Yonago Acta medica 1996;39:151–154

Selective Enhancement of Expression of Class-Mu Glutathione S-Transferase Genes during Involution of Rat Ventral Prostate Induced by Androgen Withdrawal

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cDNA clones encoding class-Mu subunits (Yb1, Yb2 and Yb3) of glutathione S-transferases were isolated from a cDNA library of a rat ventral prostate. The mRNA levels of Yb1, Yb2 and Yb3 were significantly elevated in the prostate of androgen-ablated rats. The enhancement of gene expression was characteristic for class-Mu subunits, since the relative abundance of mRNAs for class-Alpha and class-Pi was not affected by castration. The up-regulation of transcripts was repressed by immediate administration of testosterone propionate after castration.

Key words: class-Mu subunits; glutathione S-transferase; mRNA; ventral prostate

Glutathione S-transferases (GSTs) are a family of multifunctional enzymes involved in the detoxification of xenobiotic agents, drug biotransformation and protection of cells against peroxidative damage (Mannervik and Danielson, 1988; Pikett and Lu, 1989). Cytosolic GSTs from rat tissues were designated as class-Alpha (subunits Ya1, Ya2 and Yc), class-Mu (subunits Yb1, Yb2 and Yb3), class-Pi (subunit Yp) and class-Theta (subunit Yrs). Recently, we tried a subtraction hybridization cloning to isolate the genes responding to the androgen withdrawal (Hoshikawa et al., 1991). Some of the isolated clones contained mRNAs for class-Mu subunits of GSTs. It has been reported that the mRNA level of Yb1 was elevated during the regression of steroid-hormone dependent tissues (Chang et al., 1987; Flomerfelt et al., 1993). GSTs may function in the cellular process of apoptosis underlying tissue involution. In the present study, we demonstrated that the expression of class-Mu GST genes was selectively elevated during the involution of a rat ventral prostate with the specific probes for GST subunits belonging to 3 distinct classes.

Abbreviations: bp, base pair; DIG, digoxigenin; GST(s), glutathione S-transferase(s)

Materials and Methods

Isolation and sequencing of cDNA clones for rat GST subunits

cDNA clones encoding class-Mu GSTs were isolated from a cDNA library of a rat ventral prostate as described previously (Hoshikawa et al., 1991). After the second cycle of differential screening, we isolated 168 clones as candidates of the mRNAs induced after castration. Based on the results of Northern blot analyses, we selected 48 clones giving significantly different hybridization signals with mRNAs from intact and castrated rats. The 48 isolated clones were shown to represent 10 distinct mRNA species. Partial nucleotide sequences of inserted cDNA fragments were determined with the representative clones by the dideoxy chain termination method (Sanger et al., 1977).

Generation of probes for Northern blot analysis

The inserted cDNA fragments of clones 74, 64 and 3.5 were excised from plasmids with *Eco*R I and used as the probes for subunits Yb1, Yb2 and Yb3, respectively. The probe used for subunit Ya1 was the DNA fragment that was 749

Table 1. cDNA clones for class-Mu GSTs isolated from a rat ventral prostate

Clone No.	No. of related clones*	Size of mRNA† (nucleotides)	Identification
74	8	1062	Yb1
64	1	1034	Yb2
3.5	9	1264	Yb3

*Related clones were detected by cross hybridization among 168 clones selected in the second cycle of screening as candidates of gene products induced by castration.

†Sizes of the mRNAs were predicted by DNA sequence analyses of structural genes (Pickett and Lu, 1989; Abramovitz et al., 1995).

base pairs (bp) in length and contained the complete coding sequence (Lai et al., 1984). This was generated by reverse transcription-polymerase chain reaction using total RNA prepared from a rat liver and the primers 5'-AGCTGAGTGGAGAAGAAGCC-3' and 5'-AATTGGACAGTGCAGCTTCCG-3'. The probe for the rat Yp subunit was the 735-bp DNA fragment that contained the complete coding sequence. This was excised from the plasmid pGP5 (Suguoka et al., 1985) with *Eco*R I and *Sal* I. pGP5 was provided from the Riken Gene Bank. For the controls which showed that each lane contained comparable amounts of RNA, cDNA of human β-actin (Gunning et al., 1983) was used as the probe. Double-stranded DNAs were labeled with digoxigenin (DIG)-11-dUTP using the DIG-high prime labeling kit (Boehringer Mannheim Inc., Mannheim, Germany).

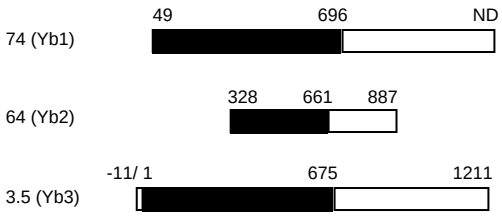

Fig. 1. Schematic representation of cDNA clones 74 (Yb1), 64 (Yb2) and 3.5 (Yb3) encoding class-Mu GSTs isolated from a rat ventral prostate. Closed and open boxes represent the protein coding and non-coding sequences, respectively. Numerals above the boxes indicate the positions of terminal nucleotides of inserted cDNA fragments and protein coding regions. Position 1 is the translational initiation site. The nucleotide sequence of 3'-terminal region of clone 74 was not determined (ND).

