Jpn. J. Cancer Res. **89**, 641–648, June 1998

Identification of Glutathione *S*-Transferase p-1 as the Class Pi Form Dominantly Expressed in Mouse Hepatic Adenomas

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To clarify which of the two genes for pi class glutathione *S*-transferases (GSTs) (p-1 and p-2) is dominantly expressed in mouse hepatic adenomas, the relative mRNA levels were examined by means of the reverse transcription-polymerase chain reaction (RT-PCR). Hepatic adenomas were induced in male and female B6C3F1 mice by diethylnitrosamine treatment. Northern blot analysis revealed that pi class mRNA levels were decreased in adenomas of male mice, but increased in those of females, with reference to the respective surrounding non-adenoma tissues. In contrast to the marked sex difference in surrounding tissues, pi class GST mRNA levels in adenomas were almost the same in both males and females. To evaluate p-1 and p-2 mRNA levels separately, the products of RT-PCR employing primers common for both cDNAs were digested with the endonuclease *Ban*I (specific for p-2) and then resolved by electrophoresis. The p-1 mRNA was thus found to be dominant in adenomas of both female and male mice. The p-2 mRNA levels were increased in the lesions as compared with those in the surrounding non-adenoma tissues. Recombinant p-1 and p-2 proteins were expressed in *Escherichia coli*. Unlike p-1, the p-2 protein did not show any significant activity towards 1-chloro-2,4-dinitrobenzene and did not bind to *S*-hexylglutathione-Sepharose despite immunological cross-reactivity. The dominant pi class form in adenomas could also be identified as p-1 by its binding to *S*-hexylglutathione-Sepharose. Single radial immunodiffusion analyses confirmed that the p-1 protein levels were in line with the mRNA findings, i.e., 1.9±0.3 mg/g adenoma as compared to 6.5±1.2 mg/g non-adenoma tissue for males and 2.2±0.6 mg/g as compared to 0.7±0.2 mg/g for females. The results thus indicated that the change of pi class forms in adenomas is caused mainly by alteration in the p-1 level and the contribution of p-2 is minimal.

Key words: Hepatocarcinogenesis — Glutathione *S*-transferase — Sex difference — Mouse — Polymerase chain reaction

Cytosolic glutathione *S*-transferases (GSTs) are a family of multifunctional dimeric enzymes that catalyze the conjugation of glutathione to electrophilic xenobiotics.[1, 2] On the basis of nucleotide and amino acid sequence similarities, mammalian GSTs have been grouped into four classes, alpha, mu, pi and theta.[3] Multiple forms of the alpha and mu classes are encoded by individual genes.[4, 5] The pi class forms have attracted particular attention because their expression is associated with malignant transformation.[6] The rat pi class form, GST-P, is specifically induced at an early stage of chemical hepatocarcinogenesis.[7]

Unlike rat GST-P, the mouse pi class form, GST-II, is expressed constitutively in livers of males under testosterone regulation.[8] Our previous study revealed that the pi class form is increased in hepatic preneoplastic lesions of female mice, but repressed in those of males, relative to the background.[9, 10] Although the pi class forms in the rat and man are considered to be encoded by single genes,[11, 12] recent studies have revealed the presence of two genes in the mouse.[13, 14] One gene (*Gst p-1* or *mGSTpiB*) encodes GST-II while transcription from the other (*Gst p-2* or *mGSTpiA*) results in a highly homologous protein with substitutions in only 6 of 210 amino acid residues but lacking enzymatic activity. The *Gst p-1* gene is transcribed at higher levels in normal livers of both sexes than the *Gst p-2* gene.[13] However, it has hitherto remained unclear which of the genes is dominantly expressed in hepatic nodules and adenomas. Furthermore, the question of whether the amounts of pi class forms in preneoplastic and neoplastic lesions differ between female and male mice requires an answer.

In the present study, we therefore examined relative p-1 and p-2 mRNA levels in hepatic adenomas by means of reverse transcription-polymerase chain reaction (RT-PCR) and protein amounts by means of immunological and bio-

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chemical methods. It was found that p-1 mRNA and protein are dominantly expressed in the lesions of both sexes to similar extents.

