Abnormal expression of hepatoma-derived γ-glutamyltransferase subtyping and its early alteration for carcinogenesis of hepatocytes

Deng-Fu Yao, Zhi-Zhen Dong, Deng-Bing Yao, Xin-Hua Wu, Wei Wu, Li-Wei Qiu, Hong-Mei Wang and Xian-Yong Meng

Nantong, China

BACKGROUND: **Although the hepatoma-specific band of gamma-glutamyltransferase (GGT) is a highly sensitive marker in diagnosis of hepatocellular carcinoma, the kinetic expression and the early alterations of GGT in the development of hepatoma remain unclear. In this study, we investigated the expression and the alterations of GGT multiple molecular forms in hepatotumorigenesis.**

METHODS: **The expression of GGT in a chemically induced hepatocarcinogenesis model was examined by giving 0.05% of 2-fluoenylacetamide in diet for 12 weeks. The expression levels of total RNA and GGT, and the changes of liver pathology, GGT multiple molecular forms and sugar-chain heterogeneity were investigated at the different stages of rat hepatoma development.**

RESULTS: **Pathological examination and biochemical analysis found that liver GGT was over-expressed and secreted into blood during canceration. Serum total GGT and liver GGT specific activities (IU/g) including soluble and membrane-combined GGT were significantly higher ($P<0.05$) in experimental groups than those in control group, respectively. A highly positive correlation was found between total GGT activities and total RNA levels ($r=0.90$, $P<0.05$) of the liver. Both were higher six weeks later than before. Con A-non-reactive-GGT was increased consistantly during the development of rat hepatoma. GGT multiple molecular forms in the liver and sera of experimental rats showed that fetal liver-type GGT bands were associated with the development of hepatoma.**

CONCLUSIONS: **Fetal liver-type GGT in sera and the liver of rats is closely related to hepatotumorigenesis. It can be used as a sensitive enzymatic marker for the early diagnosis of liver cancer.**

(*Hepatobiliary Pancreat Dis Int 2004; 3: 564-570*)

KEY WORDS: **gamma-glutamyltransferase; multiple molecular forms; rat hepatoma; expression; sugar-chain heterogeneity**

Introduction

Hepatocellular carcinoma (HCC) is one of the most common and rapidly fatal malignancies worldwide, and has been ranked as the second cancer killer in China since the 1990s, particularly in the eastern and southern areas, including the inshore area of the Yangtze River.[1,2] Major risk factors for HCC in these areas are exposure to aflatoxin B1 (AFB1) and infection by hepatitis viruses.[3,4] The prognosis of HCC is poor and early detection is of the utmost importance.[5] Its treatment options are severely limited by the presence of metastasis.[2] Although serum α-fetoprotein (AFP) is a useful tumor marker for the detection and monitoring of HCC development, the false-negative rate with AFP level alone may be as high as 40% for patients with small size HCC.[5] However, if hepatocyte-specific mRNAs are detected in the circulation, it is possible to infer the presence of circulating, presumably malignant, liver cells and to predict the likelihood of haematogenous metastasis.[6]

Enzyme gamma-glutamyltransferase (GGT) frequently overexpressed in cancer cells and tissues has been significantly used as a cancer marker. It is a membrane-bound ectoenzyme that catalyzes the degradation of glutathione and other gamma-glutamyl compounds by hydrolysis of gamma-glutamyl moiety or by its transfer to a suitable acceptor.[7,8] Serum GGT activity as a sensitive marker of hepatobiliary disorders, exhibits a tissue specific expression under various physiological and pathological conditions. The activity of GGT is the highest in

Author Affiliations: Research Center of Clinical Molecular Biology, Affiliated Hospital of Nantong University, Nantong 226001, China (Yao DF, Dong ZZ, Wu XH, Wu W, Qiu LW, Wang HM and Meng XY); Institute of Neurosciences, Nantong University, Nantong 226001, China (Yao DB)

Corresponding Author: Deng-Fu Yao, MD, PhD, Research Center of Clinical Molecular Biology, Affiliated Hospital of Nantong University, Nantong 226001, China (Tel: 86-513-5861539; Fax: 86-513-5052523; Email: dfyrcmb@yahoo.com)

