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SHORT COMMUNICATIONS

Expression of glutathione-related enzymes in human bladder cancer cell lines

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The role of glutathione (GSH) peroxidase and GSH *S*-transferase (GST) in detoxification processes is well documented [1–3]. GSH peroxidase provides protection from oxidants [3], whereas GST isoenzymes detoxify electrophilic xenobiotics either by catalyzing their conjugation to GSH or through non-catalytic binding of electrophiles [4]. Mammalian GST isoenzymes have been grouped into three major classes α, μ, and π [2].

A π type GST has been shown to be elevated in rat preneoplastic nodules [5, 6] and in some human carcinomas [7, 8], suggesting that it may be used as a marker for human carcinomas. A uniform elevation of GST π, however, was not observed in human lung tumors [9, 10]. These results suggest that overexpression of GST π in human tumors may be tissue specific and necessitate further screening of different human tumors and cell lines. These studies may be important in identifying human carcinomas where GST π may be used as a tumor marker. In the present study, we have determined GST π expression in five human bladder cancer cell lines and in a cell line established from the normal bladder of a male fetus. The levels of GST and GSH peroxidase were also determined because these enzymes have been implicated recently in cellular resistance to chemotherapeutic agents [11–14]. The significance of the findings is discussed.

Materials and Methods

Human bladder cancer cell lines, J82 (ATCC-HTB 1), HT-1197 (ATCC-CRL 1473), SCaBER (ATCC-HTB 3), and 5637 (ATCC-HTB 9) were obtained from the American Type Culture Collection, Rockville, MD. Cell line FHs 738 Bl established from the normal bladder of a male fetus in second trimester was also obtained from the ATCC. Cell line FCCB-1 (Florida Cancer Center Bladder-1) was established in our laboratory from a bladder tumor biopsy. All these cell lines grow as monolayer. Cells were harvested by exposure to trypsin-EDTA. Harvested cells were washed three times with phosphate-buffered saline (PBS) and suspended in 10 mM potassium phosphate buffer, pH 7.0, containing 1.4 mM 2-mercaptoethanol. The cells were disrupted by sonication (2 × 30 sec), and the supernatant fraction was obtained by centrifugation at 14,000 *g* for 45 min.

GST activity with 1-chloro-2,4-dinitrobenzene (CDNB) was determined according to the method of Habig *et al.* [15]. GSH peroxidase activity was determined by the method of Beutler [16]. Protein content was determined by the method of Bradford [17].

Western blotting was performed by the method of Towbin *et al.* [18]. Details of Western blotting have been described previously [19]. Chromatofocusing was performed as described by Ishikawa *et al.* [20].

Results and Discussion

GST activity towards CDNB in 14,000 *g* supernatant fractions of five human bladder cancer cell lines as well as a normal bladder cell line is presented in Table 1. This activity in cell line J82 was similar to that of the normal bladder cell line (FHs 738 Bl). On the other hand, GST activity was higher by 4.1-, 2.2-, and 1.5-fold in cell lines SCaBER, 5637, and HT-1197, respectively, when compared with that of cell line FHs 738 Bl (Table 1). Interestingly, GST activity in FCCB-1 was about 82% of that in FHs 738 Bl.

The expression of individual GST isoenzymes in these cell lines was studied by Western blotting using antibodies raised against α, μ, and π classes of human GST isoenzymes. Western blotting with antibodies raised against GST π of human placenta using equal amount of cytosolic protein (25 μg) revealed a markedly higher level of GST π type antigen in cell lines SCaBER (Fig. 1, lane 4), 5637 (lane 5), and HT-1197 (lane 6) as compared to that of FHs 738 Bl (Fig. 1, lane 7). GST π content in cell line J82 (Fig. 2, lane 3), on the other hand, was similar to that of the normal bladder cell line. Similar to enzyme activity data, Western blotting also revealed slightly lower GST π content in cell line FCCB-1 (Fig. 1, lane 8) as compared to that of cell line FHs 738 Bl. Even though molecular weight markers were not included in this gel, an examination of Fig. 1 revealed that the M_r values of π class GST subunit in normal human bladder tissue, normal human bladder cell line FHs 738 Bl, and bladder cancer cell lines J82, SCaBER, 5637, HT-1197 and FCCB-1 were similar to that of GST π from human placenta, i.e. M_r 22,500 [9, 21, 22].

