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Expression and alterations of different molecular form γ-glutamyl trans ferase and total RNA concentration during the carcinogenesis of rat hepatoma *

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INTRODUCTION

Carcinogenesis is closely related with DNA synthesis and hypermetabolism of nucleic acid. γ-glutamyl transferase (GGT, EC 2. 3. 2. 2) is a plasma-embrane-combined heterodimeric glycoprotein which initiates the degradation of extracellular glutathione and its conjugates. The cleavage of glutathione by GGT into cysteinylglycine and a γ-glutamyl residue provides a mechanism for the recovery of cystein by the cell and thus for renewed glutathione synt hesis. *In vivo*, GGT is either highly correlated with biotransformation, nucleic acid metabolism and tumorigenesis, or the sensitive enzymatic marker reflecting hepatocyte parenchymatous lesions[1,2]. However, the expression and alterations of GGT are not yet clear during the course of hepatocyte damage and canceration. As a consequence, in the present study, the pathological changes, G GT activities and total RNA concentration of rat livers were investigated on experimental rat hepatomas induced with a chemical carcinogenic agent, 2-Fluoenylacetamide (2-FAA), to explore the clinical significance of GGT in showing the extent of hepatocyte damage and in diagnosing hepatoma.

MATERIALS AND METHODS

Animal model

Forty-eight male Sprague-Dawley (SD) rats weighing 140 g - 180 g wereobtained from the Experimental Animal Centre, Nantong Medical College. The rats were divided into 8 groups at random, 6 in each group. All the rats were housed in controlled surroundings. One of these groups was randomly chosen as a control group. The control group was fed with ordinary natural granular forage, and experimental groups with forage containing 0.05% 2-FAA. One group was killed every two weeks respectively after feeding. Peripheral blood was collected for biochemical assay and a part of the liver tissues was used for liver homogenate preparation and histopathological examination (HE staining).

Preparation of hepatic tissue homogenate

Fresh rat hepatic tissues were washed with 0.9% NaCl solution, and dried with filter paper and cut into pieces. Two portions (part A and part B, 1g each) of the tissues were weighed: to part A was added 5mL homogenate solution (pH 8.6, 0.1mol/L Tris-HCl buffer solution) to extract soluble GGT, to part B was added 5ml homogenate solution containing 0.5% Triton X 100 to extract total GGT. It was porphyrized on ice, then homogenized at 12 000r/min in YQ-3 type homogenizer for 5 times (stopped 5min every 30 second), and followed by centrifugalization at 15 000r/min for 45min at 4℃. In the end, the supernatants of liver homogenates were taken and stored at -20℃ for analysis.

Total RNA extraction and detection of its concentration

Fifty mg of hepatic tissue was weighed exactly in analytical balance, and was put in a homogenizer free from RNAase to which 1.0mL of RNAzole reagent was added for homogenizing 2 minutes. Total RNA was extracted according to the assay described by Chirgwin, *et al*. RNA absorptance was detected by a ultraviolet spectrophotometer of Shimadzu UV-2201 type and converted to total RNA concentration (μg/mg wet tissue). The GGT activities (U/L) in superna tant of rat hepatic tissue homogenate were detected by a γ-glutamyl p-nitroanilinum directed assay, GGT protein

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concentrations were detected by a Follin-phenol reagent assay. GGT specific activities (U/g) were converted according to the ratio of the GGT activity (U/L) to the GGT protein concentration (g/L).

Statistical analysis

Data were input to the computer, and processed with analysis of variance and analysis of correlation and expressed as $\bar{x}\pm s$. Comparison was made between every two groups. $P<0.05$ was considered to be statistically significant.

RESULTS

Histopathological changes during the induction of rat hepatomas

At the end of the second week, the livers turned to grey-yellow and scabrous, all the hepatocytes manifested granular degeneration. At the end of the fourth week, half of hepatic tissues became precancerous lesions, the histological manifestation was normal liver lobules existing in most part of areas, but hyperplastic small round cells or oval cells or hyperplastic nodules existing in local areas. At the end of the tenth week, most parts of hepatic tissues were already cancerized. At the end of the 12th week, all the hepatic tissues were cancerized, the normal structure of hepatic lobule was completely destroyed and the liver tissues showed diffuse patchy necrosis, a lot of small round cells and overall patchy nodules of cancer nests were the main histological alterations. Histological type of hepatoma was well differentiated hepatocellular cancer. The pathological changes in different stages of rat livers after administration of 2-FAA are shown in Table 1.

Table 1 The pathological changes of rat livers during different stages of liver cancer induced with 2-FAA

Groups	*n*	Pathological feature (HE staining)		
		Degeneration	Precancerosis	Cancerization
Control	6	0	0	0
2nd week	6	6	0	0
4th week	6	3	3	0
6th week	6	0	5	1
8th week	6	0	4	2
10th week	6	0	1	5
12th week	5[a]	0	0	5

[a]One rat died during the experiment.

Changes of total RNA concentration and GGT specific activities

The total RNA level and GGT activity were observed at the different stages during the induction of rat hepatomas (Table 2). Both total RNA concentration and GGT specific activities increased gradually during the administration of 2-FAA. GGT specific activities were positively correlated with total RNA concentration (r = 0.90, P < 0.01). Total RNA concentration of hepatic tissues was also positively correlated with serum GGT specific activities (r = 0.79 , P < 0.01).