This study was supported by a grant from Key Projects of Medical Sciences Department of Health, Jiangsu Province, China (RC2003100).

embryo liver and decreases rapidly to the lowest level after birth, and it is a widely distributed enzyme that has been extensively studied in relation to hepatocarcinogenesis.[9,10] GGT is a heterodimeric glycoprotein, and several experiments have demonstrated that these increases are often associated with structural changes in the sugar-chains of the enzyme, as evidenced by a variation in the pattern of GGT isoforms in serum. Previous investigations suggested that the evaluations of GGT multiple forms might improve the specificity of GGT measurement, indicating that there might be a correlation between specific patterns and different disease states.[11,12]

GGT is mainly presented on hepatocyte cytoplasm and epithelial cells of the bile duct. Total GGT activities make differential diagnosis difficult, because GGT is over-expressed in hepatobiliary diseases. Recently, analysis of GGT isoforms has shown that the incidence of GGT hepatoma-specific band (HSB) is 87% in patients with HCC.[13,14] However, the kinetic alterations and expression of GGT at the early stage of hepatoma development remain unclear. In the present study, expression and kinetic alterations of GGT and its sugar-chain heterogeneity were observed in rats with hepatoma induced with 2-fluoenylacetamid (2-FAA) at different stages of HCC development.

Methods

Hepatoma models

Forty-two closed colony male Wistar rats weighing 140-180 g obtained from the Experimental Animal Center (Shanghai, China) were divided randomly into seven groups, one normal control group (n=6) and six experimental groups (n = 6 for each group). All rats were housed under bio-clean conditions at a temperature-controlled (22±2 ℃) environment with a 12-hour light/dark cycle and 55% humidity, and the control group was given basal diet and the diet contained 0.05% 2-FAA (Sigma, USA). The rats were then monitored daily for survival and weight loss, while recording their clinical signs. They were sacrificed at different time. One group was killed in every two weeks, and surgical procedures were conducted under deep ether anaesthesia. Blood was drawn from the rats, their livers were collected, and histological examination was performed with hematoxylin and eosin (HE) staining. The procedures were conducted in accordance with the guidelines for experimental animals approved by the Animal Care and Use Committee of Nantong University, China.

Purification of liver GGT

Fresh adult rat livers and fetal rat (18 days) livers were washed with 0.9% NaCl solution, dried with filter paper, and cut into pieces. The rat liver was added with 5 ml homogenate solution (pH8.6, 0.1 mol/L Tris-HCl buffer Ⅰ) to extract soluble GGT or added with 5 ml homogenate solution including 0.5% Triton-X 100 (buffer Ⅱ) to extract total GGT, homogenized at 4 ℃ at 12 000 r/min in a YQ-3 type homogenizer (Shanghai, China), stopped 5 minutes in every 30 seconds for 5 times, then centrifuged at 15 000 r/min for 45 minutes at 4 ℃. The supernatants were taken and stored at -20 ℃ for GGT activity, electrophoresis, and affinity analysis.

Isolation of liver total RNA

Total RNAs were isolated from rat liver homogenates with RNAzole reagent (Gibco BRL, USA) and purified according to the described protocol.[15] The total RNA concentrations of rat livers were assessed by measurement of optical density at 260 nm in an ultraviolet spectrophotometer (Shimadzu UV-2201 type, Japan).

Detection of GGT activities and multiple forms

Analyses of total GGT activity or GGT fractions were performed within one day of blood collection. The total activities (IU/L) of serum and liver GGT were measured by a p-nitroanilide direct assay.[16] The protein concentrations of the supernatants were detected by a Follin-phenol reagent assay. The specific activity (IU/g) of liver GGT was calculated according to the ratio of GGT activity (IU/L) to protein concentration (g/L). To compare with the alterations of GGT levels in sera of the experimental rats, the activities of alanine aminotransferase (ALT), adenosine deaminase (ADA) and glutathione S-transferase (GST) were simultaneously determined.[17,18]

