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Elevation of π Class Glutathione S-Transferase Activity in Human Breast Cancer Cells by Transfection of the GSTπ Gene and Its Effect on Sensitivity to Toxins

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SUMMARY

Increased expression of the glutathione *S*-transferase (GST; E.C.2.5.1.18) π class isozyme is associated with both malignant transformation and drug resistance, as well as with decreased estrogen receptor content in breast cancer. In order to further characterize the role of this enzyme in drug resistance, we cloned the cDNA encoding the human isozyme GSTπ and developed two eukaryotic expression vectors using this cDNA and either the human metallothionein IIa or cytomegalovirus immediate-early promoters. These GSTπ expression vectors were cotransfected with pSV2neo into drug-sensitive MCF-7 human breast cancer cells, which have low amounts of GST activity and which do not express GSTπ. The transfected cells were selected for G418 resistance and individual clones were screened for GST activity. Three clones that demonstrated increased GST activity were selected for further study. Immunoprecipitation studies demonstrated that the increase in GST activity in these clones was due to expression of GSTπ. Although the total GST activity of the positive clones was increased as much as 15-fold over that in wild-type MCF-7 cells, there was no change in glutathione peroxidase activity, as measured using cumene hydroperoxide as a substrate. Immunoblot studies revealed that the increased GST enzyme produced in the transfected cells was identical in size to endogenous GSTπ. Southern blot analysis demonstrated the incorporation of the GSTπ expression vector into the genome of the positive clones and Northern blot analysis showed that the transfected genes made a hybrid GSTπ RNA that was slightly larger than the endogenous GSTπ RNA. Primer extension studies demonstrated that this increase in length corresponded to the added length of the 5′ leader sequence of the expression vector. The effect of increased GSTπ activity on the sensitivity of the transfected clones to several cytotoxic agents was assessed by colony-forming assay. The transfected clones were slightly more resistant (1.3–4.1-fold) to benzo(*a*)pyrene and its toxic metabolite benzo(*a*)pyrene-(*anti*)-7,8-dihydrodiol-9,10-epoxide, as well as to ethacrynic acid (3.1- to 4.4-fold). Although increased GSTπ expression is found in MCF-7 cells selected for doxorubicin resistance, the transfected clones were not consistently more resistant to doxorubicin than control cells. In addition, the transfected cells were not resistant to either melphalan or (*cis*)-platinum, even though conjugation with glutathione is known to play a role in the detoxification of both of these drugs. Thus, increased GSTπ expression, in isolation from other intracellular changes, provides a modest level of protection from the cytotoxic effects of some lipophilic carcinogens but does not markedly contribute to resistance against doxorubicin, melphalan, or (cis)-platinum.

GSTs are multifunctional proteins that can detoxify foreign compounds through several mechanisms. GSTs have the capacity to catalyze the conjugation of electrophilic toxins with glutathione resulting in more polar and readily excreted metabolites, to sequester toxins through high affinity binding, and to remove toxic peroxides through intrinsic organic peroxidase activity (1). There are at least four distinct GST gene families, the basic or α class, the neutral or μ class, the acidic or π class, and a microsomal form (2). Overall GST activity is increased in several cell lines selected for resistance to different antineoplastic agents (3–7) and GSTs appear to provide protection to cells when added exogenously into the cell culture medium (8) as well as when the enzyme activity is induced by exposure to antioxidants (9) and low dose alkylating agents (10).

Increased expression of the human π class GST isozyme, GSTπ, has recently been associated with malignant transformation in rat (11) and human (12) tumors. Elevated levels of GSTπ protein and RNA have been found in human colon and gastric carcinomas, in comparison with normal surrounding mucosa by immunocytochemical studies (13) and RNA slot blot analysis (12). Increased levels of GSTπ enzyme activity and RNA have also been found in lung tumors, in comparison with

ABBREVIATIONS: GST, glutathione *S*-transferase; MDR, multidrug resistance; SSC, saline-sodium citrate (20×, 3 M sodium chloride, 0.3 M sodium citrate, pH 7.0); SDS, sodium dodecyl sulfate; MOPS,3-(*N*-morpholino) propanesulfonic acid; BaP, benzo(*a*)pyrene; BaPDE, benzo(*a*)pyrene-(*anti*)-7,8-dihydrodriol-9,10 epoxide; WT MCF-7, wild-type MCF-7 cells; Adr^R MCF-7, multidrug-resistant MCF-7 cells originally selected for resistance to doxorubicin (Adriamycin); bp, base pairs; kb, kilobases.

normal surrounding lung tissue, as determined by direct measurement of enzyme activity (14) and by RNA slot blot analysis (12). Tumors that exhibit increased overall GST activity appear to have increased levels of the GSTπ isozyme that account for the overall change (15). Furthermore, we have recently shown an inverse relation between GSTπ RNA levels and the estrogen receptor content in primary breast cancer, a finding that links expression of this drug-detoxifying enzyme with a clinically important prognostic variable (16).

