Expression Pattern of Chemoresistance-related Genes in Human Malignant Brain Tumors: a Working Knowledge for Proper Selection of Anticancer Drugs

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Background: In addition to traditional modalities such as surgical intervention and radiotherapy, chemotherapy is a common therapeutic method for human malignant brain tumors. However, the effectiveness of chemotherapy is frequently hampered by cancer cell chemoresistance, resulting in an unsatisfactory outcome. To overcome this disadvantage, the proper selection of efficacious anticancer agents is required.

Methods: The expression levels of chemoresistance-related genes, MGMT, mdr1, MRP, MTIIA and GST-π, in 28 surgical specimens of human brain tumors and in 10 human glioma cell lines were examined by Northern blot analysis. In addition, the SD10 values of human glioma cell lines against ACNU, CDDP, ADM and VP16 were estimated by a cell survival assay.

Results: The expression levels of each of the chemoresistance-related genes, except MRP, were generally higher in brain tumors than those in non-neoplastic brain tissues. MGMT expression correlated exclusively with ACNU resistance in all glioma cell lines examined (p = 0.0002). The transcriptional level of mdr1 in the tumor cells correlated with the SD10 values of VCR (p = 0.04) and ADM (p = 0.034). In contrast, the expression levels of MTIIA and GST-π did not correlate with resistance to any of the drugs tested. A correlation of MRP mRNA expression with multidrug resistance was not apparent in the 10 cell lines tested.

Conclusions: The data indicate that knowledge of the expression levels of MGMT and mdr1 may be particularly useful for a more rational selection of drugs which are not influenced by these resistance genes and which have improved efficacy against human brain tumors.

Key words: chemoresistance – brain tumors – MGMT – mdr1

INTRODUCTION

The persistent invasiveness of malignant gliomas into the surrounding normal brain parenchyma including eloquent regions (1) renders surgical intervention and/or radiotherapy insufficient for curative therapy. In addition, the brain is also known as an immunologically privileged organ, inaccessible to the immune surveillance system. Little progress has been made in improving the survival rate in recent decades and the 5-year survival rate still remains below 10% (2). Therefore, the treatment of malignant gliomas remains unsatisfactory.

These specific features of malignant brain tumors lead to the notion that chemotherapy for the malignant tumors should play a key role in achieving complete remission or in preventing early recurrence. A variety of chemotherapy protocols have been tested, yet these regimens have not contributed to improve prognosis for the patients. The ineffectiveness of brain tumor chemotherapy could result in part from the low efficiency of the penetration of a drug through the blood–brain barrier (BBB), resulting in a reduced, non-cytotoxic local drug concentration (3). Another potential reason for chemotherapeutic ineffectiveness could lie in the tumor cell chemoresistance, either intrinsic or acquired, and further in the
effective and optimal drugs for therapy of the patients with intertumoral variation of chemosensitivity to anticancer agents.

Recently, various mechanisms of chemoresistance have been elucidated at the molecular level (4–11). Several molecules may be responsible, at least in part, for the resistance to chemotherapeutic agents which are widely used for treatment of malignant gliomas, such as cis-diaminedichloroplatinum(II) (CDDP), vincristine (VCR), etoposide (VP16) and cis-diaminedichloroplatinum(II) (CDDP). The candidate molecules for the chemoresistance to these drugs include 02-methylguanine-DNA methyltransferase (MGMT) (12), p-glycoprotein 10, multidrug-resistance-associated protein (MRP) (5), thiol-rich detoxification enzymes [metallothioneins (MTs)] (13) and glutathione S-transferase (GST)-π (14). MGMT is a key DNA repair enzyme which removes the toxic alkyl group adducts at the O6-position of guanine in DNA which are induced by chloroethylnitrosoureas (CENU) such as ACNU, thereby conferring resistance to the cytotoxic effect of these drugs (12,15). p-Glycoprotein, the product of the mdrl gene, confers multidrug resistance (MDR) in mammalian cells where it acts as a transmembrane transporter resulting in the efflux of intracellular substrates, especially natural toxins such as VCR, VP16 and anthracyclines (10,16,17). It has also been postulated that p-glycoprotein is expressed in capillary endothelial cells of the BBB, preventing the influx of cytotoxic drugs into the brain parenchyma (18,19). MRP, which has been isolated from an adriamycin (ADM)-resistant small-cell lung carcinoma cell line (H69AR), is a possible alternative candidate leading to MDR (5). MRP is localized predominantly in the plasma membrane where it mediates transport of the natural toxins as well as negatively charged compounds that are not classical multidrug resistance drugs (20,21). Some glioma cells expressing MRP mRNA display an MDR phenotype in the absence of mdrl gene expression (22). Overexpression of MTs, especially the MTIIA isoform, has been observed in CDDP-resistant tumor cells (6,23,24). However, the contribution of MTs to CDDP resistance is still controversial (25) because these MTs can be induced by various external stimuli such as stresses and metal ions (26) and glucocorticoids (13) which are generally used for malignant glioma patients. In human cell lines which are resistant to CDDP, elevated GST activity has frequently been observed (27). Increased GST-π mRNA, encoding an anionic isozyme of the human GST family, has also been detected in many malignant tumor cells including glioma cells (14,28). DNA-topoisomerase II is an enzyme which potentially regulates the chemoresistance of cells to drugs such as VP16 (9) and kinesin (29).