The modified separation technique for serum and liver GGT multiple molecular forms was used.[19] Briefly, a vertical slab apparatus (Beijing, China) was loaded with polyacrylamide stage gel containing one layer of concentration gel of 4.0% on the top and three layers of separation gel of 7.7%, 11.5% and 15.0% in the ascending order. Then, serum or liver supernatant (20 μl) was mixed with an equal volume of 40% sucrose bromophenol blue and introduced into sample holes in the concentration gel. These samples were electrophoresized at 60 V until the sample migrated into the separation gel, and then, at a constant voltage of 200 V for 4 hours. Thereafter, the gel plate was taken out from the electrophoresis apparatus and wrapped with cellulose acetate sheets wetted through by GGT substrate solution and incubated at 37 ℃ for 45 minutes. Finally, the cellulose acetate sheets were immersed in a 50 ml solution containing 10% trichloroacetic acid and 25% glycerol, within two minutes, the red bands of GGT isoenzymes appeared on the cellulose acetate sheets.

Affinity analysis of rat liver GGT

GGT sugar-chain heterogeneity was detected by a mi-

crocolumn chromatography with concanvalin A (Canavalia Ensiformis, Con-A, Sigma, USA)-Sepharose 4B affinity.[20-21] In brief, Con-A-Sepharose 4B gel was filled into the micro-column (0.8 cm×8 cm) with natural sedimentation and balanced with buffer Ⅰ (0.1 mol/L Tris-HCl buffer containing 1 mmol $MgCl_2$, 1 mmol $CaCl_2$, 0.1 mol NaCl, 1% Triton X-100, pH 8.0), the supernatant was added into the column, which was drawn, and washed with 0.5 ml buffer Ⅰ, and discarded. The first part of GGT was washed with 3.0 ml buffer Ⅰ and collected in one test tube, and the second part of GGT was collected into the other test tube with 6.0 ml buffer Ⅱ (in buffer Ⅰ containing 0.2 mol α-methyl-D-seminose). The activities of the two parts of GGT were detected and the percentage of GGT was calculated, respectively.

Statistical analysis

All rats were divided into seven groups. The results were evaluated statistically by the Student's *t* test, the chi-square test and the Fisher's exact probability test. Probability values less than 0.05 were considered significant.

Results

Histopathological findings

The pathological changes of rat livers are shown in Table 1. The rats were administered with 0.05% of 2-FAA. At the end of the 2nd week, the appearance of the livers turned to be grey-yellow and scabrous. Hepatocytes showed granular degeneration at the early stage of liver cancer, with normal liver tissue structure, a few hyperplastic oval cells, and initial nodule formation. At the end of the 4th week, precancerous lesions appeared in half of hepatic tissues. Histologically normal liver follicles existed in most part of areas but hyperplastic small round cells or oval cells or hyperplastic nodules in local areas. After six weeks, the rat liver entered canceration stage, and the structure of hepatic lobules was completely destroyed, with necrosis and a large number of small oval cells and cancer nests. Histological finding of the rat liver comfirmed diffuse patchy necrosis and highly differentiated HCC.

Changes of liver total RNA and GGT levels

The levels of total RNA and hepatic GGT were observed at the different stages during the induction of rat hepatomas (Fig. 1). Both total RNA concentration and GGT specific activities increased gradually during the administration of 2-FAA. The activities of the rat liver and serum GGT were significantly higher in the experimental rats than those in controls. In the early stages of rat hepatoma development, GGT activities increased 5 to 10 times in the rat liver, and 4 to 8 times in rat sera compared with those in the controls. The expression levels of liver GGT in hepatoma rats approach to those in fetal rat livers (Fig. 2). A positive correlation was noted between GGT specific activities and total RNA concentration ($r=0.90$, $P<0.01$) or between serum GGT activities of the experimental rats and liver total RNA concentration ($r=0.79$, $P<0.01$). The kinetic alterations of serum GGT average levels at different stages and the comparison with other hepatic enzymes (ALT, ADA and GST) are shown in Fig. 3. The activities of rat serum ADA, GST and ALT were significantly higher than those of GGT at the stage of hepatocyte degeneration, and subsequently they decreased to normal, but increased consistently six weeks later.

