\pi\) expression vector to Chinese hamster ovary cells and found that the cells were resistant to CDDP but not to ADR. Nakagawa et al. (15) transfected a GST-\(\pi\) expression vector to NIH3T3 cells that had been transformed with H-ras and found that although the resistance to ADR had been increased, the cells did not acquire resistance to alkylating agents. In yet another study, Black et al. (16) transfected a GST-\(\pi\) expression vector to Saccharomyces cerevisiae and reported that resistance was acquired to ADR and chlorambucil. However, the results of these experiments involving the transfection of GST-\(\pi\) gene are not entirely unambiguous.

In an attempt to explain these various findings, Tew (17) proposed a number of hypotheses, including: (a) the possibility that the level of reduced GSH in the target cells was below that necessary for the activation of the GSH/GST detoxification system; (b) the possibility that the level of the cell membrane-bound GSH-conjugate export pump was insufficient; (c) the possibility that the level of native GST expression by the cells used was already at a maximum; (d) the possibility that the transcription efficiency of the transected GST-\(\pi\) gene was insufficient or that the turnover was accelerated; and (e) the possibility that the importance of the GSH/GST detoxification system in the overall anticancer drug resistance mechanism was low. Tew thus suggested that the approach of transferring the GST-\(\pi\) gene to target cells and thereby causing an increase in GST-\(\pi\) activity had not been adequate.

In consideration of this background, we designed the present study on the basis of the reverse supposition that if the expression of GST-\(\pi\) by cells was inhibited, then the activity of GST-\(\pi\) should decrease without any dependence on the level of GSH in the cells or the GSH-conjugate export pump. We tested the validity of this approach by transfection of GST-\(\pi\) antisense cDNA to the M7609 human colon cancer cell line and to its ADR-resistant subline, M7609/ADR, and then testing these cell lines for multidrug sensitivity.

MATERIALS AND METHODS

Cell Lines and Cell Culture. Human colon cancer cell line M7609 (18) was kindly provided by Dr. S. Machida (Hirosaki University, Hirosaki, Japan). This cell line was established from a colon cancer patient who had not been treated with anticancer drugs. M7609/ADR, an ADR-resistant cell line, was established from M7609 cells in our laboratory according to the method of
Whelan et al. (19). M7609/ADR shows a resistance to ADR approximately six times greater than that of M7609. M7609/ADR also shows cross-resistance to CDDP, etoposide, and melphanal but not to 5-FU, VCR, and MMC. In addition, M7609/ADR shows two times greater expression of GST-π than the parent cell line but shows no increases of p-glycoprotein, MRP, and topoisomerase II. Both of these cell lines were cultured in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) containing 10% FCS (Flow Laboratories, North Ryde, Australia) in tissue-culture flasks; incubation was performed at 37°C in an atmosphere of air containing 5% CO2.

Construction of a GST-π Antisense Vector. The plasmid pGp2 (20), containing GST-π cDNA, was obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan), and the pLJ vector (21) was kindly provided by Dr. G. Wu (University of Connecticut, Farmington, CT). pGp2 digested with EcoRI, and a 0.7-kb EcoRI-EcoRI fragment containing the whole coding region for GST-π was recovered. Both of these fragments were then blunt-ended with the Klenow fragment (Takara Shuzo Co., Ltd., Kyoto, Japan). The pLJ vector was linearized with BamHI, the blunt of both terminals was similarly performed using the Klenow fragment, and was dephosphorylated with bacterial alkaline phosphatase (Takara Shuzo Co., Ltd.). Both of these processed fragments were ligated with T4 ligase (Takara Shuzo Co., Ltd.), and a clone was selected in which the GST-π cDNA was inserted in the reverse direction. This clone was named pLJ/anti-GST-π (Fig. 1). In consideration of in vivo experiments anticipated for the future, the retroviral vector (pLJ) was employed as the expression vector for GST-π antisense cDNA in the present study.