These independent observations suggest that a comprehensive analysis of the expression levels of the chemoresistance-related genes in glioma cells may be useful in predicting the extent of cellular chemoresistance and cross-resistance to chemotherapeutic agents and might allow the selection of more effective and optional drugs for therapy of the patients with brain tumors. In the present study, we investigated the expression levels of the five chemoresistance-related genes, MGMT, mdrl, MRP, MTIIA and GST-π, in a variety of human brain tumor tissues and human glioma cell lines. In addition, we estimated the SD10 values of chemotherapeutic agents in glioma cells to determine if the expression levels of the chemoresistance-related genes correlated with resistance to the anticancer drugs used to treat human brain tumors.

MATERIALS AND METHODS

DRUGS

The anticancer agents ACNU, CDDP, VCR, ADM and VP16, were supplied by Sankyo Drug Co., Tokyo, Nihon Kayaku KK, Tokyo, Eli Lilly Japan KK, Kobe, Kyowa Hakko Kogyo Co., Tokyo and Bristol-Myers Squibb KK, Tokyo, respectively. Each drug used for the cell survival assay, except VP16, was solubilized in distilled water and stored at −80°C until use. All drug solutions were diluted with an appropriate buffer just before each experiment.

BRAIN TUMOR SPECIMENS

Specimens of 28 human primary and metastatic brain tumors and three non-neoplastic brain tissues (Brain 1–3) from cortexectomy performed during surgical treatment of deep-seated large malignant gliomas were obtained and immediately frozen in liquid nitrogen. All tissues were stored in sterile containers at −80°C until use. The tumors were identified histologically as follows: 28 tissue specimens included four glioblastoma multiformes (Gbl), four grade III astrocytomas (AAs), one grade II astrocytoma (Ast), two oligodendrogliomas (Olg), two gangliogliomas (Ggl), one ependymoma (Epd), three medulloblastomas (Mdl), one neuroblastoma (Nb), five benign brain tumors including three meningiomas (Mng) and two neuroinomas (Nrn) and five brain metastases consisting of four from lung cancers (Lc) and one from colorectal cancer (Col).

CELLS AND CULTURE

Seven human glioma cell lines, U-87MG, SF-126, U-138MG, SF-188, U-251MG, U-343MG and U-373MG, were gifts from Dr M. Rosenblum (30,31). Two human glioma cell lines, NGB11V and NGB21, were established from an individual tumor of glioblastoma multiforme resected at the National Cancer Center Hospital, Tokyo. U-251AR is an ACNU-resistant cell line established from the U-251MG by daily exposure to 40 μM ACNU as described previously (32). These cells were cultivated in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 300 μg/ml of glutamine, 0.14% sodium hydroygen carbonate and 60 μg/ml of streptomycin in a humidified atmosphere of 5% CO2 at 37°C.
PROBES FOR DETECTION OF CHEMORESISTANCE-RELATED GENE mRNAs