MATERIALS AND METHODS

Reagents Diethylnitrosamine (DEN) and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Wako Pure Chemicals (Osaka), ethacrynic acid from Sigma (St Louis, MO), fetal bovine serum from JRH Bioscience (Lenexa, KS), [α-^{32}P]dCTP (3,000 Ci/mmol) from New England Nuclear (Boston, MA), nitrocellulose filters from Bio-Rad (Richmond, CA) and restriction enzymes from Toyobo (Osaka). *Taq* polymerase and *rTth* DNA polymerase were from Takara (Shiga) and Perkin-Elmer (Norwalk, CT), respectively, and the oligodeoxynucleotide primers were from Hokkaido System Science (Sapporo). All other reagents were of analytical grade.

Induction of mouse hepatic adenomas Male C3H/He and female C57BL/6 mice were obtained from Charles River Japan Inc. (Kanagawa). B6C3F1 mice, produced in our laboratory by breeding these two strains, were given a single i.p. injection of DEN, 10 mg/kg body weight on day 15 after birth to initiate hepatocarcinogenesis.[9] Male and female mice were killed at 9 and 11 months after DEN treatment, respectively, and hepatic nodules more than 6 mm in diameter were quickly excised. These lesions showed histopathological findings of hepatocellular adenoma as defined by Frith and Ward.[15] Surrounding non-adenoma tissues and normal livers of untreated animals of the same age were used as controls. Samples were stored at −80°C until use.

Northern blot analysis Total RNAs were isolated from liver tissues by the method of Chomczynski and Sacchi.[16] Samples of RNA (10 μg) denatured with formaldehyde and formamide were separated on 1.5% agarose gels containing 0.6 *M* formaldehyde and blotted on nitrocellulose filters. Hybridization was performed in 6× SSC containing 0.1% sodium dodecyl sulfate (SDS), 10× Denhardt's solution and 10% dextran sulfate with ^{32}P-labeled GST-II cDNA[17] probed at 65°C for 18 h. X-Ray films (Kodak XAR-5, Kodak, Tokyo) were exposed to the filters at −80°C with an intensifying screen, after the latter had been washed in 1× SSC-0.1% SDS at 65°C.

RT-PCR To synthesize single-stranded p-1 and p-2 cDNAs, 0.2 μg aliquots of total RNA were incubated at 70°C for 10 min with 5 units of *rTth* DNA polymerase in a 20 μl reaction volume containing 1 m*M* $MnCl_2$, 200 μ*M* of each dNTP, 1× reverse transcriptase buffer and 0.75 μ*M* 3′ primer. Subsequently, the mixture was combined with 80 μl of PCR master mixture containing 1× chelating buffer, 20 nmol of $MgCl_2$ and 15 pmol of 5′ primer. After denaturation at 95°C for 80 s, amplification was performed for 40 cycles: denaturation, 95°C for 40 s; annealing, 57°C for 40 s; extension, 72°C for 90 s. The 5′ and 3′ primers were common for p-1 and p-2 and had the sequences 5′-ATGCTGCTGGCTGACCAGGGCCAGAG-3′ (230–255 for p-1)[13] and 5′-ATCTTGGGCCGGGC-ACTGAGGCGAG-3′ (707–731), respectively. The amplified DNAs were purified by phenol-chloroform extraction and digested with *Ban*I. Subsequently, the DNAs were electrophoresed in 3% agarose gels and stained with ethidium bromide.

Construction of expression vectors for GST p-1 and p-2 For construction of expression vectors for GST p-1 and p-2, cDNAs encoding the entire open reading frames were generated by RT-PCR as described above using other primer sets designed to contain the *Bam*HI linkers. The 5′ primer specific for p-1 had the sequence 5′-GTCCG-GATCCTGAGTACCCCTCTGTCTACG-3′ (107–136) and that for p-2 had 5′-TTGGATCCCTGAGACACCTCTCT-GACTATT-3′ (129–158). The 3′ primer (5′-TTGGATC-CTTTATTAGTGCTGGGAAAAC-3′) (837–864 for p-1) was common for both p-1 and p-2. For cloning, each cDNA was re-amplified using internal primers designed to contain the *Eco*RI restriction site at the 5′ side and the *Pst*I site at the 3′ side. The 5′ primer had the sequence 5′-GCACTGGAATTCAGGACAGCAGCCATGCC-3′ (139–177 for p-1) and that for the 3′ primer was 5′-TTAT-TACTGCAGGGAAAACGGGGACAAGA-3′ (827–855 for p-1). PCR was performed for 30 cycles at 96°C for 40 s, 57°C for 40 s and 72°C for 90 s in a reaction volume of 100 μl, containing 2.5 units of *Taq* DNA polymerase, 1× Taq buffer, 200 μ*M* of each dNTP, 0.3 μ*M* of the primers and 1 μl of amplified RT-PCR mixture. Each amplified cDNA was digested with *Eco*RI and *Pst*I and the digest was fractionated on a 0.8% agarose gel. The 695 bp fragment containing the entire p-1 or p-2 open reading frames was ligated downstream of a *trc* promoter in the plasmid *p*Trc99A (Pharmacia Biotec, Uppsala, Sweden). The nucleotide sequences of p-1 and p-2 cDNAs were confirmed by DNA sequencing.[18]

