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INTRODUCTION

A major limitation to successful treatment of cancer is the existence of intrinsic or acquired mechanisms of drug resistance in tumor cells. A wide range of cellular mechanisms may lead to drug resistance, including increased drug accumulation; altered drug metabolism; and altered drug target (1). Well characterized mechanisms of drug resistance include multidrug resistance mediated by P-glycoprotein (reviewed in Refs. 2 and 3) and resistance to DNA intercalating agents and epipodophyllotoxins resulting from altered DNA topoisomerase II activity (4–7). Another clinically important form of drug resistance which is not mediated by these mechanisms is the resistance to alkylating agents. Alkylating agents have a wide spectrum of antitumor activity and are among the most commonly used anticancer agents. Gene transfer of a bacterial repair enzyme, O6-alkylguanine-DNA alkyltransferase, has been shown to increase the resistance of mammalian cells to chloroethylation agents such as N,N′-bis(2-chloroethyl)-N-nitrosourea and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (8–11). The human enzyme has also been shown to prevent the appearance of thymic lymphoma in transgenic mice treated with N-methyl-N-nitrosourea (12). While O6-alkylguanine-DNA alkyltransferase might be implicated in tumor resistance to chloroethylation agents (13), it has not been implicated in the resistance to other alkylating agents (14).

Another important mechanism of alkylator resistance involves the thiol glutathione and the enzymes which catalyze its conjugation to potentially toxic molecules, particularly the GSTs (3). Cytosolic GSTs in mammals have been divided according to their isoelectric points into four gene families termed α-, μ-, θ-, and θ-encoding subunits that form homodimers or heterodimers within the same family. Although a considerable number of isoenzymes have been described in rats, mice, and humans, the same multigene families are seen and there is significant conservation in primary structure across the three species (15). There are a number of nonhuman (16–20) and human (21, 22) experimental models of drug resistance to alkylating agents in which specific GST forms are overexpressed. Most of the experimental models implicate an α class form of GST, particularly the Yc homodimers in rat (GST 2-2 in the most recent nomenclature), which has the highest organic peroxidase activity (23, 24). GST inhibitors have also been shown to overcome the resistance of certain cell lines to alkylating agents (25, 26). Several transfection experiments have provided direct evidence for the ability of GSTs to confer modest levels of resistance to alkylating agents in yeast and mammalian cells (27–31), while other experiments have failed to confirm consistent drug resistance (32–34).

Thus, the evidence for a role of some specific forms of GST in resistance to alkylating drugs is now very strong. However, the issue that clonal variability might potentially confound the results of conventional transfection experiments has been raised (35). To circumvent this difficulty and resolve the important issue of the role of GST in drug resistance, we have studied rat GST-Yc expression and sensitivity to alkylating drugs in populations of mouse NIH 3T3 fibroblasts following either transfection or transduction with an N2-based retroviral vector. In comparison with cells treated with an antisense vector, Yc-transfected and Yc-transduced populations of NIH 3T3 cells expressed increased levels of GST-Yc mRNA (Northern blot), increased levels of immunodetectable GST-Yc (Western blot), and, respectively, 1.4- and 1.9-fold increases in total GST activity and 6.1- and 8.3-fold increases in glutathione peroxidase activity (associated with the Yc subunit). Yc-transfected and Yc-transduced cell populations were, respectively, 5.8–(P < 0.001) and 2.4-fold (P < 0.05) resistant to chlorambucil and 10.8–(P < 0.01) and 5.4-fold (P < 0.001) resistant to mechlorethamine. The range of resistance of clonal isolates from either population was 1.8–6.0-fold for chlorambucil and 4.6–6.1-fold for mechlorethamine (P < 0.05). In contrast, these cells showed unaltered sensitivity to the antimetabolite methotrexate, a nonalkylating drug. These results clearly demonstrate that the rat GTS-Yc is able to confer alkylating drug resistance in mouse fibroblasts. The ability to confer alkylating drug resistance following retrovirus-mediated gene transfer also increases the possibility of using GST-Yc somatic gene transfer to confer protection to the hematopoietic system in a gene therapy strategy applicable to cancer.

Received 1/18/94; accepted 6/16/94.