Table 1. The pathological changes of rat livers at different stages induced with 2-FAA

Group	*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*	0	0	5

*: One rat died during the experimental course.

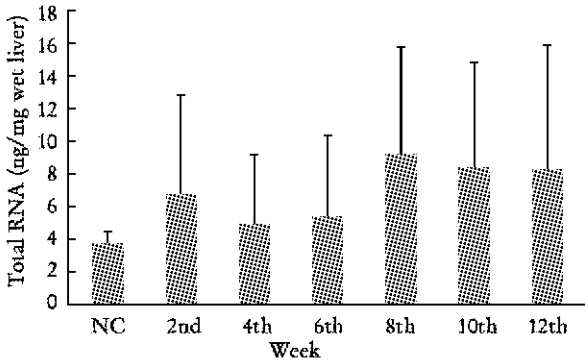

Fig. 1. The kinetic alterations of liver total RNA level (ng/mg wet liver, $n=6$ for each group except for the 12th group) at different stages during rat hepatoma development. NC: normal control rats.

Changes of different-type GGT and its sugar-chain heterogeneity

The specific activity alterations of GGT of different molecular form and its sugar-chain heterogeneity in rat livers are shown in Table 2. The levels of hepatic soluble and membrane-combined GGT increased markedly during the course of liver cancer induction, especially in the late phase. A significant difference was found between the experimental groups and the control group ($P<$

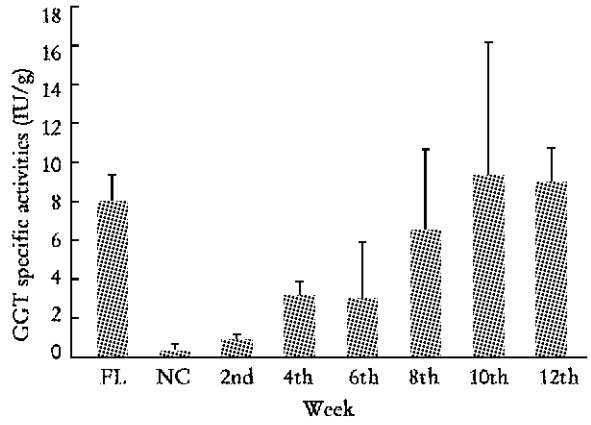

Fig. 2. The kinetic alterations of liver GGT specific activities (IU/g wet liver, n =6 for each group except for the 12th group) at different stages of rat liver cancer development and comparison with fetal rat liver GGT. FL: fetal rat livers; NC: normal control rat livers; GGT: gamma-glutamyltransferase.

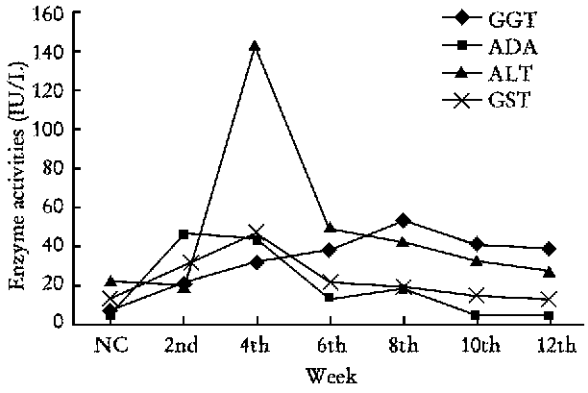

Fig. 3. The kinetic alterations of serum GGT average levels (IU/L, n =6 for each group except for the 12th group) at different stages of rat liver cancer development and comparison with three hepatic enzymes. NC: normal control; GGT: gamma-glutamyltransferase; ALT: alanine amino-transferase; ADA: adenosine deaminase; GST: glutathione S-transferase.