Expression of recombinant GST p-1 and p-2 *Escherichia coli* JM105 cells transformed with the above expression vectors were grown overnight to confluence in 10 ml of Luria-Bertani (LB) medium supplemented with 50 μg/ml ampicillin at 37°C with vigorous shaking. The medium was then mixed with 250 ml of fresh LB medium, and cultured for about 2 h. At the log phase, isopropyl-β-thiogalactopyranoside (IPTG) was added at a final 1 m*M* concentration. After an additional 3 h culture, the cells were harvested by centrifugation, resuspended in 5 ml of the lysis buffer (20 m*M* Tris-HCl, pH 8.0, 1 m*M* dithiothreitol, 1 m*M* ethylenediaminetetraacetic acid (EDTA), 5% (v/v) glycerol, 22 m*M* NH_4Cl, 0.1 m*M* phenylmethanesulfonyl fluoride (PMSF)), and disrupted using a sonicator (Model 200M, Kubota, Tokyo). Cell-free extracts were obtained by centrifugation at 105,000*g* for

45 min at 0°C. Recombinant GST p-1 was further purified by *S*-hexylglutathione-Sepharose affinity chromatography as described previously.[19] The specific activity of GST p-1 thus purified was 94 units/mg protein, comparable to that of GST-II.[8]

GST activity GST activity was assayed using CDNB as the substrate by the method of Habig *et al.*[20] In some experiments, ethacrynic acid was also used as a substrate.

Purification of GSTs from mouse liver Mouse liver tissue was homogenized in 9 vol. of 10 m*M* Tris-HCl, pH 7.8, containing 0.2 *M* NaCl. The homogenates were centrifuged at 105,000*g* for 45 min and the supernatants were applied to an *S*-hexylglutathione-Sepharose column equilibrated with the same buffer. After washing, GSTs were eluted with 5 m*M* *S*-hexylglutathione in the buffer.[8] GST-II was purified from normal livers as reported previously.[8]

Immunodiffusion Double immunodiffusion and single radial immunodiffusion were performed with anti-GST p-1 antibody according to the methods of Ouchterlony[21] and Mancini *et al.*,[22] respectively. Anti-GST p-1 antibody was raised in a rabbit using the method applied earlier for the generation of anti-GST-II antibody.[8]

Immunoblot analysis SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 12.5% acrylamide gels by the method of Laemmli[23] and immunoblotting was carried out using anti-GST p-1 antibody after Towbin *et al.*[24] Two-dimensional gel electrophoresis was carried out as reported previously.[25]