The abbreviations used are: GST, glutathione S-transferase; IC50, 50% inhibitory concentration; cDNA, complementary DNA; BSA, bovine serum albumin; PBS, phosphate-buffered saline; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
MATERIALS AND METHODS

Cell Lines. Retrovirus-packaging cell lines GP+E-86 and GP+envAm-12 were obtained from Dr. Arthur Bank (Columbia University) (36, 37). These cell lines are derived from mouse NIH 3T3 fibroblasts and produce ecotropic and amphotropic retrovirus envelopes, respectively. Both cell lines were maintained in Dulbecco’s modified Eagle’s medium (GIBCO, Burlington, Ontario, Canada) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (GIBCO).

Construction of Plasmids pN2Yc and pN2revYc. The full-length cDNA of rat GST-Yc was isolated from pPSMYc (obtained from William E. Fahl, McArthur Laboratory for Cancer Research, University of Wisconsin, Madison, WI) (38) by BamHI and XhoI digestion, agarose gel electrophoresis separation, and purification. The human adenosine deaminase cDNA was then removed from the Moloney murine leukemia virus-based vector pN2satADA (39, 40) by BamHI digestion and replaced by the 734-base pair GST-Yc cDNA in forward or reverse orientation by blunt end ligation to produce pN2Yc and pN2revYc, respectively.

Generation of Virus-producing Cell Lines. The plasmids pN2Yc or pN2revYc were cotransfected in a 10:1 molar ratio with pSV2neo (41) into GP+E-86 ecotropic packaging cells by calcium phosphate precipitation. Stable transfectants were then selected with G418 (Genetetic, GIBCO), a neomycin analogue, at 300 mg/ml (active drug), starting 48 h posttransfection. This concentration of drug was shown to completely inhibit the growth of nontransfected GP+E-86 cells (data not shown). Populations of amphotropic virus-producing cells were in turn obtained by cocultivating the transfected populations, GP+E-86/Yc or GP+E-86/revYc, with GP+envAm-12 in a 1:1 ratio for 17-21 days, similar to a previously described strategy (42). Amphotropic virus-producing cells were then selected with hygromycin (200 mg/ml). This concentration of drug had been found to completely inhibit the growth of GP+E-86 cells, but not GP+envAm-12, which were transfected with an hygromycin resistance gene in their construction (37). Single cell-derived clones of GP+E-86/Yc and GP+envAm-12/Yc were obtained by limiting cell dilutions in 24-well plates.

Determination of Virus Titters. Virus titers were determined by RNA slot blot analysis as described (43). Briefly, cell culture medium was conditioned for 16 h by subconfluent virus-producing cells; made up to 0.5% sodium dodecyl sulfate, 5 mM EDTA, 100 μg/ml of RNA, and 500 μg/ml proteinase K; incubated for 45 min at 37°C; and extracted with phenol, phenol/chloroform, and chloroform. The ethanol precipitate of the aqueous phase was resuspended in 1 μl H2O and 1 μl of the DNA solution was used for hybridization.

RNAase Protection of Vector-specific Transcripts. The correct structure and integrity of the vector sequence integrated in populations of virus-packaging cells were verified by Southern blot analysis following digestion of genomic DNA with restriction enzymes which cut twice in the N2Yc and N2revYc sequence but outside the GST-Yc cDNA. Fragments were separated by agarose gel electrophoresis and transferred to Zeta Probe membrane (Bio-Rad, Mississauga, Ontario, Canada) and used as probe for Southern and Northern blots. Hybridization was at 42°C with 50% formamide; final washes of blots were with 0.2× SSC-0.1% SDS at 37°C. Membranes were then hybridized with a 32P-labeled GST-Yc cDNA insert of pPSMYc and then washed with 0.2× SSC-0.1% SDS at 37°C. Membranes were then hybridized with a 32P-labeled GST-Yc cDNA insert of pPSMYc.

RESULTS

Integrity and Copy Number of the GST-Yc Vectors. The integrity of the vector sequence integrated in populations of virus-packaging cells was verified by Southern blot analysis following digestion of genomic DNA with restriction enzymes which cut twice in the N2Yc and N2revYc sequence but outside the GST-Yc cDNA. Blots were then hybridized with a 32P-labeled GST-Yc cDNA insert of pPSMYc. This resulted in a labeled fragment of predictable size independently of the integration site(s), in contrast to single “cutters,” which would result in a multiplicity of fragment sizes corresponding to the diversity of integration sites present in transfected or transduced cell populations. Fig. 1 shows the results of Southern blot analysis following EcoRI digestion; the expected 1.1-kilobase Yc-containing fragment is seen in both GP+E-86/Yc and GP+envAm-12/Yc. Similar analysis confirmed the absence of gross rearrangement or deletion of the vector sequence in the packaging cell line populations transfected or