The π class isozymes of GST are also strikingly elevated in two distinct models of broad cross-resistance to multiple toxic agents. First, in the Solt-Farber model of hepatocellular carcinogenesis, preneoplastic liver nodule cells that develop resistance to the toxic effects of the carcinogen that induced the malignant transformation also become cross-resistant to the cytotoxic effects of other, structurally unrelated, carcinogens (17). These preneoplastic nodules have markedly increased levels of the rat π class GST, GST-P, in comparison with normal surrounding hepatocyes (11). Second, our labortory has isolated a multidrug-resistant MCF-7 human breast cancer cell line by selection of drug-sensitive cells with doxorubicin (Adr^R MCF-7) (18). These cells display the typical MDR phenotype of cross-resistance to many natural product cytotoxins, decreased intracellular accumulation of drug, and overexpression of the P-glycoprotein (*mdr1*) gene (19). In addition, Adr^R MCF-7 cells demonstrate a large increase in expression of GSTπ (3).

Because GSTs constitute a diverse family of distinct isozymes with different substrate specificities, it is not clear whether the expression of the GSTπ isozyme directly contributes to drug resistance. In order to further characterize the role of GSTπ in drug resistance, we cloned a cDNA for GSTπ that contains the entire protein-coding region of the gene and we used it to construct expression vectors containing either the human metallothionein IIA promoter or the cytomegalovirus immediate-early promoter. Following transfection of these vectors into drug-sensitive MCF-7 cells, which have very low levels of GST activity and which do not express the GSTπ isozyme, individual clones that expressed the transfected gene were selected and examined for changes in sensitivity to cytotoxins.

Materials and Methods

Cell culture. WT MCF-7 and the transfected sublines were maintained in improved minimal essential medium with 2 mg/liter *l*-proline and 50 μg/ml gentamycin (GIBCO, Grand Island, NY) in 5% fetal bovine serum (GIBCO). Adr^R *MCF-7* cells were grown in medium containing 10 μM doxorubicin. When used in specific studies, Adr^R MCF-7 cells were grown in drug-free medium for 2–6 weeks before study.

Construction of expression vectors. The GSTπ cDNA GSTπ-1 was isolated from a λgt10 cDNA library constructed from Adr^R MCF-7 RNA as described (16). Nucleotide sequence analysis demonstrated that the 725-bp GSTπ-1 cDNA contained the entire protein-coding region of the gene, along with the complete 3′ untranslated region and polyadenylation signal (12, 20).

The plasmid vector pMTP-5H was the generous gift of Drs. Dwight Kaufman and Neal Rosen (National Cancer Institute). This vector contains an 832-bp genomic fragment of the human metallothionein IIA gene, which spanned bases −761 to +71 (21), cloned into pUC8. A polylinker had been inserted into this expression vector 3′ to the promoter, and an 800-bp SV40 sequence containing the SV40 polyadenylation signal was inserted 3′ to the polylinker. The plasmid vector pHD1013 was the generous gift of Drs. Michelle Davis and Eng-Shang Huang (22). This plasmid was constructed by inserting a 2.1-kb *PstI* fragment of the human cytomegalovirus immediate-early promoter enhancer region into the multiple cloning site of pGEM2.

GSTπ-1 was ligated into the *Eco*RI site of the multiple cloning sites of pMTP-5H and pHD1013 by standard procedures. The resulting plasmids, pMTG and pHDG, respectively (Fig. 1), were transformed into a competent *Escherichia coli* strain, amplified, and isolated by standard procedures (23). Proper orientation of the cDNA in relation to the promoter was determined by restriction enzyme analysis.

