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HIGH LEVEL OF GST-P mRNA IN ISLAND TYPE OF RAT ASCITES HEPATOMA CELL LINES

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Key words: *GST-P, albumin, ascites hepatoma, mRNA*

Abstract

Several cell lines (18) of poorly differentiated rat ascites hepatomas which grown intraperitoneally were analyzed with respect to their mRNA levels of GST-P and albumin. The expression of albumin was dramatically decreased and became undetectable in all of the examined hepatomas compared with normal rat liver. The GST-P mRNA level in the hepatomas was correlated with their ability for formation of cellular islands in ascites. The island types, expressed GST-P at high level and the free type ones showed very low level of GST-P mRNA.

Introduction

Glutathion S-transferases (GSTs; EC 2.5.1.18) are a family of isoenzymes found in the various organs of different species from metazoans to mammalians. The enzyme catalyzeds a nucleophilic substitution reaction between glutathion, where sulfied is the nucleophile, and any of a large number of electrophilic substrates. The GSTs are active in drug detoxification and hormone binding (9,12). A multigene family controls expression of the different of the GSTs are encoded by three gene families named alpha, mu, and pi (10). The neutral form from rat placenta was purified and named it the placental (GST-P)(9). In normal rat tissues including liver, placenta, and fetal liver, the protein content of GST-P was generally low but significantly high in kidney and pancrease (9). The amount of the GST-P protein content and its mRNA level, remarkably increased in the all hyperplastic nodules (a precancerous legion) and in hepatocellular carcinoma examined, compared with normal liver (4,5,8,9). The amount of GST-P and its mRNA in transplantable Morris hepatoma 5123D, 7316A, and 7794A cells (6,9) and rat Zajdela hepatoma cells (11), kept at high level as hyperplastic nodules but dramatically decreased to undetectable low level in transplantable Yoshida ascites hepatoma AH-130, AH-66F, and AH-13 cells (4,5,9). In order to test the

hypothesis that GST-P decreased in all of poorly differentiated Yoshida ascites hepatoma cell lines, the mRNA levels of GST-P and albumin(an indicator for differentiation of hepatocytes) were determined in the various hepatomas and compared with those of the normal rat liver.

Materials and methods

Hepatoma cells: Male Donryu rats weighing 150 to 200 g (5 weeks old), and 18 rat ascites hepatoma cell lines (15) AH-13, AH-13NMOR, AH-13-6MPR, YS, YS-6MPR, YS-5FUR, AH-130, AH-7974F, AH-131A, AH-371A, AH-41B, AH-143A, AH-311, AH-66F, AH-109A, AH-225A, AH272, and AH-60C were used in the experiment. Thses cell lines were different from each other in several respects (15). The ascites hepatomas were inoculated intraperitoneally into rats. The rats were sacrificed for harvest of rapidly growing hepatomas cells, 6-14 days after inoculation, depending on cell growth rate, and washed with physiological saline three times at 4°C to remove erythrocytes.

RNA extraction and Northern Blotting: Total RNA was extracted and purified from frozen samples using a single step method of acid guanidium thicoyanate-phoenol-chloroformextraction (1). The amount of RNA was determined from the absorbance at 260 nm using a Beckman DU-50 spectrophotometer.

Total RNA (20μg/lane) were electrophoresed on a 1.2% agarose gel containing 6% formaldehyde and transferred onto nitrocellulose membrane (Schleicher & Schulle, Dassel, Germany). After being baked at 80°C for 2h under vacuum, the filters were prehybridized in 5x SSPE (1x SSPE; 0.15M NaCl, 0.01M NaH_2PO_4, 1.0M EDTA), 5x Denhardt's solution, 100μg/ml denatured DNA, and 50% formamide at 42°C for 2h. For hybridization, each cDNA probe was labeled with (α-^{32}P) dCTP using the random primer method, and incubated with membranes in the same solution at 42°C overnight. Following hybridization, the filters were washed as described in the figure legends. Washed and dried filters were exposed to XRP-5 X-ray film at - 80 C in cassettes with intensifying screens. The filters were reused after the probes had been removed by washing in 0.1xDenhardt's solution containing 2mM EDTA and 5mM Tris-HCl, pH 8.0, at 70°C for 2h. The full lenghts

of GST-P (10) and albumin (3) cDNAs were used as probes.

Other methods: The indensity of specific bands of the autoradiography of the blots was evaluated with a Scanning densitometer to determine the relative amounts of GST-P mRNA. The relative levels of GST-P mRNA defined as the ratio of intensity of GST-P mRNA bands in hepatomas to that of in normal liver.

The significant differences of relative mRNA levels between two groups of samples were analyzed using Students unpaied t-test (7). The Spearman's rank correlation test was used (2). A probability of $p<0.05$ was considered statistically significant.