A fragment of 266 bp from MGMT cDNA (32), an EcoRI-PstI fragment of 2.2 kbp from pGem4 carrying the human mdr1 cDNA (a gift from K. Ueda, Kyoto University) and an Rsal fragment of 405 bp from pGP2 containing the human GST-π cDNA (a gift from M. Muramatsu, Saitama University) were used as the MGMT, mdr1 and GST-π probe for Northern blot analysis, respectively. A BamHI fragment of 400 bp and a BamHI/PvuII fragment of 272 bp from exon 2 and 3 of the MTIIA gene cloned in hMT-II (American Type Culture Collection, Rockville, MD), respectively, were used as MTII probes. The MRP probe was prepared by the reverse transcription (RT)-polymerase chain reaction (PCR) amplification method as described previously (32). Briefly, based on the nucleotide sequence of MRP cDNA reported by Cole et al. (5), the 552 bp MRP cDNA fragment corresponding to nucleotides 2552-3104 was synthesized by RT-PCR using SF-188 mRNA as a template and the following primers, 5'-GCTGACATTTACCTCCTTCAGTGTCAGTA-3' (sense)/5'-TGGCCTTCTAGTATCC-CAGTA-3' (antisense). The PCR product was subcloned into the SalI site of phagemid pTZ19R for amplification in Escherichia coli JM-109, verified by DNA sequencing and digested with EcoRI and BamHI. The digested fragment was used as an MRP cDNA probe for Northern blot analysis. The rat β-actin cDNA of 2.0 kbp was used as a control probe to standardize the mRNA samples loaded. All probes were labeled with [α-32P]dCTP (specific activity 2000 Ci/mmol) by random priming (Oligolabeling kit, Pharmacia Japan, Tokyo).

NORTHERN BLOT ANALYSIS

Total cellular RNAs from tumor tissues and exponentially growing tumor cells were independently prepared as described previously (32). A 2 μg amount of poly(A)⁺ RNA was fractionated on a 1% agarose-formaldehyde gel and transferred to a nylon filter. Sequential hybridizations using the same filter were performed with 32P-labeled human MGMT, mdr1, MRP, GST-π and MTIIA probes prepared as described in Materials and Methods. The expression level of β-actin was shown as an internal control for RNA loaded.

RESULTS

CELL SURVIVAL ASSAY

The survival assay was performed as described previously using a colony-formation assay (33). Briefly, 250 or 500 cells were seeded into 60 mm dishes and cultured for 24 h. The cells were treated for 1 h with various concentrations of each drug indicated, followed by replacement with fresh, drug-free medium and incubated for an additional 7–14 days. The cells were then fixed and colonies consisting of 50 or more cells were scored. The surviving fraction was calculated as the ratio of the colony-forming efficiency of the cells with or without drug treatment. Drug resistance was measured by the SD10 value (μM), the dose resulting in a 10% survival.

Figure 1. Expression of chemoresistance-related gene transcripts in (a) 28 surgical specimens of human brain tumors and (b) 10 human glioma cell lines. A 2 μg amount of poly(A)⁺ RNA was fractionated on a 1% agarose-formaldehyde gel and transferred to a nylon filter. Sequential hybridizations using the same filter were performed with 32P-labeled human MGMT, mdr1, MRP, GST-π and MTIIA probes prepared as described in Materials and Methods. The expression level of β-actin was shown as an internal control for RNA loaded.

EXPRESSION OF CHEMoresistance-RELATED Genes IN HUMAN Brain Tumors

To predict the chemoresistance of brain tumors, we examined the expression levels of the five chemoresistance-related genes
**Chemoresistance of human brain tumors**

**a**

- **MGMT**
  - ACNU Resistance $S_{D0} (\mu M)$ vs. MGMT mRNA (%U-87MG)
  - $y = 14.137x^{0.844} r = 0.914$
  - ***$p = 0.0002$***

- **GST-π**
  - SD$_{D0}$ (\mu M) vs. GST-π mRNA (%U-87MG)
  - $y = 32.316x^{0.759} r = 0.213$
  - $p = 0.555$

**b**

- **MTIIA**
  - CDDP Resistance $S_{D10} (\mu M)$ vs. MTIIA mRNA (%U-87MG)
  - $y = 7.156x^{1.304} r = 0.123$
  - $p = 0.734$