GST isoenzyme profiles in normal human bladder tissue and one of the bladder cancer cell lines, SCaBER, were determined by chromatofocusing. Upon chromatofocusing, two peaks of GST activity corresponding to isoelectric points (pI) of 6.6 and 4.9 were observed in normal human bladder tissue (Fig. 2). GST isoenzyme having pI of 4.9 cross-reacted with antibodies against GST π of human placenta and constituted more than 95% of total GST activity in this tissue. Immunological interrelationship of GST isoenzyme with pI 6.6 could not be established due to very low protein content. Since complete adsorption of GST activity occurred on a PBE 94 (pH 7–4) column, the possibility of any basic isoenzyme in bladder tissue was ruled out. Chromatofocusing of GST in the bladder cancer cell line SCaBER in the pH range 7–4 also revealed two peaks of GST activity corresponding to pI of 6.0 and 4.8 (Fig. 3). Similar to normal bladder tissue, GST isoenzyme

Table 1. GST and GSH peroxidase activities in bladder cancer cell lines and a normal bladder cell line

Cell line	Enzyme activity (nmol/min/mg protein)	
	GST*	GSH peroxidase†
J82	102 ± 10	34 ± 3
SCaBER	459 ± 5	55 ± 2
5637	240 ± 30	70 ± 6
HT-1197	166 ± 8	73 ± 2
FCCB-1	91 ± 5	93 ± 10
FHs 738 Bl	111 ± 7	79 ± 3

* GST activity was determined using CDNB as substrate at 25°.

† GSH peroxidase activity was determined using cumene hydroperoxide as substrate at 37°.