Transfection of WT MCF-7 cells. WT MCF-7 cells were cotransfected with 18 μg of GSTπ expression vector and 2 μg of pSV2neo in 10-cm tissue culture dishes (Costar), using a standard calcium phosphate precipitation method (24), and were then selected for resistance to G418 (0.75 mg/ml) (GIBCO). Individual colonies were isolated and screened for GST enzyme activity as described below. Another population of WT MCF-7 cells was transfected with pSV2neo and pUC8 and similarly selected for G418 resistance to serve as an experimental control (MCF-7/pSV2neo).

Protein studies. Clones surviving G418 selection were lysed by sonication at 4° in 50 mM Tris·HCl, pH 7.5, 5 mM EDTA buffer. After centrifugation in a microfuge, cytosolic GST activity was determined using 1-chloro-2,4 dinitrobenzene as substrate (25). One unit of GST enzyme activity is defined as the amount catalyzing the conjugation with glutathione of 1 nmol of substrate/min. Cytosolic glutathione peroxidase activity with both cumene hydroperoxide and H_2O_2 substrates was determined as described (26). One unit of glutathione peroxidase activity is defined as the amount catalyzing the reduction of 1 nmol of peroxide substrate/min. For immunoprecipitation studies, cytosol or purified GST (10–20 units) was incubated with affinity-purified goat polyclonal anti-GSTπ antibody, which is specific for the GSTπ isozyme (16). This antibody reacts with GSTπ purified from human placenta but not with the GST isozymes found in liver (16), and it does not react with affinity-purified α or μ class isozymes (data not shown). After incubation with fixed *Staphylococcus aureus* cells (Pansorbin) and centrifugation in a microfuge, supernatants were assayed for GST activity with the 1-chloro-2,4-dinitrobenzene assay

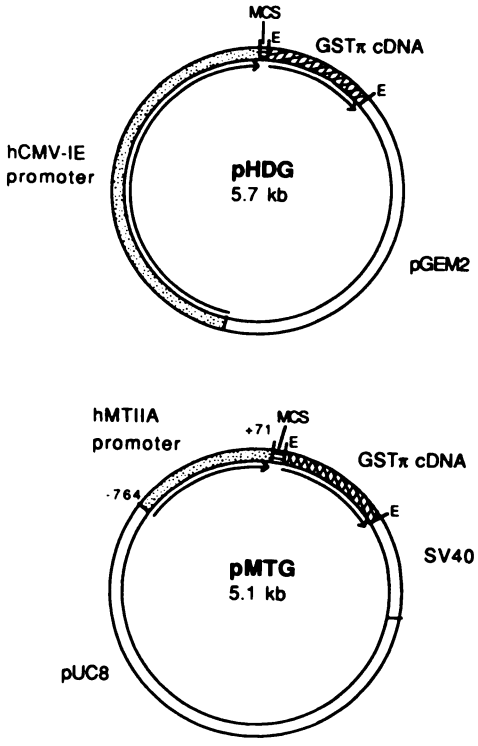

Fig. 1. Maps of the eukaryotic expression vectors pHDG and pMTG. *MCS*, multiple cloning site; *E*, *Eco*RI restriction enzyme site.

and the precipitated activity was expressed as a percentage of the control sample (mock-precipitated with preimmune serum).

For immunoblot studies, cells were harvested in late log phase growth. Samples of cytosolic protein (100 μg/lane) were resolved on a 1-mm thick 14% SDS-polyacrylamide gel and were transferred to a nitrocellulose membrane (Schleicher and Schuell, Keene, NH) using an LKB Novablot semidry electroblotter. The blot was blocked in a 5% nonfat milk solution (Blotto) and probed with the affinity-purified goat anti-GSTπ polyclonal antibody (1:500) (16, 27).

Nucleic acid studies. For Southern analysis, high molecular weight DNA was extracted by standard procedures, digested to completion with *Eco*RI endonuclease, and size fractionated on a 1% agarose gel. The DNA was transferred to a Nytran membrane, hybridized overnight with GSTπ-1 DNA that was ^{32}P-labeled by nick-translation, and washed with a final stringency of 0.1× SSC and 5% SDS at 60°. For Northern analysis, RNA was isolated by guanidine isothiocyanate-cesium chloride gradient centrifugation (23) and size fractionated on a 1% agarose gel that contained 2% formaldehyde with a 20 mM MOPS buffer containing 1 mM EDTA and 5 mM sodium acetate. The RNA was transferred to a nitrocellulose membrane, hybridized overnight to a ^{32}P-labeled GSTπ-1 probe, and washed with a final stringency of 0.1× SSC and 0.1% SDS at 50°.