Results and discussion

Figure 1 shows the expression patterns of the GST-P and albumin mRAN levels in normal liver (Donryu rat) and ascites hepatomas. The expression of GST-P was variable; the maximal and the minimal mRNA levels were observed in AH-143A, and normal liver, respectively (Fig. 1a). The GST-P was expressed at low level in AH-13, AH-66F, and AH-130 compared with rat liver, confirming the previous findings of other investigators who have used the Yoshida hepatomas (4,5,9). In contrast to GST-P, mRNA of ablumin dramatically decreased to undetectably low level in all of the examined ascites hepatomas compared with normal rat liver (Fig. 1b).

It should be noted that the ability for formation of cellular island in ascites is a characteristic for each hepatoma cell lines. So far ascites hepatomas were classified as free and island types (15). Considering that, the comparison between free and island types of hepatomas for mRNA levels of GST-P revealed that the island type hepatomas expressed GST-P significanctly at high level compared with the free type ones ($t_{df=17}$= 3.88; $p<0.05$). In view of the correlation between island formation and GST-P mRNA, AH-272 and AH-225A are exceptions (Table 1). These two cell lines contain about 75% and 90% free cells, respectively, but highly expressed GST-P (Table 1 and Fig.1).

It is well established that the AFP, another tumor marker, is highly expressed in the island type of ascites hepatomas compared with the free type ones (13,14). Using these data and the present data, there are significant correlation between GST-P mRNA level and AFP production in examined ascites hepatomas (Rs 0.603, P<0.05). Taken together, it is suggested that there is a close association between expression of AFP and GST-P in poorly differentiated rat

ascites hepatomas. Further experiments at molecular level may elucidate the biological significance of this concordance.

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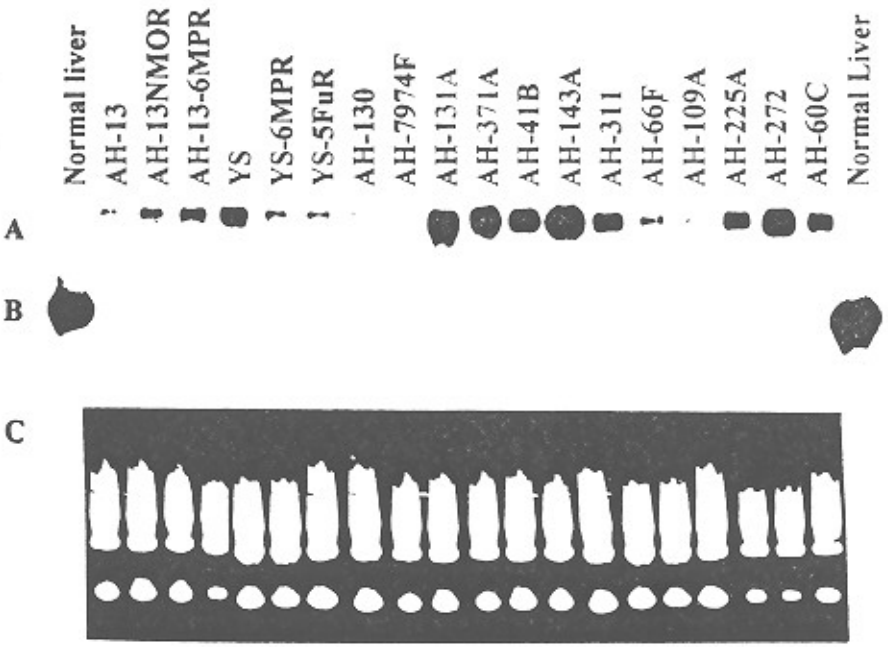

Fig. 1- Northern blot analysis of expression of GST-P and albumin in Yoshida ascites hepatoma cells and normal rat liver. Experimental conditions in A and B were essentially identical, expect that in (a) using the GST-P probe and in (b) using albumin probe. Washing condition: twice 5x SSPE, room temperature, 10 min; 2x SSPE, 42°C, 10 min; 2x SSPE + 0.1% SDS, 65°C, 6 min. Exposed for 30hr. Specific activities of the probes were approximately $5x10^8$ cpm/μg DNA. (c) The ethidium bromide stained gel represents that the equal amounts of total RNA were used.

Table 1- Relative levels of GST-P mRNA in poorly differentiated rat ascites hepatma cell lines used in the present study

Cell line	Island or Free Type *	Relative levels of GST-P mRNA**
AH-13	Free	1.80
AH-13NMOR	Free	2.80
AH-136MPR	Free	3.10
YS	Free	3.30
YS-6MPR	Free	1.95
YS-5FuR	Free	2.20
AH-130	Free	1.72
AH-7974F	Free	1.90
AH-131A	Island	22.25
AH-371A	Island	24.47
AH-41B	Island	14.50
AH-143A	Island	31.90
AH-311	Island	7.10
AH-66F	Free	2.50
AH-109A	Free	2.00
AH-225A	Free	7.95
AH-272	Free	27.97
AH-60C	Island	9.00

*: See Ref. 15.

The intensity of specific bands of the autoradiography of the blots was evaluated with a scanning densitometer.

**: The relative levels of GST-P mRNA defined as the ration of intensity of GST-P mRNA bands in hepatomas to that of in normal liver.

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