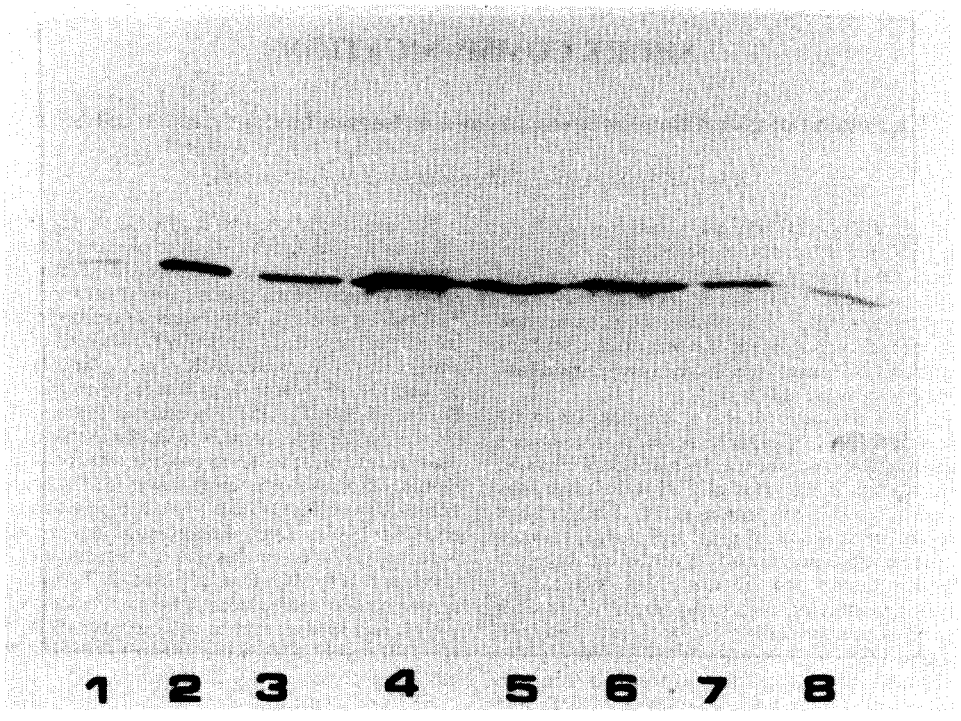

Fig. 1. Western blot analysis using antibodies raised against GST π of human placenta. Lane 1 contained purified GST π from human placenta (positive control); Lanes 2–8 contained an aliquot of 14,000 g supernatant fraction containing 25 μg protein. Key: Lane 2, normal human bladder tissue; Lane 3, bladder cancer cell line J82; Lane 4, bladder cancer cell line SCaBER; Lane 5, bladder cancer cell line 5637; Lane 6, bladder cancer cell line HT-1197; Lane 7, normal human bladder cell line FHs 738 Bl; and Lane 8, bladder cancer cell line FCCB-1.

with pI of 4.8 represented more than 95% of total GST activity and was immunologically similar to GST π of human placenta (data not shown). Due to low protein content, immunological characterization of GST isoenzyme with pI of 6.0 could not be performed. Similar to normal bladder tissue, any basic isoenzyme was not detected in this cell line. Even though chromatofocusing studies with other cell lines could not be performed due to low GST levels, these results taken together with Western blot data suggested that GST π was the major form in these samples.

Using 25 μg cytosolic protein and antibodies raised against α or μ class human GSTs, a detectable cross-reactivity was not observed in Western blot analysis (data not shown). These results suggest that μ or α class GST isoenzymes are either absent or present in very low concentrations and that GST π is the major isoenzyme in all these cell lines.

Data on GSH peroxidase activity are also presented in

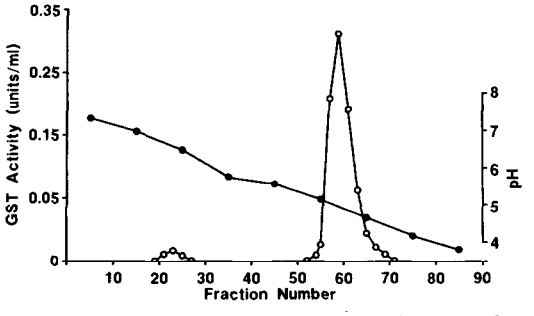

Fig. 2. Chromatofocusing of affinity purified GST from normal human bladder tissue in pH range 7–4 on a PBE 94 column. The column was equilibrated with 25 mM imidazole-HCl (pH 7.4) buffer. Elution was carried out with polybuffer 74-HCl (pH 4.0), diluted 1:8 with deaerated water. Fractions of 1.5 mL each were collected and monitored for pH (●) and GST activity (○) with CDNB. The flow rate was 20 mL/hr. One unit of enzyme catalyzed the conjugation of 1 μmol of CDNB to GSH/min at 25°.

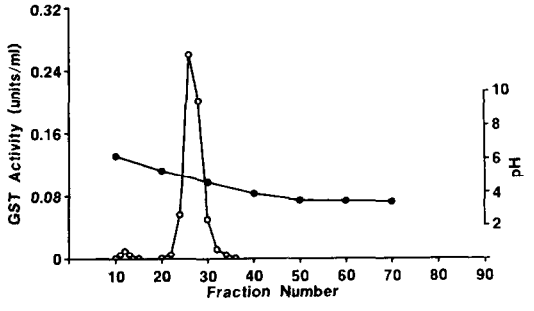

Fig. 3. Chromatofocusing of GST in 14,000 g supernatant fraction of human bladder cancer cell line SCaBER in the pH range 7–4 on a PBE 94 column. Fractions of 2.2 mL each were collected and monitored for pH (●) and GST activity (○) with CDNB. The flow rate was 22 mL/hr. Other conditions were similar to those described in the legend to Fig. 2.

Table 1. GSH peroxidase activity in cell lines J82, SCaBER, 5637, and HT-1197 was 43, 69, 88, and 92% of that in the FHs 738 Bl cell line (Table 1). Interestingly, this activity was about 1.2-fold higher in cell line FCCB-1 (Table 1).