Gene Transfer. The transfection of the pLJ/anti-GST-π into the M7609 and M7609/ADR cells was performed by the lipofection method (22). Briefly, 2.5 x 10⁵ cells were dispersed in a 3.5-cm culture dish and were incubated for 24 h. The attached cells were then washed three times with RPMI 1640 (Life Technologies, Inc.), followed by the addition of 3 ml of the same culture medium to the dish. Next, 100 μl of plasmid lipofectin reagent (Life Technologies, Inc.) were mixed with 3 μg of pLJ/anti-GST-π and were incubated at room temperature for 15 min. This mixture was then added to each culture dish, and the dishes were incubated at 37°C for 6 h in an atmosphere of air containing 5% CO2. RPMI 1640 (3 ml) containing 10% FCS was added to each culture dish, and incubation was continued for another 72 h. G418 (Life Technologies, Inc.) was added to the culture medium in each dish to a concentration of 400 μg/ml, and the cells were cultured for approximately 2 weeks at 37°C in an atmosphere of air containing 5% CO2. The G418-resistant strains of each cell line were obtained and designated M7609/anti and M7609/ADR/anti, respectively. The M7609/anti cells were further cloned by limiting dilution, and 2 of the 12 resultant clones, M7609/anti-1 and M7609/anti-2, were selected for subsequent experiments. pLJ vector without GST-π antisense cDNA was transfected to M7609 and M7609/ADR cells to obtain each vector control transfectants, M7609/pLJ and M7609/ADR/pLJ, respectively.

Southern Blotting. Genomic DNA was extracted from each cell line as described by Hoggan et al. (23). Each DNA sample (10 μg) was digested with KpnI, which was subjected to 0.8% agarose gel, and was electrophoresed at 45 V for 6 h. The DNA fragments were then transferred to nitrocellulose filters by the method of Southern (24), followed by baking at 80°C for 2 h. The GST-π cDNA (0.7-kb EcoRI-EcoRI fragment) was labeled with 32P by the random primer method (25) and was used as probes. Hybridizations and washings were performed according to the procedures described in “Southern Blotting.”

GST-π Quantitation by ELISA. After washing each cell preparation two times in cold PBS (10 mM sodium phosphate buffer containing 0.9% NaCl), the cells were adjusted to a concentration of 1 x 10⁶/ml in the same buffer and were homogenized with a Dounce homogenizer. The lysates were then centrifuged at 12,000 rpm for 15 min, and the concentration of GST-π in the supernatant was measured by the sandwich ELISA established in our laboratory as described previously (27, 28).

Sensitivities to Various Anticancer Drugs. ADR, 5-FU, and MMC were purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan), whereas etoposide and CDDP were obtained from Nippon Kayaku Co., Ltd. (Tokyo, Japan), and VCR was purchased from Shionogi Co., Ltd. (Tokyo, Japan). Melphanal and ethacrynic acid were obtained from Sigma Chemical Co. (St. Louis, MO), and Taxol was supplied by Bristol-Myers (Tokyo, Japan). The sensitivities of each cultured cell line to these various anticancer drugs were determined by the dye-uptake method (29). Briefly, cultured cells were suspended at a density of 1 x 10⁵ cells/ml in RPMI 1640 containing 10% FCS, 100-μl aliquots were dispensed to each well of 96-well culture plates, and the plates were incubated for 24 h at 37°C in an atmosphere of air containing 5% CO2. In some experiments, to obtain positive controls, 3.3 μM ethacrynic acid, a known substrate for GST-π (30), were added to M7609 or M7609/ADR with the anticancer drugs. Otherwise, various anticancer drugs were added without ethacrynic acid to the wells at 10 different concentrations. Then the cells were incubated for another 48 h at 37°C. Next, 25 μl of a 25% glutaraldehyde solution was added to each well to fix the cells, and the plates were then washed with water, were dried, were stained with a 0.05% methylene blue solution, and were eluted with 0.33 N HCl. The absorbance at 665 nm was then measured with an ELISA reader (MS-3096F; SLT-LAB Instruments Co., Salzburg, Austria). For each well on the culture plates, the cell survival rate was calculated by taking the absorbance value of the control well (to which no anticancer drug had been added) as 100%.

Statistical Analysis. Paired Student’s t tests were used to compare GST-π concentrations and IC₅₀ values for each cell type.
Results

Southern and Northern Blot Analyses of M7609/anti-1 and M7609/anti-2 Cells. Southern blot analysis by KpnI digestion was performed on M7609/pLJ, M7609/anti-1 cells, and M7609/anti-2 cells, using GST-\(\pi\) cDNA (0.7-kb EcoRI-EcoRI fragment) as a probe (Fig. 2A). In addition to a band at approximately 11 kb that represented the cellular GST-\(\pi\) gene, a band at 5.1 kb was observed in M7609/anti-1 and M7609/anti-2 cells. This band was considered to represent the transfected antisense vector.