Isolation of RNA and Northern blot analysis

Ventral prostates of male Wistar rats (body weight 300–350 g) were used after castration at 24 or 48 h. As a replacement therapy, 5 mg of testosterone propionate dissolved in 0.1 mL of cotton seed oil was administrated immediately after castration. Total cellular RNA was isolated from prostate tissues by the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). Ten micrograms of RNAs were electrophoresed on 1.2% agarose gels containing 2.2 M formaldehyde and transferred to Biodyne Plus membrane (Pall Biosupport Co., New York, NY). After hybridization DIG-labeled probes were detected by enzyme immunoassay using a DIG luminescent detection kit (Boehringer Mannheim).

Results and Discussion

Cloning of class-Mu GST genes from a cDNA library of a rat ventral prostate

In searching for the gene products involved in the castration induced involution of the rat ventral prostate, we isolated cDNA clones containing mRNAs for class-Mu GSTs (Table 1). The nucleotide sequences of clones 74, 64 and 3.5 were determined for about 200 bp in both terminal regions of inserted cDNA fragments. By searching the sequence homology with the registered sequences in the GenBank database (Release 95.0), the nucleotide sequences of clones 74, 64 and 3.5 revealed 98% identity to those of previously reported cDNAs for Yb1 (Ding et al., 1985), Yb2 (Lai and Tu, 1986) and Yb3 (Abramovitz and Listowsky, 1987), respectively. The mRNA sequences of clones 74, 64 and 3.5 were schematically represented in Fig. 1. Among rat class-Mu GSTs, the Yb3 expression is restricted in some tissues.

The Yb3 gene was originally cloned from a rat brain cDNA library and shown to be selectively expressed in brain and testis (Abramovitz and Listowsky, 1987; Abramovitz et al., 1995). In addition we demonstrated that the Yb3 gene was also expressed in the rat ventral prostate by cDNA cloning.

The mRNA levels of Yb1, Yb2 and Yb3 were elevated during the involution of prostate tissue

Northern blot analyses were performed for total cellular RNA prepared from ventral prostates of intact and castrated rats using the cDNA fragment of clones 74, 64 and 3.5 as probes (Figs. 2A–2C). The mRNAs for 3 class-Mu subunits were detected in the prostate tissues from intact rats (lane 1). The expressions of mRNAs for Yb1, Yb2 and Yb3 were enhanced by castration (lanes 2 and 3). Chang and colleagues (1987) have identified the mRNA for Yb1 to the androgen-repressed 29-kDa protein by cDNA cloning. The induction of the Yb1 gene was also observed early in the apoptotic pathway in T-lymphocytes treated with dexamethasone or calcium ionophore A23817 (Flomerfelt et al., 1993). We demonstrated that the expressions of not only Yb1 but also Yb2 and Yb3 were induced in the regressing prostate. By the administration of testosterone propionate which has androgenic hormonal activity (Izawa, 1990), the levels of mRNA for class-Mu GSTs were reduced to the levels observed in the intact rats (lane 4). It was consistent with a conclusion that the up-regulation of transcripts was dependent on the androgen withdrawal induced by castration. The elevation of mRNA levels was not observed in other male accessory sex organ, seminal vesicle (Hoshikawa et al., 1991). These results suggest that the effects of androgen withdrawal on the expression of mRNA for class-Mu subunits were variable among androgen-dependent tissues. It is not clear at present whether the increase in the relative abundance of mRNA for Yb1, Yb2 and Yb3 is due to an increase in the rate of transcription or caused by changes at the posttranscriptional level.

Expressions of class-Mu genes, as opposed to -Alpha nor -Pi GST genes, were selectively enhanced in the prostates of androgen-ablated rats

To examine whether the expression of mRNA for the other class-GSTs to class-Mu is also enhanced in the prostates of castrated rats, Northern blot analysis was performed using specific probes for Ya1 and Yp that are belonging to class-Alpha and -Pi, respectively. The mRNA for Ya1 (about 900 nucleotides in length) was not detected in the prostate (Fig. 2D), although the significant concentration of mRNA was detected in the liver cells (data not shown). The mRNA for Yp (about 1,000 nucleotides in length) was expressed in the