- **GST-π**
  - SD$_{D10}$ (\mu M) vs. GST-π mRNA (%U-87MG)
  - $y = 0.640x^{0.840} r = 0.172$
  - $p = 0.635$

- **mdr1**
  - SD$_{D10}$ (\mu M) vs. mdr1 mRNA (%U-87MG)
  - $y = 6.774x^{0.256} r = 0.524$
  - $p = 0.120$

**c**

- **VCR**
  - SD$_{D10}$ (\mu M) vs. mdr1 mRNA (%U-87MG)
  - $y = 0.046x^{0.425} r = 0.654$
  - *$p = 0.04$*

- **ADM**
  - SD$_{D10}$ (\mu M) vs. mdr1 mRNA (%U-87MG)
  - $y = 0.148x^{0.216} r = 0.670$
  - *$p = 0.034$*

- **VP-16**
  - SD$_{D10}$ (\mu M) vs. mdr1 mRNA (%U-87MG)
  - $y = 5.262x^{0.167} r = 0.174$
  - $p = 0.635$

**d**

- **VCR**
  - SD$_{D10}$ (\mu M) vs. MRP mRNA (%U-87MG)
  - $y = 0.045x^{0.364} r = 0.216$
  - $p = 0.549$

- **ADM**
  - SD$_{D10}$ (\mu M) vs. MRP mRNA (%U-87MG)
  - $y = 0.127x^{0.477} r = 0.470$
  - $p = 0.170$

- **VP-16**
  - SD$_{D10}$ (\mu M) vs. MRP mRNA (%U-87MG)
  - $y = 3.856x^{0.587} r = 0.403$
  - $p = 0.148$
by Northern blot analysis in 28 surgical specimens of human brain tumors with various histopathological characteristics. As shown in Fig. 1, the expression levels of MGMT, MTIIA, mdr1 and GST-π were generally higher in tumor tissues than non-neoplastic brain tissues. For instance, high MGMT expression was observed in ependymoma (EpD2), neuroblastoma (NbL2), neurinomas (Nrn4, 5), metastasis from lung (Lc1, 8) and colorectal cancers (Col1) and glioblastomas (Gbl1, 8), although low levels of MGMT expression were detected in oligodendrogliomas and most of the astrocytomas. A similar restricted expression of mdr1 was observed in human brain tumors. Substantial expression of mdr1 was observed in gangliogliomas, colorectal cancer metastasis and meningioma (Mng10), but not in lung cancer metastases, oligodendrogliomas or glioblastomas. MTIIA was expressed at significant levels in glioblastomas, an ependymoma, astrocytomas, gangliogliomas and neurinomas, but MTIIA expression was scarce in meningiomas, oligodendrogliomas, lung and colorectal cancer metastases and in two of three medulloblastomas (Md1, 3). Interestingly, MRP expression was undetectable in non-neoplastic brain tissues and all tumor specimens except one glioblastoma (Gbl1). In contrast, the significant expression of GST-π was detected in most of the brain tumors examined.

**Expression of Chemoresistance-Related Genes in Human Glioma Cell Lines**

To confirm previous data indicating that the ACNU resistance of glioma cells correlates with the expression level of MGMT mRNA in tumor cells (32) and to test whether this concept could provide foreknowledge of the chemoresistance of human brain tumors to anticancer drugs other than ACNU, we first analyzed the expression levels of the five chemoresistance-related genes in 10 human glioma cell lines. As shown in Fig. 1b, although the MGMT expression was restricted, a high level of MGMT expression was detected in SF-188 and also in U-251AR, an ACNU-resistant subclone of U-251MG. A similar restricted expression of mdr1 was observed in human glioma cells. mdr1 expression was detectable in SF-126 and NGB11V cell lines, but was undetectable in the other eight cell lines. Interestingly, eight of 10 glioma cell lines examined expressed significant levels of MRP mRNA, although it is not expressed in most human brain tumors, as shown in Fig. 1a. This finding suggests that transcription of MRP may be modulated and enhanced during establishment of the glioma cell lines. On the other hand, as found in human brain tumors, high levels of MTIIA and GST-π mRNA were detected in all glioma cell lines except U-373MG. Surprisingly, U-373MG cells had no detectable expression of all chemoresistance-related genes examined. The intensities of the bands corresponding to the chemoresistance-related gene transcripts from tumor samples were quantified and the expression level of each mRNA was calculated as a ratio of chemoresistance gene to β-actin mRNA.