Overexpression of GST π has been reported in certain human tumors [7, 8]. In the present study, out of five human bladder cancer cell lines, significant overexpression of GST π was observed in only three cell lines: SCaBER, 5637, and HT-1197. These results are similar to those of our previous study [19] with three human small cell lung cancer cell lines where we found that GST π was elevated in only two cell lines. In a different study, on the other hand, GST π was found to be absent in six other small cell lung cancer cell lines [23], although this enzyme was shown to be overexpressed in non-small cell lung cancer cell lines [23]. Similarly, analysis of GST π in lung tumors and adjacent normal tissues did not reveal a uniform elevation of this isoenzyme in every tumor sample [9, 10]. The results of the present study suggest that GST π may not be an ideal marker for human bladder carcinoma. However, before proving conclusively that GST π may not be a marker for this tumor type, comparison of this isoenzyme must be carried out on a larger number of normal and neoplastic bladder tissues.

GSH-dependent enzymes, particularly GST and GSH peroxidase, have been implicated recently in cellular drug resistance [11–14]. The key role of these enzymes in the detoxification process suggests that their relative expression may be an important factor in determining tumor sensitivity to chemotherapeutic agents [10]. Recent studies provide direct evidence for the involvement of GSH-related enzymes in drug resistance [12, 13, 24]. For example, doxorubicin resistance in the MCF-7 human breast cancer cell line has been attributed to elevated GSH peroxidase activity [12]. Even though we have not determined a cytotoxicity profile in this study, the results presented suggest that these bladder cancer cell lines may exhibit differential sensitivity to various chemotherapeutic agents due to differences in GSH peroxidase and GST activities. In addition, it is interesting to note that GSH peroxidase activity was found to be lower in cell lines which exhibited elevated GST activity. The reasons and significance of down-regulation of GSH peroxidase activity in cell lines with high GST activity, however, remain to be elucidated.

In summary, results of the present study suggest that π class GST isoenzyme(s) is the major form in normal human bladder, a normal bladder cell line, and bladder cancer cell lines used in this study. These results also suggest that whereas π class GST is uniformly overexpressed in several human tumors such as renal and colon carcinomas [7, 8, 25], this isoenzyme may not be uniformly overexpressed in bladder carcinomas. Even though the present study suggests that overexpression of π class GST in human tumors may be tissue specific, analysis of this isoenzyme in normal and malignant tissues of different tissue origin will be needed not only to substantiate this contention but also to identify human tumors where π class GST can be used as a tumor marker. In addition, studies are also needed to understand the mechanisms by which the expression of GSH-related enzymes is affected in various carcinomas and in cell lines resistant to chemotherapeutic agents. These studies will be important in determining the role of GSH-related enzymes in carcinogenesis and drug resistance.