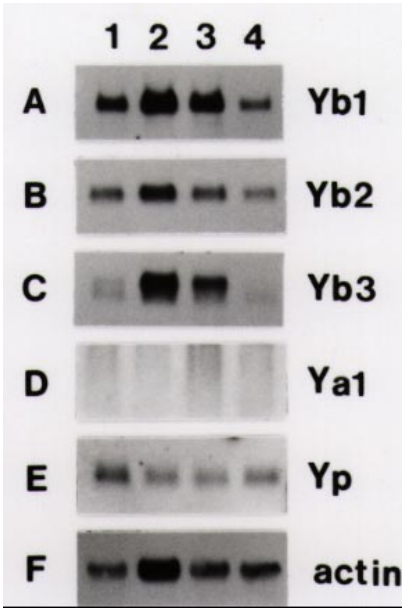

Fig. 2. Effects of castration and testosterone propionate on the expressions of GST genes in the rat ventral prostate. Ten micrograms of total cellular RNAs from the ventral prostates of intact (lane 1) and rats castrated for 24 h (lane 2) and 48 h (lane 3), and treated immediately after castration with testosterone propionate for 48 h (lane 4) was electrophoresed on 1.2% denaturing agarose gels. RNAs were transferred onto nylon membranes and hybridized with DIG-labeled probes for rat GSTs; Yb1 (**A**), Yb2 (**B**), Yb3 (**C**), Ya1 (**D**) and Yp (**E**). cDNA for human β-actin (**F**) was used as a probe for RNA amounts in each lane.

prostates of intact rats (Fig. 2E, lane 1). However, the effect of castration on the expression of mRNA for Yp was insignificant (Fig. 2E, lanes 2 and 3). These results demonstrated that the selective enhancement of expression of class-Mu GST genes was induced in the rat ventral prostate following androgen withdrawal. The functions of GSTs were well characterized in the liver which is the major site for the metabolism and detoxification of drugs and chemical carcinogens (Mannervik and Danielson, 1998; Pikett and Lu, 1989). However, very little is known about the roles of GSTs in extrahepatic organs. GSTs are known to function as as cellular detoxifying enzymes by removing harmful metabolic byproducts that may result from a variety of biochemical reactions. As the cellular apoptotic pathway is expected to increase such byproducts, the selective enhancement of mRNA expression of class-Mu GSTs may be associated with the early signals of apoptosis in the regressing prostates.

Acknowledgments: This work was supported by a Grant-in-Aid for the Second-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare of Japan.

References

1 Abramovitz M, Listowsky I. Selective expression of a unique glutathione S-transferase Yb3 gene in rat brain. J Biol Chem 1987;262:7770–7773.

2 Abramovitz M, Testori A, Angelov IV, Darmon A, Listowsky I. Brain and testis selective expression of the glutathione S-transferase Yb3 subunit is governed by tandem direct repeat octamer motifs in the 5'-flanking region of its gene. Mol Brain Res 1995;28:37–46.

3 Chang C, Saltzman AG, Sorensen NDS, Hiipakka RA, Liao S. Identification of glutathione S-transferase Yb1 mRNA as the androgen-repressed mRNA by cDNA cloning and sequence analysis. J Biol Chem 1987;262:11901–11903.

4 Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–159.

5 Ding GJ-F, Lu AYH, Pickett CB. Rat liver glutathione S-transferases: nucleotide sequence analysis of a Yb1 cDNA clone and prediction of the complete amino acid sequence of the Yb1 subunit. J Biol Chem 1985;260:13268–13271.

6 Flomerfelt FA, Briehl MM, Dowd DR, Dieken ES, Miesfeld RL. Elevated glutathione S-transferase gene expression is an early event during steroid-induced lymphocyte apoptosis. J Cell Physiol 1993;154:573–581.

7 Gunning P, Ponte P, Okayama H, Engel J, Blau H, Kedes L. Isolation and characterization of full-length cDNA clones for human α-, β- and γ-actin mRNA: skeletal but not cytoplasmic actin have an amino-terminal cystein that is subsequently removed. Mol Cell Biol 1983;3:787–795.

8 Hoshikawa Y, Satoh Y, Ichii S. Isolation and characterization of cDNA clones for castration-induced mRNAs in the rat ventral prostate. Endocrinol Jpn 1991;38:619–626.

9 Izawa M. cDNA cloning of androgen-repressed mRNA in rat seminal vesicles: partial characterization of a cDNA clone, pSvr-1. Endocrinol Jpn 1990;37:233–238.

10 Lai H-CJ, Li N-Q, Weiss MJ, Reddy CC, Tu C-PD. The nucleotide sequence of a rat liver glutathione S-transferase subunit cDNA clone. J Biol Chem 1984;259:5536–5542.

11 Lai H-CJ, Tu C-PD. Rat glutathione S-transferases supergene family: characterization of an anionic Yb subunit cDNA clone. J Biol Chem 1986;261:13793–13799.

12 Mannervik B, Danielson UH. Glutathione transferases: structure and catalytic activity. CRC Crit Rev Biochem Mol Biol 1988;23:283–337.

13 Pickett CB, Lu AYH. Glutathione S-transferases: gene structure, regulation, and biological function. Annu Rev Biochem 1989;58:743–764.

14 Sanger F, Niklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977;74:5463–5467.

15 Suguoka Y, Kano T, Okuda A, Sakai M, Kitagawa T, Muramatsu M. Cloning and the nucleotide sequence of rat glutathione S-transferase P cDNA. Nucleic Acids Res 1985;13:6049–6057.

(Received September 3, Accepted September 18, 1996)