bovine serum albumin as a standard. Purified rat liver cytosolic GST (Bio-Rad), which contains Ya (GST 1), Yb (GST 3 and 4), and Yc (GST 2) subunits, was used as a positive control. Polycrylamide gel electrophoresis was performed according to the method of Laemmli (46) using a 4% polyacrylamide stacking gel layered over a 12% resolving gel. Seventy-five μg of protein from each sample were run under 50 mA for 5 h and transferred onto a nitrocellulose membrane by electroblotting (15 V for 16 h). Nonspecific binding to the membrane was blocked by incubation in 5% BSA and 0.02% sodium azide in PBS for 2 h at room temperature. Blots were then reacted with 0.5% BSA in wash buffer (0.05 μg/ml H2O2, 0.5 μg/ml NaCl, 0.1% Tween 20) and a polyclonal rabbit anti-rat GST antibody (Bio-Rad) for 1 h at room temperature. Blots were then reacted with a 1:1000 dilution of anti-Yc (Medlabs-Biotrin International, Dublin, Ireland), a 1:250 dilution of anti-rat liver GST (kindly provided by Dr. M. Clapper, Fox Chase Institute for Cancer Research, Philadelphia, PA), or a 1:1000 dilution of anti-Yp (Medlabs-Biotrin International). Following 3 washes with wash buffer for 15, 10, and 5 min at room temperature, membranes were reacted with a 1:3000 dilution of goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (Bio-Rad) and 0.5% BSA in wash buffer. The sites of antibody binding were finally revealed by incubation with 0.52 mg/ml diaminobenzidine and 0.04% H2O2 in PBS.

Enzymatic Assays. Cytosolic protein extracts were prepared as described above. Total GST enzyme activity was assayed using 2-chloro-1,3-dinitrobenzene as a substrate (47). Selenium-independent glutathione peroxidase activity was assayed using cumene hydroperoxide (48). Assays were linear functions of protein concentration and time (for up to 5 min) when the amount of enzyme used resulted in an absorbance change of 0.02-0.04 A340 min for total GST and 0.01-0.03 A340 min for GST peroxidase. Cells subjected to gene transfer with the sense construction were compared with cells transfected with the antisense vector but otherwise treated in the same manner.

Drug Sensitivity Assay. Drug sensitivity assays were performed using a colorimetric assay which measures the ability of viable cells to reduce a soluble yellow tetratiazolium salt (MTT) to an insoluble purple formazan precipitate (49, 50). Cells in the logarithmic phase of growth were resuspended at a concentration of 1–5 × 104 cells/ml; 100 μl/well were plated in 96-well microtiter plates. Plates were incubated for 24 h at 37°C in 5% CO2. Medium (100 μl) containing the drug was then added to 8 wells for each drug concentration and plates were incubated for a further 72 h. Medium (180 μl) was then removed from each well and replaced by 150 μl of medium containing 10 μM 1,4-piperazinediethanesulfonic acid (pH 7.4) and 50 mM MTT (Sigma Chemical Co., St. Louis, MO) at 2 mg/ml in PBS. Plates were then wrapped in aluminum foil and incubated for 4 h at 37°C. The formazan crystals were dissolved in 180 μl dimethyl sulfoxide (Fisher, Montreal, Quebec, Canada) and 25 μl glycine buffer (0.1 M glycine-0.1 M NaCl, pH 10.5) by mixing on a shaker for 5 min. The formazan product formed by viable cells was then quantitated by measuring the absorbance at a wavelength of 570 nm on a microplate reader (Bio-Rad Model 5500). IC50 was determined by plotting the percentage of surviving cells versus the log of drug concentration. Again, cells treated with the sense vector were compared with cells transfected with the antisense construction. Chlorambucil and mephalazine were obtained from Sigma; methotrexate was obtained from Adria Laboratories (Columbus, OH).
transduced with N2Yc and N2revYc vectors. It also revealed, as seen in Fig. 1, that the copy number of transferred vectors was severalfold greater in the transduced ecotropic packaging cells than in the transduced (infected) amphotropic packaging cells [ethidium bromide staining of the gel before transfer showed equivalent loading of both DNAs (data not shown)].