For primer extension studies, a γ-^{32}P-end-labeled 15-bp oligonucleotide primer (Midland Certified Reagent Company, Midland, TX) complementary to codons 4–8 of GSTπ-1 was annealed to whole cellular RNA from both Adr^R MCF-7 and MCF-7/pMTG-1 cells, as described (20). The primer was then extended in the 5′ direction by reverse transcriptase (International Biotechnologies, Inc., New Haven, CT) to the 5′ end of the RNA transcript. This complex was denatured and analyzed on an 8% acrylamide sequencing gel that contained 50% urea, to measure the size of the primer extension product as described (20). The same primer was annealed to GSTπ-1 subcloned into pUC8, extended using a ^{32}P-labeled dideoxy sequencing method, and run in adjacent lanes to the primer extension products in order to determine their size.

Colony-forming assays. Transfected cells were plated at the density of 500 cells/well in 6-well tissue culture dishes in improved minimal essential medium containing 5% fetal bovine serum. On the following day, the cells were exposed to varying concentrations of drug for 1 hr except for the BaPDE exposure, which was continuous. Colonies were stained with methylene blue in methanol after 8–10 days. Each colony-forming assay was performed three times in triplicate.

Results

Transfection. A total of 46 colonies of cells cotransfected with pMTG or pHDG and pSV2 were isolated and analyzed for GST activity. Of these, 8 clones demonstrated increased GST activity. The 2 clones with the highest activity had both been transfected with the pMTG plasmid (MCF-7/pMTG-5 and MCF-7/pMTG-1; Table 1). The other 6 positive clones, including pHDG-1, all had a lower and approximately similar level of GST activity, in the range of 30 units/mg of protein. These three positive clones for GSTπ enzyme activity, MCF-7/pMTG-5, MCF-7/pMTG1, and MCF-7/pHDG-1, were selected for further study.

Analysis of positive clones. The GST produced by the positive clones was analyzed by immunoprecipitation and by immunoblot to confirm the identity of the GST produced by the transfected cells. There was an overall increase of 4.5- to 14.7-fold in GST enzyme activity in the transfected cell lines, in comparison with the control MCF-7/pSV2neo cells (Table 1). Immunoprecipitation studies demonstrated that most of the GST activity in the transfected cells reacted with a polyclonal antibody that is specific for the GSTπ enzyme. The residual GST activity in the transfected cells after precipitation is

TABLE 1

GSTπ and glutathione peroxidase activity of WT MCF-7 clones transfected with GSTπ expression vectors

GST and glutathione peroxidase activity is expressed in units/mg of protein. The percentage of the total GST that was identified as the GSTπ isozyme was determined by immunoprecipitation, as described in the text.

	GST Activity	GSTπ	Total GSTπ	Glutathione Peroxidase	
				H_2O_2	Cumene
	units/mg	%		*units/mg*	
Control cell lines					
WT MCF-7	8	1.6	<1	1.7	1.3
Adr^R MCF-7	140	97	136	44	58
MCF-7/pSV2neo	6.2	3.3	<1	2.6	2.3
GSTπ-transfected clones					
MCF-7/pHDG-1	28	77	22	1.9	1.7
MCF-7/pMTG-1	54	86	46	1.5	1.3
MCF-7/pMTG-5	91	94	86	2.5	2.2

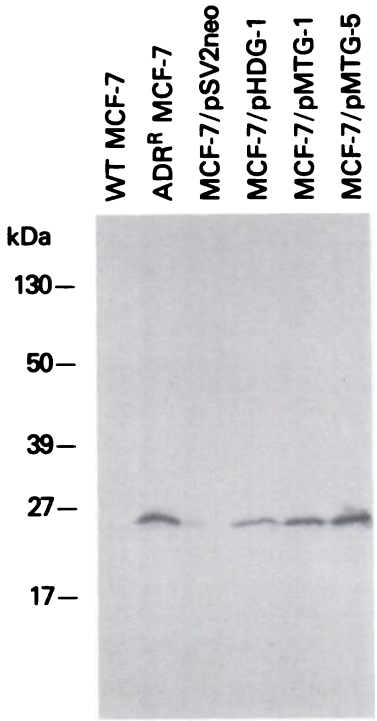

Fig. 2. Western blot analysis of GSTπ produced by the transfected cells. Cytosolic protein (100 μg/lane) was resolved on a 14% SDS-polyacrylamide gel, transblotted, and probed with a polyclonal goat anti-GSTπ antibody, as described in the text. Molecular weight markers are indicated to the *left*.

equivalent to the small amount of GST activity seen in the control cells. Immunoblot analysis (Fig. 2) confirmed that the GST species produced by the expression vectors reacts with the anti-GSTπ antibody and that the enzyme in the transfected cells has an M_r of approximately 23,000, which is identical to that of the GSTπ found in Adr^R MCF-7 cells and placenta.