Table 2. Changes of different-type GGT and affinity analysis of GGT enzyme proteins at different stages of rat hepatoma development

Group	n	Liver GGT (IU/g)		Con A-affinity (%)	
		Soluble-type	Membrane-type	GGT-R-C	GGT-N-C
Normal control	6	0.7±0.1	0.3±0.1	94.0±0.9	6.0±0.9
2nd week	6	3.4±2.8 *	1.4±0.2	93.8±1.2	6.2±1.2
4th week	6	7.1±0.9 **	2.3±1.3 **	92.7±2.0	7.1±2.0 *
6th week	6	3.5±0.7 **	2.5±1.3 **	92.7±2.0	7.1±2.0 *
8th week	6	8.7±5.2 **	2.4±1.5 **	79.4±4.3	20.6±4.3 **
10th week	6	12.2±5.5 **	3.8±1.3 **	77.9±6.2	22.1±6.2 **
12th week	5	9.6±2.3 **	6.2±4.4 **	79.1±7.7	20.9±7.7 **

GGT-R-C: Con A-reactive-GGT; GGT-N-C: Con A-non-reactive-GGT. *: P <0.05, **: P <0.01, compared with the normal control.

Table 3. Electrophoresis patterns of liver (serum) GGT isoenzymes in the experimental rats, fetal liver and normal control

Group	n	GGT isoenzyme bands on electrophoresis					
		I	II	III	IV	V	VI
Normal control	6	0(0) *	6(6)	0(0)	0(0)	0(0)	6(6) − **
Fetal livers	6	6	0	6	6	6	6 +++
2nd week	6	3(2)	6(6)	2(2)	3(2)	3(2)	6(6) +
4th week	6	3(3)	6(6)	3(3)	3(3)	4(4)	6(6) ++
6th week	6	6(6)	6(6)	4(4)	4(4)	5(4)	6(6) +++
8th week	6	6(6)	6(6)	5(5)	5(5)	6(6)	6(6) +++
10th week	6	6(6)	6(6)	6(6)	6(6)	6(6)	6(6) +++
12th week	5	5(5)	5(5)	5(5)	5(5)	5(5)	5(5) +++

* 0(0): expresses band numbers of liver GGT (serum GGT) isoenzymes; ** − or +: the low or high GGT levels on origin location of electrophoresis.

0.05). The activities of the two different molecular forms of GGT increased parallelly, and the ratio of soluble GGT to total GGT was about 0.7. During the development of rat hepatomas, the liver GGT of the normal or hepatoma rats presented two forms, that is, Con A-reactive-GGT (GGT-R-C) and ConA-non-reactive-GGT (GGT-N-C), detected by Con A-sepharose 4B micro-column affinity chromatography. The GGT-N-C levels two weeks later were significantly higher (P <0.05 or P <0.01) in the experimental groups than in the control group, showing a tendency of persistent increasing.

Electrophoresis changes of rat serum and liver GGT

Different electrophoresis patterns of serum or liver GGT isoenzymes were found among control, fetal, and experimental rats by a vertical slab electrophoresis on polyacrylamide gradient gel. GGT isoenzymes were

separated into one to six bands from the sera and liver of these rats. The changes and electrophoresis patterns of the sera and liver GGT isoenzymes in the experimental rats with fetal or control rats were observed (Table 3). The fetal liver-type patterns of GGT isoenzymes were isoenzyme-Ⅰ, Ⅲ, Ⅳ, Ⅴ, and Ⅵ. Only one band (isoenzyme-Ⅱ) was found in the sera and livers of the controls; however, mixed six bands (isoenzyme-Ⅰ, Ⅱ, Ⅲ, Ⅳ, Ⅴ, and Ⅵ) were detected in the sera and livers of the experimental hepatoma rats. The data indicated that the patterns of GGT isoenzymes in the hepatoma rats were different from those in the normal rats, and that the fetal-type GGT isoenzymes were associated with hepatotumorigenesis and appeared in the early stage of hepatoma development.