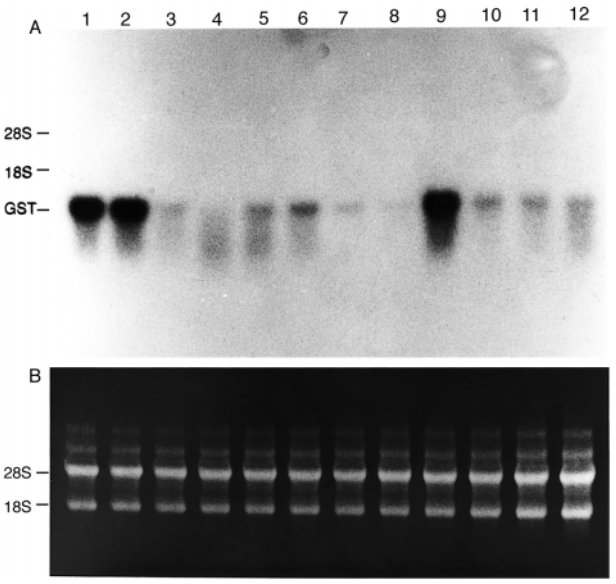

Fig. 1. Northern blot analysis of pi class GST mRNA in hepatic adenomas of female and male mice. Ten microgram aliquots of RNA were analyzed by denaturing agarose gel electrophoresis and hybridized with ^{32}P-labeled GST-II cDNA as described in the text (A) or stained with ethidium bromide (B). Lanes 1 and 2, surrounding non-adenoma tissues of males; lanes 3–6, hepatic adenomas of males; lanes 7 and 8 surrounding non-adenoma tissues of females; lanes 9–12, hepatic adenomas of females. 28S and 18S, 28S and 18S rRNA, respectively; GST, the position of pi class GST mRNAs.

RESULTS

GST p-1 and p-2 mRNAs in mouse hepatic adenomas Pi class GST mRNA levels in mouse hepatic adenomas were examined by northern blotting using the GST-II cDNA as a probe. As indicated in Fig. 1A, mRNA levels were decreased in adenomas of male mice, but increased in those of females as compared with the respective surrounding non-adenoma tissues. The levels in surrounding non-adenoma tissues of male and female mice were similar to those in normal livers of the respective sexes (data not shown). In contrast to the sex difference in the surrounding tissues, pi class GST mRNA levels in adenomas were similar between females and males, although some variations were observed among the adenoma samples and one female case exhibited enhanced expression (lane 9, Fig. 1). To evaluate p-1 and p-2 mRNA levels separately, the products of RT-PCR employing 5′ and 3′ primers common for both p-1 and p-2 cDNAs were digested with the endonuclease *Ban*I (specific for p-2) and then resolved by agarose gel electrophoresis (see "Materials and Methods"). Under these conditions, the 512 bp PCR product derived from the p-2 cDNA was completely converted to a 256 bp fragment (lane 1, Fig. 2), while that from the p-1 cDNA was unaltered (lane 8). Addition of decreasing amounts of p-2 cDNA to p-1 cDNA as templates resulted in corresponding decreases of the 256 bp band (lanes 2–7). When the p-2/p-1 cDNA ratio was 1/31 or below (lanes 6–8), the 256 bp band was undetectable. Only the 512 bp band (p-1 cDNA) was evident in the surrounding non-adenoma tissues of both sexes (males, lanes 1 and 2 in Fig. 3; females, lanes 7 and 8). Although it was dominant in adenomas of both female and male mice, only small, but significant, amounts of the 256 bp band (p-2 cDNA) were also detected. A strong 256 bp band was detected in the one case (lane 9, Fig. 3) that showed a high pi class GST mRNA level by northern blotting (Fig. 1, lane 9).

Properties of recombinant p-2 protein expressed in *E. coli* cDNAs encoding p-1 and p-2 proteins were cloned downstream of a *trc* promoter (see "Materials and Methods") and these clones were termed *p*GTp1 and *p*GTp2, respectively. The nucleotide sequences of p-1 and p-2 cDNAs thus prepared were confirmed to be identical with the respective sequences reported previously.[13] When *E. coli* JM105 transformed with *p*GTp1 was grown in the presence of IPTG, cultures expressed functional GST p-1