Southern blot analysis (Fig. 3) demonstrated that *Eco*RI digestion of all the MCF-7 sublines produced a fragment of approximately 11 kb, which represents the endogenous GSTπ gene (20), and a fainter signal of 6 kb, which may represent a GSTπ pseudogene (20). The Southern blot also demonstrates the presence of the 725-nucleotide GSTπ-1 cDNA in the three stably transfected positive clones and the absence of a fragment of this size in the control cells, MCF-7/pSV2neo, or in the Adr^R

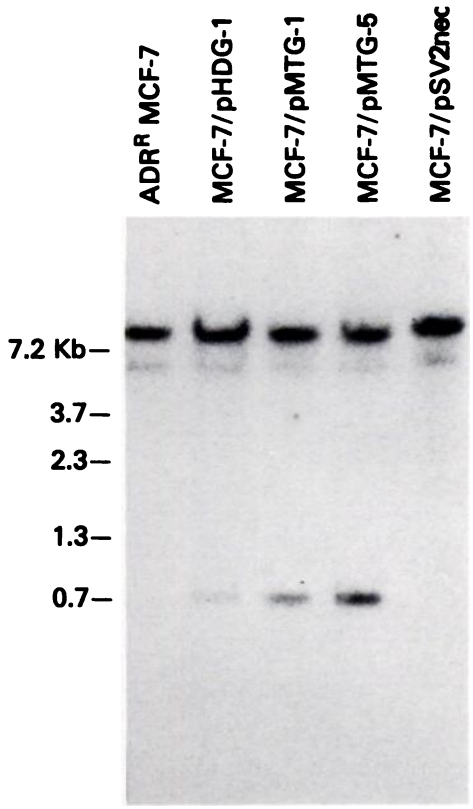

Fig. 3. Southern blot analysis of genomic DNA from transfected clones. DNA was isolated as described in the text and digested with *Eco*RI endonuclease. DNA (15 μg/lane) was size fractionated on a 1% agarose gel, transferred to a Nytran membrane, and hybridized to a radiolabeled GSTπ-1 cDNA probe, as described in the text. The 0.7-kb GSTπ-1 cDNA insert is seen in the three transfected cell lines that demonstrated increasd GSTπ expression, indicating that the transfected cDNA was incorporated into the genome of the transfected cells. This fragment is not seen in either Adr^R MCF-7 cells, which express high amounts of GSTπ, or in the control MCF-7/pSV2neo cells, which show no GSTπ activity.

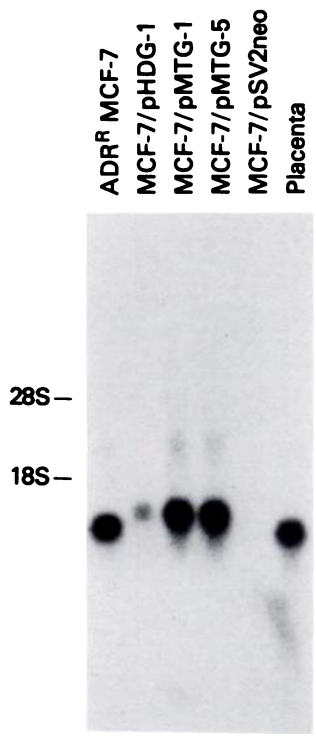

Fig. 4. Northern blot analysis of whole cellular RNA isolated from transfected clones. RNA (15 μg/lane) was size fractionated, transferred onto nitrocellulose, and hybridized with a radiolabeled GSTπ-1 cDNA probe, as described in the text. RNA from Adr^R MCF-7 cells and from placenta is shown to demonstrate the difference in size between endogenous RNA and the RNA produced by the hybrid genes. No GSTπ RNA is seen in the control MCF-7/pSV2neo cells.