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REFERENCES

1. Jakoby WB and Habig WH, Glutathione transferases. In: *Enzymatic Basis of Detoxication* (Ed. Jakoby WB), Vol. 2, pp. 63–94. Academic Press, New York, 1980.
2. Mannervik B, Alin P, Guthenberg C, Jensson H, Tahir MK, Warholm M and Jornvall H, Identification of three classes of cytosolic glutathione transferase common to several mammalian species: Correlation between structural data and enzymatic properties. *Proc Natl Acad Sci USA* **82**: 7202–7206, 1985.
3. Meister A and Anderson ME, Glutathione. *Annu Rev Biochem* **52**: 711–760, 1983.
4. Litwack G, Ketterer B and Arias IM, Ligandin: A hepatic protein which binds steroids, bilirubin, carcinogens, and a number of exogenous organic anions. *Nature* **234**: 466–467, 1971.
5. Satoh K, Kitahara A, Soma Y, Inaba Y, Hatayama I and Sato K, Purification, induction, and distribution of placental glutathione transferase: A new marker enzyme for preneoplastic cells in the rat chemical hepatocarcinogenesis. *Proc Natl Acad Sci USA* **82**: 3964–3968, 1985.
6. Meyer DJ, Beale D, Tan KH, Coles B and Ketterer B, Glutathione transferases in primary rat hepatomas: The isolation of a form with GSH peroxidase activity. *FEBS Lett* **184**: 139–143, 1985.
7. Di Ilio C, Del Boccio G, Aceto A and Federici G, Alteration of glutathione transferase isoenzyme concentrations in human renal carcinoma. *Carcinogenesis* **8**: 861–864, 1987.
8. Kodate C, Fukushi A, Narita T, Kudo H, Soma Y and Sato K, Human placental form of glutathione *S*-transferase (GST-π) as a new immuno-histochemical marker for human colonic carcinoma. *Gann* **77**: 226–2199, 1986.
9. Singh SV, Haque AK, Ahmad H, Medh RD and Awasthi YC, Glutathione *S*-transferase isoenzymes in human lung tumors. *Carcinogenesis* **9**: 1681–1685, 1988.
10. Carmichael J, Forrester LM, Lewis AD, Hayes JD, Hayes PC and Wolf CR, Glutathione *S*-transferase isoenzymes and glutathione peroxidase activity in normal and tumour samples from human lung. *Carcinogenesis* **9**: 1617–1621, 1988.
11. Wang AL and Tew KD, Increased glutathione *S*-transferase activity in a cell line with acquired resistance to nitrogen mustards. *Cancer Treat Rep* **69**: 677–682, 1985.
12. Sinha BK, Katki AG, Batist G, Cowan KH and Myers CE, Differential formation of hydroxyl radicals by Adriamycin in sensitive and resistant MCF-7 human breast tumor cells: Implications for the mechanism of action. *Biochemistry* **26**: 3776–3781, 1987.
13. Singh SV, Nair S, Ahmad H, Awasthi YC and Krishan A, Glutathione *S*-transferases and glutathione peroxidases in doxorubicin-resistant murine leukemic P388 cells. *Biochem Pharmacol* **38**: 3505–3510, 1989.
14. Gupta V, Singh SV, Ahmad H, Medh RD and Awasthi YC, Glutathione and glutathione *S*-transferases in a

human plasma cell line resistant to melphalan. *Biochem Pharmacol* **38**: 1993–2000, 1989.
15. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferases: The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
16. Beutler E, Glutathione peroxidase, In: *Red Cell Metabolism: A Manual of Biochemical Methods* (Ed. Beutler E), p. 74. Grune & Stratton, New York, 1984.
17. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
18. Towbin H, Staehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* **76**: 4350–4354, 1979.
19. Awasthi YC, Singh SV, Ahmad H, Moller PC and Gupta V, Expression of glutathione *S*-transferase isoenzymes in human small cell lung cancer cell lines. *Carcinogenesis* **9**: 89–93, 1988.
20. Ishikawa T, Tsuchida S, Satoh K and Sato K, The subunit structure of a major glutathione *S*-transferase form, M_T, in rat testis: Evidence for a heterodimer consisting of subunits with different isoelectric points. *Eur J Biochem* **176**: 551–557, 1988.
21. Singh SV, Ahmad H, Kurosky A and Awasthi YC, Purification and characterization of unique glutathione *S*-transferases from human muscle. *Arch Biochem Biophys* **264**: 13–22, 1988.
22. Ahmad H, Singh SV, Medh RD, Ansari GAS, Kurosky A and Awasthi YC, Differential expression of α, μ, and π classes of isoenzymes of glutathione *S*-transferase in bovine lens, cornea, and retina. *Arch Biochem Biophys* **266**: 416–426, 1988.
23. Eimoto H, Tsutsumi M, Nakajima A, Yamamoto K, Takashima Y, Maruyama H and Konishi Y, Expression of the glutathione *S*-transferase placental form in human lung carcinomas. *Carcinogenesis* **9**: 2325–2327, 1988.
24. Dulik DM, Fenselau C and Hilton J, Characterization of melphalan-glutathione adducts whose formation is catalyzed by glutathione transferases. *Biochem Pharmacol* **35**: 3405–3409, 1986.
25. Mekhail-Ishak K, Hudson N, Tsao M-S and Batist G, Implications for therapy of drug metabolizing enzymes in human colon cancer. *Cancer Res* **49**: 4866–4869, 1989.