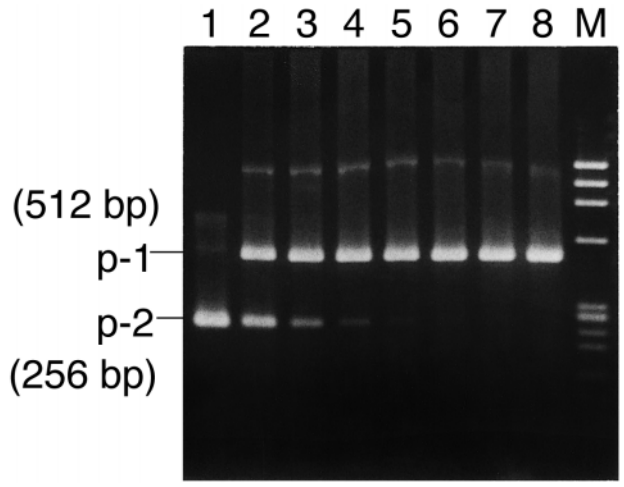

Fig. 2. Separation of p-1 and p-2 cDNAs by digestion with a p-2-specific restriction enzyme. Mixtures of p-1 cDNA and p-2 cDNA prepared for expression of recombinant proteins were amplified by PCR using common primers as described in the text. PCR products were then digested with *Ban*I, subjected to electrophoresis in a 3% agarose gel, and visualized with ethidium bromide. Lanes 1 and 8 represent the results for p-2 cDNA and p-1 cDNA alone, respectively. Mixtures of constant amounts of p-1 cDNA and decreasing amounts of p-2 cDNA were used as templates and the p-2/p-1 ratios are 1, 1/3, 1/7, 1/15, 1/31, 1/61 for lanes 2–7, respectively. M, size markers of ϕX174 DNA digested with *Hae*III. p-1, p-1 cDNA (512 bp); p-2, p-2 cDNA (256 bp).

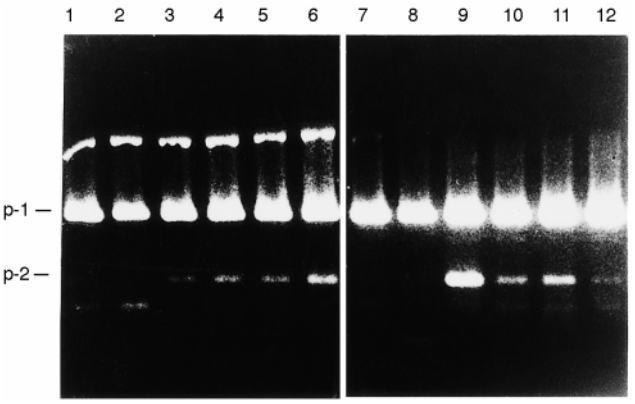

Fig. 3. Relative levels of p-1 and p-2 mRNAs in hepatic adenomas of female and male mice. RT-PCR was carried out using total RNA and common primers as described in the text. PCR products digested with *Ban*I were subjected to electrophoresis as described for Fig. 2. p-1 and p-2 are the same as in Fig. 2. Lane numbers are the same as in Fig. 1.

with activity toward CDNB. In contrast, the p-2 protein did not show significant activity toward CDNB or ethacrynic acid. The p-1 protein bound to *S*-hexylglutathione-Sepharose (Fig. 4A), while p-2 did not bind to it (Fig. 4B) or to glutathione-Sepharose, confirming the result reported by Xu and Stambrook.[14] Ouchterlony double immunodiffusion was carried out using crude extracts of *E. coli* to examine the immunological relationship between the two proteins. The precipitin line between p-1 and anti-GST p-1 antibody formed a spur with the line between p-2 and the antibody (Fig. 5A), indicating that p-1 and p-2 share a common antigenicity, but are not identical to one another. On single radial immunodiffusion with anti-GST p-1 antibody, crude extracts of *E. coli* expressing p-2 generated smaller precipitin rings than those containing p-1, in a comparison of the same amounts of extracts (Fig. 5B). A linear relationship was observed between increasing amounts of p-2 extract and the diameter of the precipitin ring with a slope of about one-third of that for the p-1 extract. Two-dimensional gel electrophoresis followed by immunoblotting with anti-GST p-1 antibody revealed that the p-2 subunit has the same isoelectric point and relative molecular mass as the p-1 subunit (data not shown).

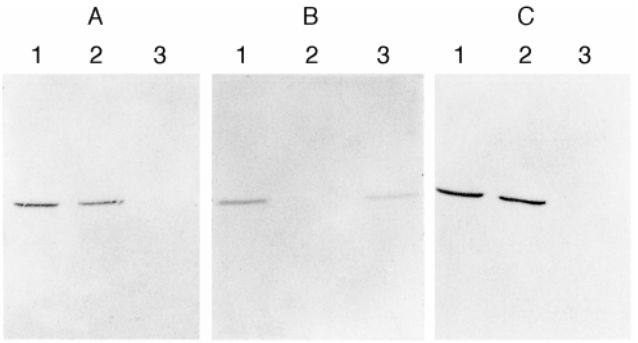

Fig. 4. Binding of recombinant p-1 and p-2 proteins expressed in *E. coli* and pi class GST in adenomas to *S*-hexylglutathione-Sepharose. Affinity chromatography using an *S*-hexylglutathione-Sepharose column was carried out as described in the text. Immunoblotting was performed with anti-GST p-1 antibody. A and B, Extracts of *E. coli* expressing p-1 and p-2, respectively; C, Supernatant from adenoma tissue from a female mouse. In each panel, lane 1 contains crude extract; lane 2, fractions bound to the affinity column; lane 3, fractions not bound to the column.