**Cellular Resistance to Chemotherapeutic Agents in Human Glioma Cell Lines**

We also examined the chemoresistance of human glioma cell lines to various chemotherapeutic agents, such as CDDP, VCR, ADM and VP16 by colony formation assays. The resistance level of each cell line to anticancer drugs is expressed as the SD10 value which is calculated as the concentration of drug required to reduce the initial population to 10% (Table 1). The results showed that the U-87MG cell line was the most sensitive cell line to all drugs examined except ACNU. The relative resistances of the glioma cell lines to each drug relative to that of U-87MG were calculated and are presented in Table 1.

**Correlation Analysis**

A correlation between the expression levels of the chemoresistance-related genes and the resistance to anticancer agents in human glioma cell lines was evaluated using Pearson’s correlation analysis method. Consistent with previous reports (32,34), the expression level of MGMT mRNA significantly and exclusively correlated with the ACNU resistance in glioma cell lines (p = 0.0002) (Fig. 2a). In contrast, the expression levels of GST-π had no association with the ACNU resistance. Regarding the CDDP resistance, we did not observe any apparent correlation with the expression level of these genes including MTIIA and GST-π, although CDDP resistance appeared to correlate weakly with mdr1 gene expression (p = 0.12) (Fig. 2b). As shown in Table 1, U-373MG cells had a 3.4-fold higher resistance to CDDP than U-87MG cells. However, the expression level of MTIIA mRNA, encoding an isoform of MTs which may play a role in cellular CDDP resistance, was significantly higher in U-87MG cells than in U-373MG cells. Thus, it is unclear if the MTIIA gene is involved in CDDP resistance. On the other hand, the expression level of mdr1 significantly correlated with the SD10 values of VCR (p = 0.04) and ADM (p = 0.034) in human glioma cell lines tested (Fig. 2c), supporting the previous conclusion that mdr1 gene expression confers multidrug resistance in human tumors (35). A correlation between MRP mRNA levels and multidrug resistance was not apparent in the 10 cell lines tested (Fig. 2d).

**Figure 2.** Correlation analysis between the expression levels of the chemoresistance-related genes and the resistance to anticancer agents in human glioma cell lines. The extent of the drug resistance was determined by colony-forming efficiency assay as described in Materials and Methods. (a) Correlation between the ACNU resistance and the expression levels of MGMT mRNA and GST-π mRNA. (b) Correlation between the CDDP resistance and the expression levels of MTIIA, GST-π and mdr1 mRNA. (c) Correlation between the expression level of mdr1 mRNA and the resistance to VCR, ADM and VP16. (d) Correlation between the expression level of MRP mRNA and the resistance to VCR, ADM and VP16.
Chemoresistance of human brain tumors