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Sex differences and endocrine regulation of morphine oxidation in rat liver

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Sex differentiation of rat liver metabolism of steroids is heavily dependent on neonatal imprinting by testicular androgens. This imprinting process has been indicated as a prerequisite for a hypothalamo-pituitary regulation of rat liver metabolism. Castration of male neonates interferes with the hypothalamic imprinting by testicular androgens [1]. The neuroendocrine effects on rat liver are mediated by the sex differentiated secretory pattern of growth hormone (GH) [2, 3] which is regulated by the hypothalamus through the combined action of somatostatin and a GH-releasing factor [4, 5]. The sexual dimorphism in GH-secretion occurs at puberty, in parallel with the development of gonadal function and maintenance of a male secretory pattern in the adult rat is dependent on circulating androgens [2, 6].

Sex differentiated rat liver metabolism has been described for a variety of drugs and steroids [7, 8]. A number of endocrine manipulations modifies hepatic metabolism [1, 6–9]. Castration of male rats at neonatal or at adult age, as well as continuous infusion of GH to male rats tends to feminize hepatic metabolism [1, 3, 4, 6, 9] and hypophysectomy of male and female rats markedly attenuates sex differences in liver metabolism [7, 9].

Perinantal exposure of rats to morphine is known to interfere with the development of sexual behaviour [10, 11] and to interact with certain endocrine parameters [10]. We recently reported on the effects of perinatal exposure to morphine on hepatic metabolism in adult rats of both sexes [12]. The microsomal capacity to perform several cytochrome P450 mediated reactions was significantly different in the morphine treated rats compared to controls and the effects were similar in males and females.

The present study was designed to investigate whether N-demethylation of morphine is regulated via the hypothalamo-pituitary-liver axis in a way similar to that previously described for metabolism of 4-androstene-3,17-dione (androstenedione) [3, 4, 6–9].

Materials and Methods

In experiment 1 male and female Sprague–Dawley rats (Møllegaard Breeding Center, Ejeby, Denmark; were killed at either 21 or at 56 days of age. Hypophysectomy was performed at Møllegaards at the age of 42 days by an intraauricular method under short anaesthesia with metohexital sodium (Brietal). These rats were killed at 56 days of age and were compared to their untreated controls.

In experiment 2 male Sprague–Dawley rats (ALAB, Stockholm, Sweden) were castrated either neonatally, within 36 hr of birth, or at 42 days of age. All rats in the experiment were killed at the age of 61 days.

Adult male rats in experiment 3 were treated with continuous infusion of bGH (6 μg/hr) in Alzet osmotic minipumps, model 2001 (Palo Alta, CA). bGH (USDA-bGH-B-1) was kindly supplied by the NIIDK (MD, U.S.A.). Treatment was started at 49 days of age and the rats, including untreated male and female controls, were killed at 56 days of age.

Methods. The animals were starved overnight before decapitation in order to reduce hepatic levels of glycogen. Liver microsomes were prepared according to Ernster *et al.* [13] and protein was measured as described by Lowry *et al.* [14]. Metabolism of androstenedione was measured as previously described [8] and N-demethylation of morphine according to Rane *et al.* [15]. 4-[4-^{14}C]Androstene-3,17-dione (59 mCi/mmol) was obtained from the Radiochemical Center (Amersham, U.K.) and unlabelled androstenedione from the Upjohn Company (Kalamazoo, NI). Morphine chloride was purchased from Apoteksbolaget (Gothenburg, Sweden).

Statistical analysis. Statistical analysis was performed using the Wilcoxon rank sum test [16] and the level of significance was set at $P < 0.05$. All values were expressed as means ± SD.