MCF-7 cells. There is an apparent relation between the GSTπ expression vector gene copy number and the level of enzyme activity in the three positive clones.

Northern blot analysis (Fig. 4) demonstrated the presence of a GSTπ transcript of approximately 1.2 kb in the positive clones. No GSTπ RNA was detected in the control MCF-7/pSV2neo cells. The hybrid RNA species is slightly larger than the approximately 1.0-kb endogenous species made by Adr^R MCF-7 cells and human placenta, consistent with the predicted additional length added to the hybrid GSTπ RNA by the 5′ leader sequences of the promoter cassettes.

To detemine whether the GSTπ RNA in the transfected cells resulted from transcription of the expression vector, primer extension analysis was peformed. In previous studies, the major start site of transcription of the endogenous GSTπ gene has been determined to be only 29 nucleotides 5′ to the start of translation (20, 28). As shown schematically in Fig. 5A, sequence data indicate that the start of transcription in the hMTIIa promoter plasmid contruct should be 153 nucleotides from the *Eco*RI insertion site of the GSTπ cDNA. As shown in Fig. 5B, the radiolabeled primer extension analysis of GSTπ RNA in MCF-7/pMTG-1 cells produced a single species, which was 153 nucleotides in length. For the purpose of comparison, the primer extension product of RNA from Adr^R MCF-7 cells is shown in the far left lane; the primer extension of the endogenous GSTπ gene transcript is shorter in length by 124 nucleotides, as would be predicted from previous analysis of the genomic GSTπ gene (20, 28). Thus, the GSTπ RNA in this transfected cell line appears to be transcribed from the expression vector and, because there is no primer extension product the size of the endogenous message in the transfected cell line, there is no evidence of GSTπ transcription from the endogenous GSTπ gene in the transfected cells. This is consistent with the longer GSTπ transcript size detected in each of the transfected cell lines by the Northern blot analysis (Fig. 4).

It has previously been reported that the π class GST produced in Adr^R MCF-7 cells appeared to possess intrinsic glutathione peroxidase activity against cumene hydroperoxide (3). We, therefore, measured glutathione peroxidase activity in the transfected clones (Table 1). Although each of these clones contained elevated levels of GSTπ, there was no increase in the glutathione peroxidase activity in any of these clones, when assayed using either cumene hydroperoxide or H_2O_2 as substrates. Thus, the GSTπ cDNA does not appear to encode cumene peroxidase activity.

Cytotoxicity studies. In order to determine whether GSTπ provided any protection against toxic substances, we first tested the sensitivity of the positive clones to ethacrynic acid, a substrate of the GSTπ isozyme (2). As seen in Table 2, increased GSTπ expression did confer resistance against ethacrynic acid to the transfected clones, relative to the control cells. The range of relative resistance extended from 3.1-fold in the clone expressing the lowest level of GSTπ to 4.4-fold in the clone containing the highest level of GSTπ.

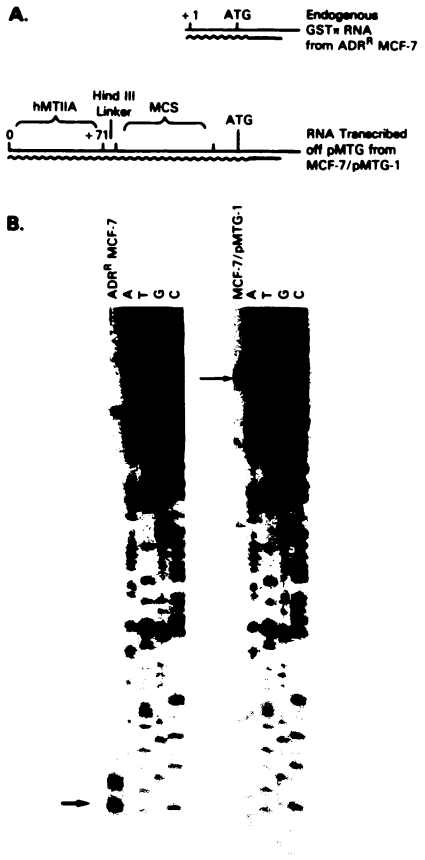

Fig. 5. A, A schematic diagram illustrating the primer extension study. An oligonucleotide primer encoding the inverse complement of the 5′ protein coding region of GSTπ-1 was annealed to RNA from Adr^R MCF-7 and RNA from the transfected clone MCF-7/pMTG-1. The primer extension product (*squiggle line*) is larger in the transfected cell line because the transcript includes leader sequences in the vector in addition to the 17 bp of the 5′ non-protein-coding region of the GSTπ-1 cDNA. B, The primer extension study itself, which demonstrates the predicted difference in size between the hybrid GSTπ RNA product (*arrow*) and the endogenous GSTπ RNA product (*bold arrow*).