Discussion

The prognosis of HCC is still poor at present. Exact diagnosis and proper treatment of HCC at early stages are required for a better prognosis.[22] Serum GGT activity is considered a sensitive marker of hepatobiliary disorders. In the rat liver, the high GGT activity observed at the fetal stage decreases rapidly at birth, being barely detectable in the adult. GGT is often re-expressed in adult rat liver-bearing hepatocarcinogenesis and this is why it has long been regarded as an early marker of cellular dedifferentiation, signaling a reversion of the hepatocyte to a fetal phenotype.[23,24] In rats with 2-FAA at early stage, their livers showed pathologically hyperplastic oval cells that were closely related to the development of hepatomas and the tendency of nodule formation. The oval cells not only had the cytotoxicity resistent to carcinogenesis and showed indifferent morphological shapes of hepatic cells, but also overexpressed GGT. In the present study, GGT was strikingly activated during the course of tumorigenesis in rat hepatoma models (Table 1), and was significantly increased at the degeneration ($P < 0.05$, Table 2) and precancerous stages ($P < 0.01$) of hepatocytes. The increase of rat serum GGT activity was associated with an elevation of liver GGT activity. The data indicated that the expression of liver GGT is related to the development of rat hepatomas.[25-27]

The alterations of GGT sugar-chain structure occurred during the development of hepatomas. Yamashita et al[28] compared the sugar-chains of hepatoma GGT and normal rat liver GGT, and found marked difference of the two kinds of GGT in enzymatic molecular composition. The sugar-chain of normal rat liver GGT was acidic while that of hepatoma GGT was 72% in acidity and 28% in alkaline. After canceration, the total saliva acid and sugar-chains of hepatoma GGT increased to 3-4 times in the hepatoma rats than in the controls. Normal liver GGT contained complete Gal1-4G LcnAc 1-outer sugar-chains, whereas hepatoma GGT showed incomplete sugar-chains, usually without galactose (Gal) outer sugar-chains.[28,29] In this study, liver GGT-N-C concentration of the experimental rats consistently increased during the carcinogenesis of the rats induced with 2-FAA (Table 3). These findings suggest that both liver GGT levels and the sugar-chain heterogeneity of GGT molecule increase apparently in the process of carcinogenesis. Therefore, the detection of GGT-N-C concentration may be useful to hepatoma diagnosis.

The measurement of serum GGT activity is used as a routine laboratory test, which is generally accepted as the most sensitive marker of cholestasis, pancreatic disease, and enzymatic induction by alcohol and drugs. Total GGT activities make differential diagnosis difficult, because GGT is over-expressed in all hepatobiliary diseases. Serum GGT levels are affected by several other factors and a variety of clinical conditions, the measurement of serum total GGT is lack of specificity, and its value in the differential diagnosis is limited because of great overlapping. In the present study, the evaluations of fetal liver-type GGT fractions improved the specificity of the GGT measurement, indicating that there might be a correlation between specific patterns and different disease states,[5,19] and that the fetal liver activity of GGT is increased in cancerous hepatocytes and the fetal forms of GGT can be detected in sera of rats with liver malignancy by analysis of electrophoresis patterns.

GGT isoenzymes of human hepatoma, non-hepatoma tissues and serum in patients with HCC were analyzed in the previous studies,[5] and GGT of the liver supernatant and sera of HCC patients showed the hepatoma specific GGT band located on α1-region of protein electrophoresis, while the non-hepatoma tissues couldn't show the band.[30] These data suggested that hepatoma specific GGT band(s) be derived from hepatoma microsome.[31] Also, the GGT activity could be found in the supernatant, granule parts of fetal liver, and experimental hepatomas. In this study, different multiple forms of rat livers or serum GGT were separated into 1 to 6 bands by a vertical slab electrophoresis using stage gradient polyacrylamide gel. Different electrophoresis patterns of serum or liver GGT were found in the control, fetal and experimental rats (Table 3). The analysis of multiple molecular forms of rat GGT showed five GGT bands (Ⅰ, Ⅲ, Ⅳ, Ⅴ and Ⅵ) in the fetal rat livers, all six bands in sera and livers of the experimental hepatoma rats, and only one band (Ⅱ) in the control rats. The fetal-type GGT appeared at the degeneration stage of rat hepatocytes, and the appearance of fetal liver-type GGT was slightly earlier in liver than in serum, showing its early diagnostic value for hepatomas. The recent albumin-GGT in cirrhotics with HCC would be a sensitive diagnostic marker of both advanced and small HCC-complicating liver cirrhosis.[32]

In conclusion, the expression levels of GGT are

significantly increased in the development of HCC, and the fetal liver-type isoenzymes could be detected in peripheral blood at the early stage of HCC. The circulating GGT isoenzymes could be a useful molecular marker for HCC diagnosis, especially in the early diagnosis of HCC. Further studies will explore the molecular mechanisms of reactivation of GGT in the development of HCC.