Table 2 Changes of total RNA concentrations and GGT specific activities at different stages of rat hepatomas

Groups	*n*	Total RNA (μg/mg tissue)	GGT specific activity	
			Liver GGT(U/g)	Serum GGT(U/L)
Control	6	3.67±0.78	1.06±0.24	6.0±4.8
2nd week	6	6.69±6.08	4.77±4.41[a]	20.2±17.8[a]
4th week	6	4.81±4.27	9.30±1.81[b]	31.2±28.1[b]
6th week	6	5.38±4.84	5.95±1.23[b]	37.8±32.1[b]
8th week	6	9.23±6.48[a]	10.73±4.93[b]	53.5±43.6[b]
10th week	6	8.37±6.51[a]	16.03±5.22[b]	40.6±23.6[b]
12th week	5	8.16±8.87[a]	15.71±3.31[b]	38.6±8.8[b]

[a]P<0.05, [b]P<0.01, compared with the control group.

Changes in activities of different molecular forms of GGT

Hepatic soluble and membrane-combined GGT activities increased markedly during the induction of liver cancer, especially in the late phases. There was also significant difference between each experimental group and the control group (P < 0.05 or P < 0.01). The activities of two different molecular forms of GGT increased in parallel, the ratio of soluble GGT to total GGT was about 0.7 in all the rats, no significant difference was found between the control group and the experimental groups. The activities of different GGT molecular forms are shown in Table 3.

Table 3 The changes in specific activities of different GGT forms at different stages of rat hepatomas

Groups	*n*	Hepatic homogenate GGT (U/g)		
		S(Soluble-type)	T-S(Membrane-type)	S/T-GGT
Control	6	0.74±0.14	0.32±0.14	0.71±0.08
2nd week	6	3.35±2.83[a]	1.42±0.23	0.72±0.14
4th week	6	7.07±0.92[b]	2.29±1.28[b]	0.76±0.10
6th week	6	3.50±0.70[b]	2.45±1.25[b]	0.60±0.15
8th week	6	8.68±5.24[b]	2.39±1.52[b]	0.74±0.18
10th week	6	12.21±5.49[b]	3.82±1.32[b]	0.73±0.13
12th week	5	9.56±2.27[b]	6.15±4.36[b]	0.64±0.24

[a]P<0.05, [b]P<0.01, compared with the control group.

DISCUSSION

The activities of GGT are rather low in normal liver tissues, expressed mainly on the border of epithelial cell membrane of biliary duct and intrahepatic cholangioles with strong secretory and absorptive functions. To explore the laws of the production and alterations of hepatoma-specific GGT in the canceration of normal hepatocytes, the diazo chemical carcinogens such as 2-FAA, DEAA, MDAA, O-AT, 3'-MC-4DAB are usually administered to establish hepatoma model in male SD rats or Wistar rats. With the feature of carcino-embryonic protein, hepatoma-related GGT is produced and secreted when the genes controlling GGT synthesis were expressed abnormally. Hence GGT is usually considered as the early enzyme marker of hepatocarcinogenesis.

Hepatocarcinogenesis is closely related with DNA synthesis and nucleic acid metablism. Hepatic GGT plays an important role in biotransformation and nucleic acid metabolism. In addition, its abnormal expression is a sensitive enzyme marker for hepatocellular paranchymal lesions. Chemical induction of cancer is a multistage course, its fundamental causes are the activation of prooncogenes or the inactivation of tumor-suppressor genes initiated by carcinogens. Thus, the abnormal regulation of genes coordinated to lead cancerization[3]. Most chemical carcinogens were turned into strong electrophilic agents by enzymes *in vivo* with high affinity with and response to membrane-combined GGT. In the course of canceration, liver tissues can synthesize and secret various kinds of hepatocarcinoma-related proteins, polynucleotides, isoenzymes such as A FP, GGT-II[4,5]. When the abnormal expression of GGT synthesized genes is controlled, hepatocarcinoma-related GGT is secreted with the characteristics of embryonic liver, which are regarded as the early enzyme marker of hepatocarc inogenesis[6].

In the present study it was obsered that in the early phases of hepatoma induced with the chemical carcinogen 2-FAA, the oval cells with the features of undifferentiated type were found to have the function to resist the cytotoxicity of the carcinogen, and could express tumor-related enzymes or proteins. In the preca ncerous and cancerous phases of hepatic tissues, significant changes occurred in total RNA concentration and GGT specific activities. As a result of the damage of hepatocytes, GGTs were released into blood to bring about the rise of their a ctivities in the serum, suggesting that a lot of GGT were expressed and secreted into blood by hepatocytes in the early phases of cancerization. Moreover, the features of GGT electrochroma-tography were different from those of normal ones. The results showed that GGT plays an important role in resisting mutagenesis and carcinogenesis. Hepatic functions in detoxification and biotransformation could be strengthened by enzymatic action which relieves toxic effect of chemical mut agens, carcinogens and other substances, and protects normal hepatocytes from the effect of cancer initiating and promoting factors[7].

During the course of canceration, different GGT molecular forms were also overexpressed. As shown in Table 3, the hepatic specific activities of both soluble and membrane-combined GGTs in the experimental groups were higher than those in the control group ($P < 0.05$ or $P < 0.01$). The ratio of soluble GGT to me mbrane-combined GGT was 0.7 in all groups. The specific activities of two GGT molecular forms increased three to five times in the early stage, ten to twenty times in the late stage, indicating that the two kinds of hepatic GGT had the parallel tendency to increase not only in the early stage but also in the late stage. Furthermore, hepatic GGT was released into the blood with high level in sera. It is concluded that hepatoma-specific GGT can be used as a tumor marker for the early diagnosis of hepatic cancer[8].

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