TABLE 2

Relative drug resistance of MCF-7 cells transfected with GSTπ expression vectors, as determined by colony-forming assay

IC_{50} values were determined by colony-forming assay as described in the text. Each toxicity study was performed at least three times in triplicate. The IC_{50} values were then normalized to the IC_{50} of the control cell line MCF-7/pSV2neo. For comparison, Adr^R MCF-7 cells are 200-fold resistant to doxorubicin (19) and 30-fold resistant to BaP (38).

	Resistance					
	Ethacrynic Acid	BaP	BaPDE	Doxorubicin	(*cis*)-platinum	Melphalan
	fold					
MCF-7/pSV2Neo	1	1	1	1	1	1
MCF-7/pHDG-1	3.1	1.4	1.5	0.4	1.1	1.1
MCF-7/pMTG-1	4.0	4.1	2.9	1.1	1.5	2.1
MCF-7/pMTG-5	4.4	1.3	1.4	1.3	1.1	0.9

Because increased anionic GST expression is associated with resistance to the toxic effects of carcinogens in rat hyperplastic nodules, we sought to determine whether the human form of this isozyme might have a protective role against the cytotoxic effects of carcinogens. Cells expressing GSTπ developed low levels of resistance to B*a*P (1.3- to 4.1-fold), relative to control cells (Table 2). Because GSTπ is the isozyme with the highest affinity for the toxic metabolite of B*a*P, (*trans*)-B*a*PDE (29), the sensitivity of the transfected clones to this toxin was examined; each of the clones displayed low levels of resistance to (*trans*)-BaPDE (1.4- to 2.9-fold), relative to control cells (Table 2).

Because marked elevation of GSTπ activity is seen in multidrug-resistant Adr^R MCF-7 cells, we examined the contribution of GSTπ to doxorubicin resistance. As shown in Table 2, we found no consistent difference between the sensitivity of the GSTπ-positive clones and the control cells, indicating that increased GSTπ activity alone does not confer a degree of resistance to doxorubicin that could be detected by comparison of IC_{50} values.

Previous studies have indicated that the alkylating agent melphalan is detoxified, at least in part, by conjugation with glutathione (30). In addition, the GSTπ isozyme is elevated in a cell line selected for resistance to (*cis*)-platinum (6). These data suggest that the GSTπ isozyme could contribute to resistance of these cells to both of these agents. We found, however, that the elevated GSTπ expression in the positive clones did not consistently result in increased resistance to either (*cis*)-platinum or melphalan, as determined by comparison of IC_{50} values (Table 2).

Discussion

The increased expression of GSTπ in models of malignant transformation and MDR prompted our investigation into the role of this isozyme in protecting cells from the toxic effects of both carcinogens and antineoplastic agents. In order to address this question, we transfected a hybrid gene that makes a functional GSTπ isozyme into drug-sensitive MCF-7 cells, which do not exhibit endogenous expression of this isozyme. By comparing IC_{50} values of these transfected cells to those of cells that were similarly selected for G418 resistance, we have determined the contribution that this isozyme makes to drug resistance in the absence of other intracellular changes.

We utilized two different eukaryotic promoters in this experiment. The highest levels of hybrid gene expression were obtained in cells transfected with the vector that contained the human metallothionein IIa promoter. Cadmium induction of this promoter led to a modest 50% increase in enzyme activity in the clones with the highest constituitive activity (data not shown). Because cadmium is involved in multiple cellular mechanisms, we elected to perform all of our studies without cadmium induction and to assess only the effects on the constitutive level of GSTπ expression in all cell lines.

Northern blot analysis demonstrated that the GSTπ RNA in the transfected cells was slightly larger than the naturally occurring species. The primer extension study demonstrated that the increase in length was attributatle to the leader sequences 5′ to the cDNA in the transfection vector. Thus, the GSTπ RNA present in the positive clones appeared to be transcribed from the transfection vector. Immunoblot analysis confirmed that the transfected cells were capable of faithfully translating the hybrid RNA to make a functional GSTπ enzyme that was indistinguishable immunochemically and electrophoretically from the GSTπ made by Adr^R MCF-7 cells.