Pi class GSTs in mouse hepatic adenomas Since the p-1 and p-2 proteins exhibited differences in binding to *S*-hexylglutathione-Sepharose, the supernatants of hepatic adenomas were applied to the column to examine which of the two was dominantly expressed in the lesions. Immunoblot analysis with anti-GST p-1 antibody revealed pi class GST in the bound, but not the unbound fractions in all cases examined (Fig. 4C). p-2 protein unbound to the column was not detected even in the sample that showed a strong p-2 cDNA band by RT-PCR. Further-

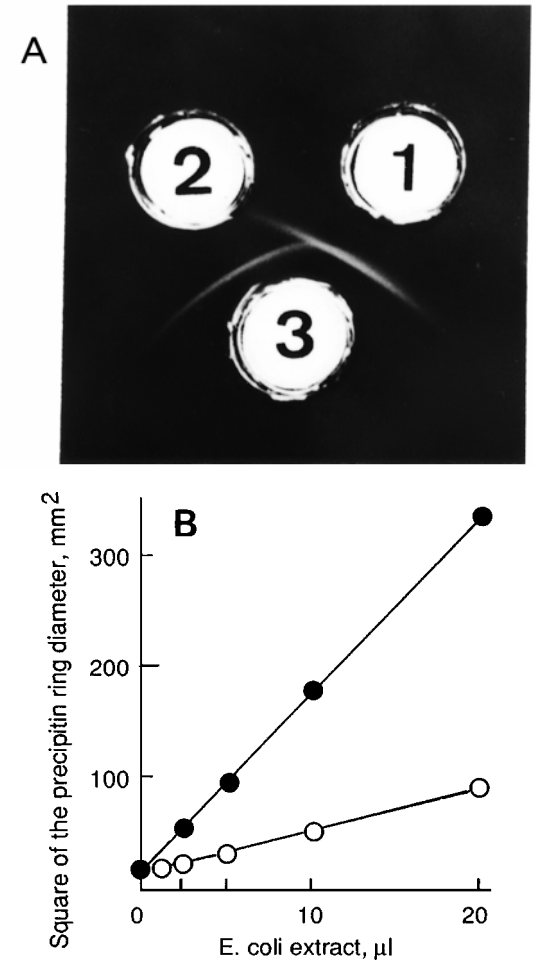

Fig. 5. Immunological analysis of recombinant p-1 and p-2 proteins. A, Double immunodiffusion. Wells 1 and 2, crude extracts of *E. coli* expressing p-1 and p-2, respectively; 3, anti-GST p-1 antibody. B, Different linearities of p-1 and p-2 proteins on single radial immunodiffusion. Squares of the diameters of precipitin rings are plotted against the amounts of crude extracts of *E. coli* expressing p-1 (closed circles) and p-2 (open circles). p-1 and p-2 protein amounts in these extracts were estimated to be comparable by SDS-PAGE. In the case of p-1, 20 μl of *E. coli* extract included 2.5 μg of p-1 protein.

more, dilution series of the supernatants exhibited linear curves parallel to the line for p-1 in single radial immunodiffusion tests (Fig. 5B). These results indicated the dominant pi class form in adenomas to be p-1. Data for p-1 contents of adenomas, surrounding non-adenoma tissues, and normal livers of untreated mice determined by this test are summarized in Table I. The p-1 level was about 9-fold higher in male non-adenoma tissues (6.5±1.2 mg/g tissue) than in female non-adenoma tissues (0.7±0.2 mg/g tissue). The values, which are similar to those in

Table I. GST p-1 Content in Hepatic Adenomas of Female and Male Mice

Sex	GST p-1 content[a)] (mg protein/g tissue)		
	Normal liver of untreated mouse	Surrounding non-adenoma tissue	Adenoma
Male (4)	6.7±1.1	6.5±1.2	1.9±0.3
Female (4)	0.6±0.1	0.7±0.2	2.2±0.6

a) Data are mean±SD values for four samples in each group.