Competing interest

The author or one or more of the authors have received or will receive benefits for personal or professional use from a commercial party related directly or indirectly to the subject of this article.

References

1 Peto J. Cancer epidemiology in the last century and the next decade. Nature 2001;411:390-395.
2 Qin LX, Tang ZY. The prognostic molecular markers in hepatocellular carcinoma. World J Gastroenterol 2002;8:385-392.
3 Yao DF, Horie C, Horie T, Shimizu I, Meng XY, Ito S. Virological features of hepatitis C virus infection in patients with liver diseases in the inshore area of the Yangtze river. Tok J Exp Med 1994;41:49-56.
4 Shimizu I, Yao DF, Horie C, Yasuda M, Shiba M, Horie T, et al. Mutations in a hydrophilic part of the core gene of hepatitis C virus from patients with hepatocellular carcinoma in China. J Gastroenterology 1997;32:47-55.
5 Yao DF, Jiang DR, Huang ZW, Lu JX, Tao QY, Yu ZJ, et al. Abnormal expression of hepatoma specific γ-glutamyl transferase and alteration of γ-glutamyl transferase gene methylation status in patients with hepatocellular carcinoma. Cancer 2000; 88:761-769.
6 Tsutsumi M, Sakamuro D, Takada A. Detection of a unique gamma-glutamyl transpeptidase messenger RNA species closely related to the development of hepatocellular carcinoma in humans: a new candidate for early diagnosis of hepatocellular carcinoma. Hepatology 1996;23:1093-1097.
7 Bellin M, Tumino E, Giordani R, Fabrini G, Costa F, Galli R. Serum γ-glutamyl transpeptidase isoforms in alcoholic liver disease. Alcohol & Alcoholism 1997;32:259-266.
8 Chen SZ, Yao DF, Huang JF. Experimental study of γ-glutamyl transpeptidase isoenzyme in early diagnosis of hepatoma. Chin Liver 1996;1:201-204.
9 Kitten O, Ferry N. Mature hepatocytes actively divide and express gamma-glutamyl transpeptidase after D-galactosamine liver injury. Liver 1998;18:398-404.
10 Countay L, Heisterkamp N, Siest G. Expression of multiple γ-glutamyl transferase genes in man. Biochem J 1994;297:503-508.
11 Goodspeed DC, Dunn TJ, Miller CD, Pitot HC. Human γ-glutamyl transpeptidase cDNA: comparison of hepatoma and kidney mRNA in the human and rat. Gene 1989;76:1-9.
12 Huang JF, Yao DF, Wei Q. Purification of γ-glutamyl transferase from human fetal liver and preparation of its antiserum. Acta Acad Med NT 1995;15:163-166.
13 Huang JF, Yao DF, Meng XY, Wei Q, Zhang H. The value of the rocket electrophoresis of hepatoma-specific GGT in the diagnosis of primary hepatic cancer. Chin J Dig 1995;15:269-273.
14 Huang ZW, Yao DF, Meng XY. Clinical significance of quantitative investigation of serum gamma-glutamyl transferase and its high molecular form. Acta Acad Med NT 1993;13:341-342.
15 Simms D, Cizdziel PE, Chomczynski P. TRIzol™: a new reagent for optimal single-step isolation of RNA. Focus 1993; 15:99-102.
16 Yi L, Yao DF, Xu KC, Meng XY. Rapid separation and detection of high molecular γ-glutamyl transpeptidase by a microcolumn chromatogram assay. J Chin Lab Sc 1987;5:175-177.
17 Kobayashi F, Ikeda T, Marumo F. Adenosine deaminase isoenzymes in liver diseases. Am J Gastroenterol 1993;88;266-268.
18 Ray DC, Aldridge LM, Spens HJ, Drummond GB, Howie AF, Beckett GJ. Biological variation and the effect of fasting and halothane anesthesia on plasma glutathione S-transferase concentrations. Clin Chem 1995;41:668-671.