Transcriptional Activity of the Vector. The transcription activity of the transferred vectors was assessed by Northern blot analysis using a full-length GST-Yc cDNA probe. This demonstrated efficient expression of the expected 2.8-kilobase vector mRNA transcript in GP+E-86 cells transfected with pN2Yc or pN2revYc, as well as in GP+envAm-12 transduced by the corresponding retroviral vectors (data not shown). The correct structure of vector transcripts was further verified by RNase protection assay using a riboprobe complementary to 21 base pairs of N2 vector and 99 base pairs of GST-Yc cDNA at the 5' junction of these sequences. Fig. 2 shows the results of this assay on GP+E-86 cells stably transfected with pN2Yc or pN2revYc. As expected, there was no rat GST-Yc sense transcript in nontransfected GP+E-86 or after transfection with the antisense vector (Fig. 3). In contrast, NIH 3T3-derived packaging cells expressed unchanged low levels of Yb and Yp and no Yc cross-reacting material following gene transfer of N2Yc or N2revYc (not shown).

Expression of Immunodetectable Rat GST-Yc. Western blot analysis was performed on cell populations and clones subjected to GST-Yc gene transfer using the following polyclonal rabbit anti-rat GST antibodies: anti-Yc, anti-rat liver GST (Ya, Yb, and Yc), and anti-[pi]. This revealed a marked increase in the expression of the immunodetectable Yc isoform in N2Yc-transfected or N2Yc-transduced NIH 3T3 cell populations (packaging cells) and clones in comparison with very low levels of cross-reacting murine Yc isoform in wild-type cells or cells treated with the antisense vector (Fig. 3). In contrast, NIH 3T3-derived packaging cells expressed unchanged low levels of Yb and Yp and no Yc cross-reacting material following gene transfer of N2Yc or N2revYc (not shown).

Total GST and GSH Peroxidase Activity. Cytosolic extracts from cells subjected to GST-Yc gene transfer were assayed for total GST and GSH peroxidase activity by the method of Huang et al. (10). The rat liver GST-2, the major GST isoform, was assayed separately in the cytosolic extract. The specific activities of total GST and GSH peroxidase were calculated by dividing the total GST and GSH peroxidase activities by the protein content of the extracts.
Fig. 3. Western blot detection of GST-Yc gene product. Seventy-five μg of cytosolic protein from each cell line were separated by polyacrylamide gel electrophoresis and transferred to nitrocellulose; the membrane was reacted successively with a polyclonal rabbit anti-GST-Yc antibody, a horseradish peroxidase-conjugated goat anti-rabbit antibody, and a solution of diaminobenzidine and H2O2 as detailed in "Materials and Methods." purified GST, purified rat liver GST; GP+E-86/Yc # 17; same clonal isolate referred to as GP+E-86/Yc # D17 in Fig. 2; Am12/Yc #14, clone isolate from the transduced GP+envAm12/Yc cell population. Other symbols are the same as in Figs. 1 and 2.

Table 1 Total GST activity and selenium-independent glutathione peroxidase activity of GST-Yc-transfected and GST-Yc-transduced cells

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Total GST ± SD (nmol/min/mg protein)</th>
<th>Ratio</th>
<th>GST peroxidase ± SD (nmol/min/mg protein)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP+E-86</td>
<td>88.9 ± 16.1</td>
<td>0.6</td>
<td>0.51 ± 0.43</td>
<td>0.6</td>
</tr>
<tr>
<td>GP+E-86/Yc</td>
<td>146.7 ± 34.7</td>
<td>1.0</td>
<td>0.83 ± 0.52</td>
<td>1.0</td>
</tr>
<tr>
<td>GP+E-86/Yc #17</td>
<td>199.7 ± 68.4</td>
<td>1.4</td>
<td>0.90 ± 0.28</td>
<td>1.4</td>
</tr>
<tr>
<td>GP+envAm-12</td>
<td>103.6 ± 6.2</td>
<td>1.1</td>
<td>0.45 ± 0.13</td>
<td>1.1</td>
</tr>
<tr>
<td>GP+envAm-12/Yc</td>
<td>107.3 ± 6.1</td>
<td>1.1</td>
<td>0.37 ± 0.19</td>
<td>1.1</td>
</tr>
<tr>
<td>GP+envAm-12/Yc #17</td>
<td>191.9 ± 63.4</td>
<td>1.9</td>
<td>0.37 ± 0.19</td>
<td>1.9</td>
</tr>
<tr>
<td>GP+envAm-12/Yc #2</td>
<td>1473 ± 13.6</td>
<td>1.4</td>
<td>2.07 ± 0.06</td>
<td>1.4</td>
</tr>
<tr>
<td>GP+envAm-12/Yc #4</td>
<td>205.6 ± 53.6</td>
<td>2.0</td>
<td>3.26 ± 0.95</td>
<td>2.0</td>
</tr>
<tr>
<td>GP+envAm-12/Yc #7</td>
<td>144.7 ± 15.4</td>
<td>1.4</td>
<td>2.63 ± 0.70</td>
<td>1.4</td>
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<tr>
<td>GP+envAm-12/Yc #13</td>
<td>159.4 ± 33.8</td>
<td>1.5</td>
<td>2.27 ± 0.90</td>
<td>1.5</td>
</tr>
<tr>
<td>GP+envAm-12/Yc #15</td>
<td>284.8 ± 12.1</td>
<td>2.8</td>
<td>4.41 ± 0.77</td>
<td>2.8</td>
</tr>
<tr>
<td>GP+envAm-12/Yc #17</td>
<td>119.3 ± 11.9</td>
<td>1.2</td>
<td>4.95 ± 0.56</td>
<td>1.2</td>
</tr>
</tbody>
</table>