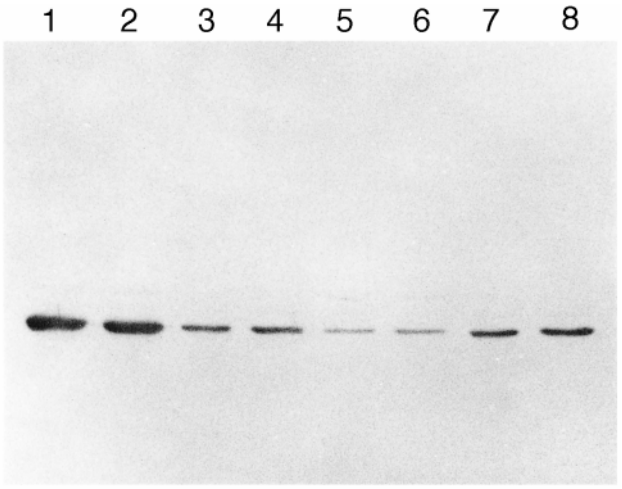

Fig. 6. Immunoblots with anti-GST p-1 antibody of supernatant fractions from hepatic adenomas and the surrounding non-adenoma tissues of both sexes. Protein samples (100 μg) from 105,000*g* supernatants were applied to each lane. Lanes 1 and 2, surrounding tissues of males; lanes 3 and 4, hepatic adenomas of males; lanes 5 and 6, surrounding tissues of females; lanes 7 and 8, hepatic adenomas of females. The sample applied to lane 7 was derived from the adenoma that exhibited a high pi class mRNA level (lane 9, Fig. 1).

normal livers of the respective sexes, were decreased in hepatic adenomas of male mice (1.9±0.3 mg/g tissue) and increased in those of female mice (2.2±0.6 mg/g tissue), as compared with the values in the respective surrounding non-adenoma tissues, but did not significantly differ between adenomas of male and female mice. These results were supported by the immunoblotting findings with the anti-GST p-1 antibody (Fig. 6). p-1 protein levels in adenomas showed similar alteration patterns to those of its mRNA (Fig. 1). However, one adenoma sample exhibiting a high mRNA level (lane 7, Fig. 6) did not show an enhanced protein level, as compared with other adenoma samples.

GST p-1 and p-2 mRNAs in normal tissues Relative amounts of p-1 and p-2 mRNAs were also investigated in normal tissues of male mice by the RT-PCR method. As

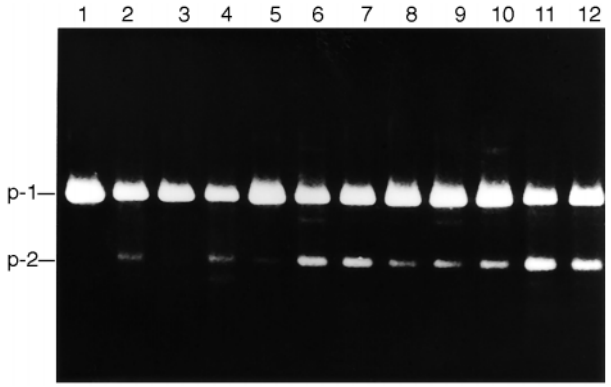

Fig. 7. Relative levels of p-1 and p-2 mRNAs in normal male tissues. RT-PCR and subsequent *Ban*I digestion were carried out using total RNA from the following tissues, as described in Fig. 3. Lane 1, liver; 2 , kidney; 3, adrenal gland; 4, heart; 5, lung; 6, stomach; 7, esophagus; 8, spleen; 9, small intestine; 10, colon; 11, tongue; 12, skeletal muscle. p-1 and p-2 are the same as in Fig. 2.

shown in Fig. 7, the p-1 mRNA was dominant in all tissues examined, with p-2 mRNA levels being relatively high in the tongue and skeletal muscle, followed by the esophagus, stomach, small intestine and colon.