Table 1. Chemoresistance to chemotherapeutic agents in human glioma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>SD\textsubscript{10} (µM) value to drugs</th>
<th>ACNU</th>
<th>CDDP</th>
<th>VCR</th>
<th>ADM</th>
<th>VP16</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-87MG</td>
<td>22.41 (1.0)</td>
<td>3.40 (1.0)</td>
<td>0.020 (1.0)</td>
<td>0.069 (1.0)</td>
<td>1.81 (1.0)</td>
<td></td>
</tr>
<tr>
<td>SF-126</td>
<td>11.33 (0.5)</td>
<td>23.24 (6.8)</td>
<td>0.404 (19.9)</td>
<td>0.228 (3.3)</td>
<td>5.33 (2.9)</td>
<td></td>
</tr>
<tr>
<td>U-138MG</td>
<td>75.46 (3.4)</td>
<td>9.20 (2.7)</td>
<td>0.121 (5.9)</td>
<td>0.451 (6.5)</td>
<td>15.92 (8.8)</td>
<td></td>
</tr>
<tr>
<td>SF-188</td>
<td>92.23 (4.1)</td>
<td>2.63 (0.8)</td>
<td>0.031 (1.5)</td>
<td>0.165 (2.4)</td>
<td>5.19 (2.9)</td>
<td></td>
</tr>
<tr>
<td>U-251MG</td>
<td>13.85 (0.6)</td>
<td>6.81 (2.0)</td>
<td>0.018 (0.9)</td>
<td>0.118 (1.7)</td>
<td>11.47 (6.3)</td>
<td></td>
</tr>
<tr>
<td>U-251AR</td>
<td>251.72 (11.2)</td>
<td>9.22 (2.7)</td>
<td>0.051 (2.5)</td>
<td>0.131 (1.9)</td>
<td>7.31 (4.0)</td>
<td></td>
</tr>
<tr>
<td>U-343MG</td>
<td>6.58 (0.3)</td>
<td>10.80 (3.2)</td>
<td>0.044 (2.2)</td>
<td>0.166 (2.4)</td>
<td>4.01 (2.2)</td>
<td></td>
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<tr>
<td>U-373MG</td>
<td>11.61 (0.5)</td>
<td>11.59 (3.4)</td>
<td>0.046 (2.3)</td>
<td>0.126 (1.8)</td>
<td>1.93 (1.1)</td>
<td></td>
</tr>
<tr>
<td>NGB11V</td>
<td>10.30 (0.5)</td>
<td>13.07 (3.8)</td>
<td>0.123 (6.1)</td>
<td>0.391 (5.6)</td>
<td>11.36 (6.3)</td>
<td></td>
</tr>
<tr>
<td>NGB21</td>
<td>14.89 (0.7)</td>
<td>2.96 (0.9)</td>
<td>0.035 (1.7)</td>
<td>0.104 (1.5)</td>
<td>3.97 (2.2)</td>
<td></td>
</tr>
</tbody>
</table>

Drug sensitivity of 10 human glioma cell lines to various chemotherapeutic agents was determined by colony-forming efficiency assays. SD\textsubscript{10} dose of drug required to reduce the initial surviving population to 10%. Numbers in parentheses indicate relative resistance index values which are determined by dividing the SD\textsubscript{10} of each cell line by that of the U-87MG cell line.

DISCUSSION

We have measured the expression levels of five chemoresistance-related genes in human brain tumors and human glioma cell lines and the SD\textsubscript{10} values of glioma cell lines treated with chemotherapeutic agents such as ACNU, VCR, ADM, CDDP, and VP16.

A clear correlation between the resistance of human brain tumors to ACNU and the level of MGMT gene expression was observed here, similar to our previous study (32). The SD\textsubscript{10} value of ACNU in each glioma cell line correlated well with the expression level of MGMT mRNA in the corresponding cell line (p = 0.0002). However, the ACNU resistance of glioma cells did not correlate with the expression levels of other chemoresistance-related genes such as GST-π (p = 0.04). These findings strongly suggest that MGMT plays a central role in the acquisition of the ACNU resistance of glioma cells. Northern blot analysis demonstrated that the expression level of MGMT mRNA was elevated in some astrocytic tumors, consistent with a recent report (36), whereas oligodendrogliomas had apparently lower MGMT expression than other brain tumors including metastatic brain tumors, supporting the current use of chloroethylnitrosoureas as a chief drug in the treatment of oligodendrogliomas (37).

The level of mdr1 mRNA correlated well with resistance of human glioma cells to natural toxins such as VCR (p = 0.04) and ADM (p = 0.034). In addition, expression of mdr1 was weakly associated with the CDDP resistance of glioma cells (p = 0.121). However, the resistance of glioma cells to VP16, a substrate of p-glycoprotein, did not correlate with the expression level of mdr1 mRNA. These results suggest that other mechanisms, such as topoisomerase II-regulated atypical MDR, may be more significant in regulating VP16 resistance of human brain tumors (9).

The human glioma cell lines used in this study expressed low or undetectable levels of mdr1 mRNA, whereas non-neoplastic brain tissues and most of the brain tumors tested, especially malignant gliomas and meningiomas, expressed significant amounts of mdr1 mRNA. These results are in agreement with recent studies which have demonstrated that p-glycoprotein is expressed in normal capillaries in the brain and also in neovascularized capillaries in most malignant gliomas (38,39). Since preservation of an intact BBB within or adjacent to the tumor is one of the essential determining factors for the effectiveness of drug treatment and expression of p-glycoprotein in endothelial cells is involved in the function of BBB and confers MDR in tumors cells, these results suggest that the analysis of the expression level of mdr1 may be a practical method for the estimation of cellular resistance to VCR and ADM. These drugs could be valuable for the chemotherapy of human brain tumors like metastatic tumors from lung cancers expressing low levels of mdr1 mRNA.