- P < 0.05
- P < 0.01
- P < 0.001

DISCUSSION

We have used a retroviral vector to test the ability of the Yc isoform of rat GST to confer resistance to alkylating drugs. NIH 3T3-derived GP+E-86 ectopic retrovirus-packaging cells were stably transduced with a retrovirus plasmid construction designed to transcribe the rat GST-Yc cDNA in sense (pN2Yc) or antisense (pN2revYc) orientation from the virus promoter/enhancer in the 5' long terminal repeat. The resulting defective retroviruses were used to transduce NIH 3T3-derived GP+envAm-12 amphotropic retrovirus-packaging cells. Southern blot analysis confirmed the presence of an unarranged vector sequence in the transfected and transduced packaging cell lines (Fig. 1). It also revealed that the copy number of the vector per cell was greater in transfected than in transduced cell populations. This is not surprising since transfected plasmids tend to integrate in concatemers of large numbers of plasmids (51), whereas retroviruses tend to integrate in low copy numbers per cell (on average one copy/cell).

Both transfected GP+E-86 and transduced GP+envAm-12 cells
(populations and clonal isolates) showed efficient expression of the expected 2.8-kilobase sense or antisense transcripts following transfer of N2Yc or N2evYc. A more detailed analysis of the region of the transcript corresponding to the junction between the vector sequence and the 5′ end of cDNA insert in the sense construction by RNase protection assay revealed that there was approximately equivalent amounts of 2 predominant protected mRNA species, 1 being 5–6 base pairs shorter than expected (Fig. 2). The mechanism by which this smaller transcript arose is unclear but may relate to the presence of cryptic splice acceptor signals in N2-derived vectors (52). There was no evidence, however, that this alternate mRNA structure interfered with expression of the transferred gene. Efficient transcription of the vector was also reflected in the titer of virus-producing cells. Populations of transfected ecotropic packaging cells produced the vectors at 0.5–2 × 10⁶ particles/ml, whereas populations of “superinfected” amphotropic packaging cells produced 2–5 × 10⁶ particles/ml. Titers >10⁶ viral particles/ml will generally permit efficient gene transfer in a wide range of target cell types.

Western blot analysis demonstrated the efficient expression of immunoreactive GST-Yc isoform in packaging cells transfected or transduced with N2Yc (Fig. 3). Likewise, expression of functional enzyme following gene transfer was evident in the 1.1–2.8-fold increase in total cytosolic GST activity and, more clearly, in the 4.8–11.5-fold increase in glutathione peroxidase activities (Table 1). Most of these changes reached statistical significance (unpaired Student’s t-test). The slightly higher total GST activity of parental nontransduced GP + envAm-12 cells in comparison to cells transduced with the antisense vector could conceivably be due to an antisense effect on endogenous GST expression. However, the glutathione peroxidase activity was equivalent in both cell populations and there was no detectable increase in any isoform of GST on Western blot.