To examine the expression of endogenous GST-\(\pi\) and antisense mRNA, Northern blot analysis was performed using the GST-\(\pi\) cDNA probe (Fig. 2B). Endogenous GST-\(\pi\) mRNA was detected as a 0.7-kb band for all three cells. However, the amounts of the 0.7-kb mRNA in M7609/anti-1 and M7609/anti-2 cells were apparently less than that in M7609/pLJ cells. Moreover, an additional minor band at 4.0 kb was detected in the two transfectants that was considered to represent antisense RNA. There have been some reports that antisense RNA of a transfected gene cannot be detected at times because the sense-antisense hybrids are rapidly degraded (31, 32). Our results suggest that the expression of antisense mRNA is responsible for lowering the levels of endogenous mRNA (perhaps by hybridization), resulting in the degradation of double-stranded RNA.

Intracellular GST-\(\pi\) Concentration in M7609/anti-1 and M7609/anti-2 Cells. The intracellular concentrations of GST-\(\pi\) in the M7609 cells and the M7609/pLJ cells were 1.4 ± 0.2 pmol/10^6 cells and 1.3 ± 0.2 pmol/10^6 cells, respectively, with no statistically significant difference between them. On the other hand, the intracellular concentrations of GST-\(\pi\) in the M7609/anti-1 cells and the M7609/anti-2 cells were 0.8 ± 0.1 pmol/10^6 cells and 0.8 ± 0.1 pmol/10^6 cells, respectively. These levels decreased to approximately half of the GST-\(\pi\) concentrations detected in the parent M7609 cells and the M7609/pLJ cells (Table 1).

Anticancer Drug Sensitivities of M7609/anti-1 and M7609/anti-2 Cells. Both the M7609/anti-1 and the M7609/anti-2 cells showed significantly elevated sensitivity to ADR, CDDP, melphalan, and etoposide in comparison to the M7609/pLJ cells. When M7609 cells were incubated in the presence of ethacrynic acid, a known inhibitor of GST-\(\pi\), they showed similar patterns of elevated sensitivities to ADR, CDDP, melphalan, and etoposide. Neither M7609/anti-1 nor M7609/anti-2 cells showed statistically significant differences in sensitivity between each drug in the presence of ethacrynic acid.

Table 1: GST-\(\pi\) concentration in M7609 cells, M7609 transfected with GST-\(\pi\) antisense cDNA, and M7609 treated with ethacrynic acid

<table>
<thead>
<tr>
<th>Cell line</th>
<th>GST-(\pi) concentration (pmol/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M7609</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>M7609/pLJ</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>M7609 + EA</td>
<td>0.8 ± 0.1*</td>
</tr>
<tr>
<td>M7609/anti-1</td>
<td>0.8 ± 0.1*</td>
</tr>
<tr>
<td>M7609/anti-2</td>
<td>0.8 ± 0.1*</td>
</tr>
</tbody>
</table>

* Each value is the mean ± SD of three separate experiments.