MRP mRNA encoding the multidrug-resistance-associated protein was expressed in most of the glioma cell lines tested, although the expression levels did not correlate significantly with resistance to any of the drugs examined. This result suggests that MRP may not be as potent as p-glycoprotein in mediating drug resistance in glioma cells, although it has been implicated in an MDR phenotype working as a drug efflux pump. Interestingly, in contrast to significant levels of MRP mRNA in glioma cell lines, MRP expression in all surgical specimens of human brain tumors, except Gb11, was undetectable. This converse observation might involve amplification-induced activation of MRP gene expression, during the establishment of a cell line following gene amplification (5,40) or may involve alteration of the promoter activity of the gene. Little expression of MRP mRNA in brain tumors suggests that MRP may not be expressed in capillary endothelial cells of the brain and in contrast to p-glycoprotein, MRP may not play a
significant role in construction of the BBB, although MRP has been shown to be expressed in murine and bovine brain capillary endothelial cells (41,42). Thus, to understand the role of this gene product on chemoresistance of brain tumors, the tissue localization of the MRP protein could be determined by immunohistochemical analysis.

It has been shown that glutathione and its catalyzing enzyme GST, especially the π isozyme, play important roles in acquisition of CDDP resistance through decreased intracellular accumulation of the drugs, enhancement of detoxification of the drugs and/or stimulation of repair of the DNA damage induced by the drugs (14,27,43). However, we did not find any correlation between the expression level of GST-π mRNA and the extent of the CDDP resistance in human glioma cell lines (p = 0.635). Our data also showed that in both human brain tumors and glioma cell lines, GST-π mRNA expression was extremely high, in contrast to that in normal brain tissues. These findings, therefore, suggest that the expression level of GST-π mRNA may not be useful for the estimation of cellular resistance to drugs such as CDDP, but may be useful as a tumor marker for diagnosis of brain tumors, as reported by Hida et al. (44).

Thiol-rich metallothioneins and the isoform MTIIA are also factors mediating CDDP resistance (6,13,23,24). Although the expression level of MTIIA mRNA did not correlate with CDDP resistance in human glioma cells, its gene expression was associated well with the extent of the CDDP resistance in resected tumor tissues. For instance, an MTIIA mRNA-negative recurrent glioblastoma (Gbl8) responded significantly to post-operative chemotherapy using CDDP as a chief drug (46); in contrast, a patient with medulloblastoma (Mdl2) having high MTIIA expression had repeated recurrence following CDDP-based chemotherapy; a recurrent ependymoma (Epd2) with an increased level of MTIIA mRNA regrew immediately despite post-operative CDDP treatment. The discrepancy between the data for the statistical analysis performed in this study and the clinical relevance may be that the expression level of MTIIA mRNA does not always reflect the intracellular amount of the MTIIA protein and MTIIA activity. It is also possible that other molecular mechanisms which confer CDDP resistance may be involved. These mechanisms include reduction of intracellular accumulation of the drugs, elevation of DNA repair activity (47,48), increase in Bcl-2 expression that suppresses the killing effect of the drugs (49) and induction of isoforms of MTs by various external stimuli.

In summary, five chemoresistance-related genes were expressed independently in human glioma cell lines and human brain tumor specimens. Among these genes, the expression level of MGMT and mdr1 correlated well with the strength of the cellular resistance to chemotherapeutic agents, ACNU and VCR and ADM, respectively. We recently examined the expression levels of MGMT, mdr1, GST-π and MT in a patient with recurrent glioblastoma multiforme and succeeded in regression of the tumor by selecting the most suitable anticancer agents (45). The present study and this success establish the clinical utility of investigating the expression level of chemoresistance-related genes in human brain tumors for the determination of optimal anticancer agents for patients. Furthermore, by combining approaches to downregulate the expression of these genes by specific substrates (50,51), antibodies (52) and/or antisense nucleotides (53), it may be possible to overcome drug resistance and to improve the efficacy of chemotherapy for brain tumors.

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