The most important and interesting effect of GST-Yc gene transfer was its impact on cellsensitivity to alkylating agents (Table 2). Populations of NIH 3T3-derived packaging cells transfected with the retrovirus N2Yc construction were 5.8-fold resistant to chlorambucil and 10.8-fold resistant to mechlorethamine. Likewise, populations of packaging cells superinfected with the vector but not preselected for successful gene transfer were 2.4-fold resistant to chlorambucil and 5.4-fold resistant to mechlorethamine. The range of resistance in clonal isolates from either population was 1.8–6.0-fold for chlorambucil and 4.6–6.1-fold for mechlorethamine. These changes in sensitivity to chlorambucil and mechlorethamine were all statistically significant. There was no significant difference in drug sensitivity between parental cells and cells transduced or transduced with the antisense vector, again arguing against a significant inhibition of GST expression by the antisense vector.

These results clearly indicate that the rat Yc isoform of GST is able to confer alkylating drug resistance in mouse-derived fibroblasts. The reasons for contradictory results from previous transfection experiments (27–29, 32–34) are not entirely clear but may relate to substrate specificities of the isoenzymes that were studied or to differences in endogenous expression of GST isoenzymes or of cofactors between the cell lines that were transfected. We have obtained relatively high levels of drug resistance by transferring the Yc isoform of GST in MatB rat mammary carcinoma cells which normally express low levels of GST-Yc (31). Stably transfected cell clones expressing increased levels of GST activity and immunoreactive Yc isoform were resistant to melphanal (6–12-fold), mechlorethamine (10–16-fold), and chlorambucil (7–30-fold). Late passage transfectants showed decreased GST activity concurrent with a partial reversion toward wild-type drug sensitivity. The present study extends those findings and addresses the issue that clonal variability could potentially confound the results of conventional transfection experiments (35). To circumvent this problem, we have studied rat GST-Yc expression and alkylating drug sensitivity of populations of mouse NIH 3T3 fibroblasts transfected with a GST-Yc expression vector or transduced by a Yc retrovirus vector. The effect of GST-Yc gene transfer on the sensitivity of cell populations to alkylating agents excludes potential artifacts related to clonal variability and thus confirms and extends our earlier findings.

The ability of GST-Yc to confer alkylating drug resistance following retrovirus-mediated gene transfer supports the implication of Yc in naturally occurring resistance. It also raises the possibility of using GST-Yc somatic gene transfer to confer protection to the hematopoietic system in a gene therapy strategy applicable to cancer. Autologous transplantation of drug-resistant bone marrow cells could reduce life-threatening toxicities from subsequent courses of chemotherapy and might permit safe dose intensification. Contaminating tumor cells could be excluded from the bone marrow or later killed by the inclusion of a safety “suicide gene” in the vector. The feasibility of “bone marrow protection” has been demonstrated in mice using a human MDR1-transducing retrovirus and clinical trials are now being considered. Although the efficiency of gene transfer must be directly evaluated in hematopoietic cells, we have evidence for efficient gene transfer with the N2Yc vector since all 20 clones derived from single cells of transfected GP + envAm-12/Yc were found to contain at least 1 copy of integrated provirus and 14 of them (70%) overexpressed glutathione peroxidase activity (data not shown). Despite the relatively modest but most likely clinically significant levels of drug resistance that appear to be mediated by GST isoenzymes, there are several potential advantages to transferring these genes into the hematopoietic system. Alkylating drugs have a broad spectrum of anti-tumor activity, maintain a dose-related effect through multiple logs of tumor cell kill, and are in most cases limited by their hematotoxicity (53–55). Thus, they are among the most important and useful antitumor agents. While the levels of drug resistance obtained following gene transfer of GST may appear modest compared to MDR genes, relative resistance to alkylating agents observed clinically is of the same or lower magnitude (22), suggesting that resistance factors of 1.5–3.0-fold are important in chemotherapy. Finally, we have recently demonstrated that there is no detectable constitutive expression of GST-Yc in either human and rodent mononuclear bone marrow cells (56), indicating a potential benefit from heterologous gene expression. Future experiments will address the feasibility of using GST-Yc to confer drug resistance to the hematopoietic system.

ACKNOWLEDGMENTS

We thank Dr. William E. Fahl (McArdle Laboratory for Cancer Research, University of Wisconsin) for providing the plasmid pPSMYc containing the GST-Yc cDNA.

REFERENCES

RETROVIRUS TRANSFER OF GST Yc


Retrovirus-mediated Gene Transfer of Rat Glutathione S-Transferase Yc Confers Alkylating Drug Resistance in NIH 3T3 Mouse Fibroblasts

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