Although the transfected cell lines demonstrated increased GSTπ activity comparable to that of Adr^R MCF-7 cells, there was no change in the cumene peroxidase activity in these cells. Thus, the transfected GSTπ cDNA apparently encodes an isozyme with little or no peroxidase activity, and the increase

in peroxidase activity in Adr^R MCF-7 cells is apparently encoded by another gene. Indeed, using a specific cDNA probe for the human selenium-dependent glutathione peroxidase gene (E.C. 1.11.1.9) (31) we have found markedly increased levels of glutathione peroxidase RNA levels in Adr^R MCF-7 cells, when compared with WT MCF-7 cells (data not shown). Other studies have indicated that the increase in glutathione peroxidase activity in Adr^R MCF-7 cells is due predominantly to an increase in the selenium-dependent glutathione peroxidase enzyme (32, 33).

In this report, increased expression of GSTπ activity was associated with moderate increases in resistance to ethacrynic acid, BaP, and BaPDE. Previous studies on purified GSTπ enzyme have shown that both ethacrynic acid and BaPDE are good substrates for this isozyme (2, 28). However, the low level of resistance conferred by GSTπ to BaP and BaPDE indicates that other mechanisms of detoxification, such as glucuronidation and peroxide reduction, may also be involved in cellular resistance to these toxins.

Other GST isozymes may also play a protective role. Fahl and co-workers (34) have shown that transient transfection of a rat α class GST ya gene conferred resistance to BaPDE in monkey Cos cells. It is difficult to compare the relative effectiveness of the different isozymes in protecting cells from BaPDE toxicity, because the study by Fahl and co-workers used pooled, transiently transfected cells, whereas this study examined the resistance of three stably transfected clones.

Increased GSTπ expression has been associated with MDR in Adr^R MCF-7 cells. However, in this report, cells transfected with the GSTπ gene did not demonstrate any increased resistance to doxorubicin. Thus, the development of MDR in Adr^R MCF-7 cells appears to be due predominantly to overexpression of the *mdr1* gene (18). Although transfection into drug-sensitive cells of the *mdr1* cDNA isolated from Adr^R MCF-7 cells is sufficient to confer MDR,[1] transfection of the GSTπ gene did not affect resistance to doxorubicin, which is associated with the phenotype of MDR. These findings are consistent with the study of Tsuruo and co-workers (35), who could not demonstrate a direct relation between GST levels and MDR in several pairs of sensitive and MDR cell lines.

Previous studies have shown increased GSTπ expression in cells selected for resistance to (*cis*)-platinum (6), whereas other studies have shown that the alkylating agent melphalan can be conjugated with GSH (30). However, MCF-7 cells transfected with the GSTπ gene did not demonstrate a significant increase in resistance to (*cis*)-platinum or melphalan. Other GST isozymes may be more effective in the glutathione-mediated detoxification of these compounds.

The apparent inability of elevated GSTπ activity to enhance cellular resistance to these antineoplastic agents does not necessarily prove that the isozyme is not involved in drug resistance. We conclude that GSTπ expression alone, without other concurrent cellular changes, is insufficient to influence cell survival in the presence of certain anticancer drugs. However, it is possible that the GSTπ enzyme may function in concert with other drug resistance genes to enhance their activity. For example, the deduced amino acid sequence of *mdr1* has revealed that the P-glycoprotein is structurally similar to bacterial transport proteins that carry out their function in concert with other periplasmic substrate-binding proteins (36, 37). The combined transfection of both the *mdr1* and the GSTπ genes into drug-sensitive cells will permit analysis of whether a similar interaction occurs between GSTπ and P-glycoprotein.

In addition, other questions remain in regard to the relevance of the increase in GSTπ expression observed in the related processes of carcinogenesis and drug resistance. The demonstration that GSTπ RNA levels are elevated in some tumors, when compared with normal tissue, suggests that increased expression of GSTπ may serve as a useful clinical marker either of carcinogen-induced premalignant changes or of inherent drug resistance. Finally, the finding that GSTπ expression is related to estrogen receptor levels in breast cancer is an indication that the mechanisms regulating expression of the GSTπ gene may be related to other processes that affect tumor biology and clinical outcome.

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