Table 2: Comparison of the sensitivities of M7609, M7609/pLJ, M7609/anti-1, M7609/anti-2, and ethacrynic acid-treated M7609 cells to various anticancer drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC_{50} (\muM)</th>
<th>IC_{50} pLJ</th>
<th>IC_{50} anti-1</th>
<th>IC_{50} anti-2</th>
<th>M7609 + EA (\muM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADR</td>
<td>1.53 ± 0.02</td>
<td>1.40 ± 0.01</td>
<td>0.42 ± 0.02*</td>
<td>0.42 ± 0.02*</td>
<td>0.22 ± 0.01* (6.5)</td>
</tr>
<tr>
<td>CDDP</td>
<td>8.9 ± 0.2</td>
<td>9.9 ± 0.1</td>
<td>4.0 ± 0.2*</td>
<td>4.0 ± 0.2*</td>
<td>1.71 ± 0.02* (5.2)</td>
</tr>
<tr>
<td>Melphalan</td>
<td>16.8 ± 0.9</td>
<td>17.2 ± 0.8</td>
<td>7.8 ± 0.9*</td>
<td>7.8 ± 0.9*</td>
<td>5.8 ± 0.5* (2.9)</td>
</tr>
<tr>
<td>Etoposide</td>
<td>2.1 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>1.0 ± 0.1*</td>
<td>1.0 ± 0.1*</td>
<td>0.8 ± 0.1* (2.6)</td>
</tr>
<tr>
<td>Taxol</td>
<td>0.087 ± 0.001</td>
<td>0.063 ± 0.002</td>
<td>0.054 ± 0.002</td>
<td>0.054 ± 0.002</td>
<td>0.066 ± 0.004 (1.0)</td>
</tr>
<tr>
<td>VCR</td>
<td>4.0 ± 0.3</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.3*</td>
<td>4.0 ± 0.3*</td>
<td>3.8 ± 0.2 (1.0)</td>
</tr>
<tr>
<td>5-FU</td>
<td>73.7 ± 3.2</td>
<td>74.8 ± 4.1</td>
<td>74.2 ± 2.4</td>
<td>74.2 ± 2.4</td>
<td>74.2 ± 3.2 (1.0)</td>
</tr>
<tr>
<td>MMC</td>
<td>6.3 ± 0.2</td>
<td>6.4 ± 0.6</td>
<td>6.2 ± 0.4</td>
<td>6.2 ± 0.4</td>
<td>6.5 ± 0.3 (1.0)</td>
</tr>
</tbody>
</table>

\* IC_{50} drug concentration that inhibits cell growth by 50%. Each value is the mean of three independent experiments.

\* RR, relative resistance, IC_{50} for control cells (M7609/pLJ)/IC_{50} for transfecants or ethacrynic acid-treated M7609 cells.

\* EA, ethacrynic acid, using a nontoxic concentration of ethacrynic acid in combination with each drug.

The value was significantly lower than that of M7609/pLJ cells; \(P < 0.01\).
significant changes in their sensitivity to Taxol, VCR, 5-FU, or MMC (Table 2).

Southern and Northern Blot Analyses of M7609/ADR/anti Cells. Southern blot analysis by KpnI digestion was performed on M7609/ADR/anti, M7609/ADR/plJ, and M7609/ADR cells, using GST-\(\pi\) cDNA (0.7-kb EcoRI-EcoRI fragment) as a probe (Fig. 3A). Besides an 11-kb band representing the cellular GST-\(\pi\) gene, a 5.1-kb band was observed in the M7609/ADR/anti cells. This band was considered to represent the transfected antisense vector. In Northern blot analysis, endogenous GST-\(\pi\) mRNA was detectable as a 0.7-kb band in all three cell lines (Fig. 3B). However, the amount of endogenous mRNA in M7609/ADR/anti was apparently decreased compared to that in the M7609/ADR/plJ and M7609/ADR cells. In addition to the band at 0.7 kb, a minor band at 4.0 kb was detected in M7609/ADR/anti cells that was considered to represent the antisense mRNA. These results suggest that the expression of antisense mRNA is responsible for lowering the levels of endogenous mRNA.

Intracellular GST-\(\pi\) Concentration in M7609/ADR/anti Cells. The intracellular concentrations of GST-\(\pi\) in M7609/ADR and M7609/ADR/plJ cells were 2.5 ± 0.1 pmol/10⁶ cells and 2.3 ± 0.2 pmol/10⁶ cells, respectively. The difference between these values was not statistically significant. In contrast, the intracellular concentration of GST-\(\pi\) in antisense transfectant M7609/ADR/anti was 0.8 ± 0.1 pmol/10⁶ cells, which is approximately half of the GST-\(\pi\) concentrations detected in M7609/ADR cells and M7609/ADR/plJ cells (Table 3).

Anticancer Drug Sensitivities of M7609/ADR/anti Cells. There were no statistically significant differences in the IC₅₀ values of ADR and 5-FU for M7609/ADR and M7609/ADR/plJ. Sensitivity to ADR of M7609/ADR/anti and that of M7609/ADR incubated in the presence of ethacrynic acid was elevated by approximately 4.4- and 6.1-fold compared with that of M7609/ADR/plJ or M7609/ADR cells, respectively. Both cell types showed no elevation in their sensitivities to 5-FU (Table 3).