DISCUSSION

A previous study utilizing primers specific for p-1 or p-2 cDNAs demonstrated p-2 mRNA to be expressed in certain tissues,[14] but did not clarify the relative proportions of p-1 and p-2. The p-1 and p-2 mRNAs are not separable by northern blotting using the GST-II cDNA as a probe because of similar mobilities on electrophoresis, but since the p-2 cDNA has a nucleotide sequence sensitive to *Ban*I endonuclease,[13] the proportion of the two mRNAs could be examined by RT-PCR using primers common for both cDNAs, followed by digestion with the restriction enzyme and electrophoresis. Thus, it was revealed that p-1 mRNA predominates in hepatic adenomas of both male and female mice (Fig. 3), although p-2 mRNA levels were increased as compared to the surrounding tissues. Since the 5′ and 3′ primers used were common for p-1 and p-2 cDNAs, amplification of the two cDNAs should be closely related. However, we could not rule out the possibility that the RT-PCR for p-1 mRNA may reach a plateau under PCR conditions, because the intensities of p-1 bands were similar in surrounding tissues of females and those of males.

Since pi class GST is known to be expressed in several cancer cell lines,[2] we examined the relative proportions of p-1 and p-2 mRNAs in 4 mouse cell lines, NCTC1469, Balb/3T3, F9 and Ehrlich ascites cells. The p-1 mRNA was dominant in all cases while p-2 mRNA was detected in NCTC1469 and F9 cells as a minor component (data not shown). These results also support the view that low p-2 expression in adenomas is unlikely to be due to surrounding non-adenoma tissues that might contaminate the samples.

The dominant pi class protein in adenomas was also identified as p-1 on the basis of its *S*-hexylglutathione-Sepharose binding (Fig. 4), so that the results indicate that the change of pi class forms in the lesions is caused mainly by alteration of p-1 expression and the contribution of p-2 is minimal. The p-1 protein levels were proportional to pi class mRNA levels in surrounding tissues of both sexes and most adenomas (Fig. 6). However, an increase in protein amount was not evident in one adenoma sample that exhibited a high mRNA level, comparable to that of non-adenoma tissue of males. Although p-2 mRNA was strongly expressed in this case, the protein was not detected (Fig. 4C), suggesting that both p-1 and p-2 protein levels might be repressed at post-transcriptional levels.

Previous immunohistochemical investigations indicated that the pi class form is increased in nodules of females but repressed in those of males, relative to the background.[9, 10] However, p-1 protein levels in hepatic adenomas were not different between females and males (Table I), despite 9-fold higher expression in surrounding non-adenoma tissues of males than those of females. The present results appear contrary to our earlier finding that small foci in female mice are more strongly stained for pi class GST than those in males,[9] but only large hepatic adenomas were used to isolate RNA and protein in the present study and staining intensities in the large lesions were generally found to be similar in the two cases. Since GST-II in male normal livers is under the influence of testosterone,[8] these findings suggest that p-1 expression in adenomas is not affected by the hormone, but rather is regulated by factor(s) independent of the sex. Our previous study revealed that the oncogene product c-Jun is expressed in most nodules and adenomas of both females and males.[10]

In the present study, the p-1 and p-2 proteins expressed by the respective plasmids migrated to the same position as authentic GST-II from mouse liver on two-dimensional gel electrophoresis (data not shown). Although the recombinant p-2 protein did not exhibit activity toward CDNB or other substrates, it shared some cross-reactivity with p-1. In addition, the nucleotide sequences of p-1 and p-2 cDNAs were identical with those previously reported for the respective clones.[13, 14] Thus, the recombinant p-1 and p-2 proteins were authentic products, and the former was found to be very similar to GST-II in all properties examined. The properties of the p-2 protein were consistent with those reported by Xu and Stambrook.[14] Although

Bammler *et al.* reported that the p-2 protein bound with 4-fold lower affinity to a glutathione-agarose column, as compared with the p-1 protein,[26] we could not confirm the binding of p-2 to the column. The selective distribution of the p-2 mRNA, with high values for skeletal muscle and the gastrointestinal tract, but low expression elsewhere, was in clear contrast to the ubiquitous distribution of the p-1 mRNA, being essentially in accordance with the results reported earlier.[14] The function of the p-2 protein is not clear.

In conclusion, the present results indicated that change of pi class forms in mouse hepatic adenomas is caused mainly by alterations in p-1 expression and the contribution of p-2 is minimal. The regulation of p-1 in hepatic adenomas was also found to be independent of the sex of the animal, suggesting the existence of some intrinsic mechanism limited to the lesions.

ACKNOWLEDGMENTS

This study was supported in part by the Karoji Memorial Fund of Hirosaki University School of Medicine and by a grant from the Ichiro Kanehara Foundation.

(Received January 21, 1998/Revised March 23, 1998/Accepted March 27, 1998)

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