19 Yao DF, Huang ZW, Chen SZ, Xiao MB, Meng XY. Diagnosis of hepatocellular carcinoma by quantitative detection of hepatoma-specific bands of serum γ-glutamyl transferase. Am J Clin Pathol 1998;110:743-749.
20 Okuyama K. Separation and identification of serum gamma-glutamyl transpeptidase isoenzymes by wheat germ agglutinin affinity electrophoresis: a basic analysis and its clinical application to various liver diseases. Keio J Med 1993;42:149-156.
21 Saitoh S, Ikeda K, Koida I, Chayama K, Murashima N, Kumada H. Diagnosis of hepatocellular carcinoma by concanavalin an affinity electrophoresis of serum alpha-fetoprotein. Cancer 1995;76:1139-1144.
22 Khamzina L, Eraiser T, Borgent P. α-Fetoprotein (AFP) expression in clones of McA-RH 777 rat hepatoma: correlation with occurrence of homogeneously staining regions on chromosome 14. Cancer Res 1995;55:3615-3622.
23 Jacob JR, Sterczer A, Toshkov IA, Yeager AE, Korba BE, Cote PJ, et al. Integration of woodchuck hepatitis and N-myc rearrangement determine size and histological grade of hepatic tumors. Hepatology 2004;39:1008-1016.
24 Cui R, He J, Zhang F, Ding H, Shen H, Li Y, et al. Diagnostic value of protein induced by vitamin K absence (PIVKAII) and hepatoma-specific band of serum gamma-glutamyl transferase (GGTII) as hepatocellular carcinoma markers complementary to alpha-fetoprotein. Br J Cancer 2003;88:1878-1882.
25 Carrasco-Legleu CE, Marquez-Rosado L, Fattel-Fazenda S, Arce-Popoca E, Perez-Carreon JI, Villa-Trevino S. Chemoprotective effect of caffeic acid phenethyl ester on promotion in a medium-term rat hepatocarcinogenesis assay. Int J Cancer 2004;108:488-492.
26 Thapliyal R, Naresh KN, Rao KV, Maru GB. Inhibition of nitrosodiethylamine-induced hepatocarcinogenesis by dietary turmeric in rats. Toxicol Lett 2003;139:45-54.
27 Ha WS, Kim CK, Song SH, Kang CB. Study on mechanism of multistep hepatotumorigenesis in rat: development of hepatotumorigenesis. J Vet Sci 2001;2:53-58.
28 Yamashita K, Totani K, Iwaki Y. Comparative study of the sugarchain of γ-glutamyl transpeptidase purified from human hepatocellular carcinoma and from human liver. J Biochem 1989;105:778-785.
29 Sekiya C, Ono M, Yamada M. Measurment of immune-reactive gamma-glutamyl transpeptidase in human sera and liver tissue of patients with various liver disease. Gastroenterology Jpn 1989;24:378-385.
30 Yao DF, Huang JF, Wei Q. Preparation of hepatoma specific

γ-glutamyl transferase isoenzmeⅡantibody and clinical application of its electrophoresis. Med J Commun 1997;11:145-147.
31 Pettersen I, Andersen JH, Bjornland K, Mathisen O, Bremnes R, Wellman M, et al. Heterogeneity in gamma-glutamyltransferase mRNA expression and glycan structures. Search for tumor-specific variants in human liver metastases and colon carcinoma cells. Biochim Biophys Acta 2003;1648:210-218.
32 Pompili M, Addolorato G, Pignataro G, Rossi C, Zuppi C, Covino M, et al. Evaluation of the albumin-gamma-glutamyl transferase isoenzyme as a diagnostic marker of hepatocellular carcinoma-complicating liver cirrhosis. J Gastroenterol Hepatol 2003;18:288-295.

Received June 23, 2004
Accepted after revision August 15, 2004

Those who can take in necessary nutrition are healthier than those who eat more; similarly, a real schloar is not always the man who reads widely but the one who reads useful books.

—— (Ancient Greece) Aristippus