DISCUSSION

In this study, an antisense RNA method to selectively inhibit the expression of GST-\(\pi\) in human colon cancer cell lines was used to determine whether GST-\(\pi\) is directly involved in the drug resistance of cancer cells. Antisense methods can be broadly divided into antisense oligoDNA methods and antisense RNA methods. However, with the antisense oligoDNA methods, the resultant inhibitory effect is transient and unstable, and it is impossible to obtain permanent transfectants. In contrast, the antisense RNA method is considered to be useful in the analysis of specific gene functions (33). For these reasons, the antisense RNA method was used for the present study. Permanent transfectants were established and were studied to detect changes in their sensitivities to various anticancer agents. The degree of inhibition of gene expression by the antisense RNA method depends on many factors, including the levels of expression of the target gene, the amount of antisense RNA transcribed, the stereochemical configuration of the antisense RNA, and so on. In this study, we transfected the GST-\(\pi\) antisense cDNA to human colon cancer cell line M7609 and its ADR-resistant subline, M7609/ADR, and found that the intracellular concentration of GST-\(\pi\) in these two cell lines was reduced to approximately half. As a result of this decreased GST-\(\pi\) concentration, the sensitivities of these cell lines to ADR, CDDP, melphalan, and etoposide were increased. Accordingly, it is suggested that the increased expression of GST-\(\pi\) is not merely an incidental phenomenon occurring in parallel to the acquisition of resistance by cancer cells, but a cause of the resistance to anticancer drugs in cancer cells. On the other hand, the sensitivities to Taxol,

![Image](https://cancerres.aacrjournals.org/pars/3580.png)

Fig. 3. Southern and Northern blot analyses of M7609/ADR cells transfected with GST-\(\pi\) antisense cDNA. A, Southern blot analysis was performed as described in Fig. 2. The 5.1-kb band is from transfected GST-\(\pi\) antisense cDNA. B, Northern blot analysis was performed as described in Fig. 2. The 4.0-kb band is from transfected GST-\(\pi\) antisense cDNA. A GAPDH probe was used as an internal quantitative control for the amounts of RNA on the filter (bottom panel).

### Table 3 Comparison of the sensitivities of M7609/ADR, M7609/ADR/plJ, M7609/ADR/anti, and ethacrynic acid-treated M7609/ADR cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>GST-(\pi) concentration (pmol/10⁶ cells)</th>
<th>IC₅₀a (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M7609/ADR</td>
<td>2.5 ± 0.1</td>
<td>76.5 ± 1.2</td>
</tr>
<tr>
<td>M7609/ADR/plJ</td>
<td>2.3 ± 0.2</td>
<td>81.3 ± 3.2</td>
</tr>
<tr>
<td>M7609/ADR + EAb</td>
<td>2.5 ± 0.1</td>
<td>78.2 ± 2.1</td>
</tr>
<tr>
<td>M7609/ADR/anti</td>
<td>1.2 ± 0.1d</td>
<td>77.6 ± 5.8</td>
</tr>
</tbody>
</table>

a IC₅₀ drug concentration that inhibits cell growth by 50%. Each value is the mean of three independent experiments.

b EA, ethacrynic acid. Using nontoxic concentrations of ethacrynic acid in combination with ADR or 5-FU.

c RR, relative resistance. IC₅₀ for control cells (M7609/ADR/plJ)/IC₅₀ for transfectant or IC₅₀ for ethacrynic acid-treated M7609/ADR cells.

d The value was significantly lower than that of M7609/ADR cells; \(P < 0.01\).
VCR, MMC, and 5-FU were not changed, indicating that GST-\(\pi\) is not related to the resistance of these anticancer drugs. Previously, we found that an ADR-resistant strain (M7609/ADR) expressing high amounts of GST-\(\pi\) showed resistance not only to ADR but also to CDDP, etoposide, and melphalan, indicating that the expression of GST-\(\pi\) is related to the resistance of these anticancer drugs.\(^5\) The results of the present study are therefore in agreement with the previous reports concerning GST-\(\pi\).

To date, there have been very few reports concerning whether or not GST-\(\pi\) is involved in the intrinsic resistance of cancer cells to drugs. Using the ELISA system for GST-\(\iota\) established in our laboratory, we have demonstrated that the level of GST-\(\pi\) was elevated not only in the malignant tissues but also in the plasma of patients with digestive tract cancers, which are generally considered to be intrinsically resistant to anticancer agents (27). Thus, we suggested the possibility that the expression of GST-\(\pi\) is involved in the intrinsic drug resistance of cancer cells. Using the cultured cell line M7609 as target cells, we confirmed that GST-\(\pi\) is one of the resistance factors responsible for intrinsic resistance to anticancer drugs. It was also demonstrated that GST-\(\pi\) is directly involved in acquired resistance to anticancer agents, using the ADR-resistant M7609/ADR cells as target cells.

With regard to the mechanism of drug resistance due to GST-\(\pi\), the following four possibilities can be hypothesized based on the physiological properties of GST-\(\pi\): (a) the involvement of GST-\(\pi\) in detoxification of anticancer drugs by GSH conjugation; (b) the sequestration of anticancer drugs as a result of binding with GST-\(\pi\); (c) the chemical reduction of lipid peroxides by GST-\(\pi\); and (d) the reduction of the amounts of DNA peroxides and the repair of DNA (34). In the present research, we demonstrated that GST-\(\pi\) is a multidrug resistance factor related to ADR, CDDP, melphalan, and etoposide. Of these anticancer drugs, melphalan has been proven as a substrate for the GST-catalyzed reaction (35, 36). Bolton et al. demonstrated GST-\(\alpha\)-dependent conjugation of GSH and melphalan. However, they have not extended their study to the function of GST-\(\pi\). The only nitrogen mustard that is well characterized as a substrate for GST-\(\pi\) is chlorambucil. Ciaccio et al. (38) have shown that chlorambucil conjugation with GSH can be catalyzed by human GST-\(\pi\) with \(K_m\) of 0.002, although the catalytic efficiency is some 40- to 50-fold less than for GST-\(\alpha\). With regard to CDDP resistance, there is no evidence thus far to indicate the direct involvement of GST-\(\pi\). However, Ishikawa et al. (39) demonstrated that CDDP is first conjugated with GSH and is then expelled from the cells by an ATP-dependent GSH-conjugate export pump. Although the activity of this export pump was recently proven to be closely related to the intracellular levels of GSH and the activity of \(\gamma\)-glutamylcysteine synthetase (40), the mechanism for GSH conjugation of CDDP is still unknown. It may be of interest to investigate the possibility of conjugate formation by virtue of GST-\(\pi\) activity. As for ADR resistance, it is possible that GST-\(\pi\) confers resistance because of its ability to detoxify fatty acid peroxides that arise during lipid peroxidation caused by ADR (41, 42). In this regard, it would be a matter of great interest to determine if ADR-resistant cells are also resistant to radiation that also generates intracellular oxygen radicals. The mechanisms of etoposide resistance related to GST-\(\pi\) are a matter of conjecture and are beyond the scope of this study.

Nevertheless, it is apparent that GST-\(\pi\), like p-glycoprotein, functions as a multidrug resistance factor. Ramachandran et al. (43) studied the expression of p-glycoprotein and GST-\(\pi\) genes in five human melanoma cell lines showing resistance to ADR and reported that GST-\(\pi\) was more strongly correlated than p-glycoprotein in that resistance in cell lines showing comparatively lower (less than 10-fold) resistance to ADR. This finding is in agreement with our results, obtained from the characterization of M7609/ADR cells and the present experiment of GST-\(\pi\) antisense transfection. That is, although the activity of GST-\(\pi\) as a resistance factor may not be as effective as that of p-glycoprotein, it is surmised that GST-\(\pi\) is primarily involved in intrinsic drug resistance and in the early stage of acquired resistance. Accordingly, we speculate that, in clinical terms, GST-\(\pi\) may be a more important resistance factor than p-glycoprotein in the early stage of chemotherapy of cancer.

**ACKNOWLEDGMENTS**

We thank Dr. I. Listowsky for helpful discussion and Dr. L. W. Stiver for the correction of English in the manuscript.

**REFERENCES**

Transfection of Glutathione S-Transferase (GST)-π Antisense Complementary DNA Increases the Sensitivity of a Colon Cancer Cell Line to Adriamycin, Cisplatin, Melphalan, and Etoposide

Noriyoshi Ban, Yasuo Takahashi, Tetsuji Takayama, et al.

*Cancer Res* 1996;56:3577-3582.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/15/3577

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/56/15/3577.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.