The analysis of γ-glutamyl transpeptidase gene in different type liver tissues

Guo-Qing Han, Cheng-Yong Qin, Rong-Hua Shu

Guo-Qing Han, Cheng-Yong Qin, Rong-Hua Shu, The Center of Liver Diseases, Shandong Provincial Hospital, Clinical Medical College of Shandong University, Jinan 250021, China. Correspondence to: Dr. Guo-Qing Han, The Center of Liver Diseases, Shandong Provincial Hospital, Jinan 250021, China. hgqeq@jn-public.sd.cninfo.net Telephone: +86-531-7938911-2450 Received: 2002-09-13 Accepted: 2002-10-29

Abstract

AIM: To probe the value of γ-glutamyl transpeptidase (GGT) messenger RNA in monitoring canceration of liver cells and for early diagnosis of hepatocellular carcinoma (HCC), by researching the types of GGT messenger RNA (GGTmRNA) in liver tissues and peripheral blood of different hepatopathy.

METHODS: The three types of GGTmRNA (A, B, C) in liver tissues and peripheral blood from the patients with HCC, noncancerous hepatopathy, hepatic benign tumor, secondary carcinoma of liver, and healthy persons were detected by reverse-transcription polymerase chain reaction (RT-PCR).

RESULTS: (1) In normal liver tissues, type A was predominantly found (100.00 %), type B was not found, type C was found occasionally (25.00 %). (2) The distribution of types of GGTmRNA in liver tissues with acute hepatitis, chronic hepatitis, cirrhosis, alcoholic hepatitis was similar as in normal liver tissues (P>0.05), but type B was found in 3 of 18 patients with chronic hepatitis (16.67 %), and also in 3 of 11 patients with cirrhosis (27.27 %). (3) There was no significant difference of types of GGTmRNA between liver tissues with hepatic benign tumor, secondary carcinoma of liver and normal liver tissues (P>0.05). (4) Type B was predominant in cancerous tissues with HCC (87.75 %), the prevalence of type B in cancerous tissues was significantly higher than that in normal liver tissues (0/12) (P<0.05), but the prevalence of type A in cancerous tissues (46.88 %) was significantly lower than that in normal liver tissues (100.00 %) (P<0.05), and the prevalence of type C (6.25 %) in cancerous was the same as that in normal liver tissues (25.00 %) (P>0.05). In noncancerous tissues of livers with HCC, the main types were type A and type B, the prevalence of type A (85.71 %, 90.48 %) and type C (14.29 %, 9.52 %) in noncancerous tissues of liver with HCC was similar as that in normal liver tissues (A: 100.00 %; C: 25.00 %) (P>0.05), but the prevalence of type B (80.95 %, 76.19 %) in noncancerous tissues of livers with HCC was significantly higher than that in normal liver tissues (0/12) (P<0.05). (5) The prevalence of type B (37.5 %) in peripheral blood with HCC was higher than that in normal person (0/12) (P<0.05). In peripheral blood, type B was found in 4 of 11 cases of HCC with serum AFP negative.

CONCLUSION: The shift of types of GGTmRNA from A to B in liver tissues may be closely related to the development of HCC, and the analysis of GGT gene may provide a useful tool for early diagnosis of HCC.
paracancerous tissues (to the brim of carcinoma 3-5 cm) and
distal cancerous tissues (to the brim of carcinoma >5 cm) were
respectively obtained during surgery from 21 patients with
HCC, and the cancerous tissues and noncancerous tissues were
obtained by needle liver biopsy with help of ultrasound B from
the other patients of HCC (11 cases). Liver tissues were
obtained by needle liver biopsy in the other groups, non-tumor
tissues were merely obtained in hepatic benign tumor and
secondary carcinoma of liver. All the tissue specimen was
immediately refrigerated by liquid nitrogen for 1 hour, and
then were transferred to refrigerator at -80 °C for future use.

Blood samples: peripheral blood was obtained from each
person at morning before breakfast, anticoagulated by sodium
citrate solution, and then peripheral blood mononuclear cells
(PBMC) were separated from blood, total RNA was extracted
from PBMC, cDNA was synthesized with reverse transcription,
and then reserved in refrigerator at -80 °C.

Total RNA extraction
Total RNA was extracted by using TRIzol (from TECH-LINE
company) and the purity of RNA was tested with ultraviolet
spectrophotometer of DAOJUN UR-2201.

Detection of the types of GGTmRNA by RT-PCR
CDNA was synthesized with RNA, reverse transcriptase (MLV)
and random primer (Oligdts). then cDNAs of GGTmRNA were amplified by PCR using polymerase and
different primer sets which were specific for the three
GGTmRNA types. Nucleotide sequences of the primer sets to
each type of GGTmRNAs are: type A sense 5'-CAC AGG
GGA CAT ACA GTG AG-3', antisense 5'-GAA ATA GCT
GAA GCA CGC GC-3'; type B sense 5'-GGA TTC GCC
CAG AGA TTG CC-3', antisense 5'-GAA GGT CAA GGG
AGG GTA CC-3'; type C sense 5'-GCC CAG AAG TGA GAG
CAG TT-3', antisense 5'-TCC AGA AAG CAG CTA GAG
GG-3'. The condition of reaction: in advance denaturalization
of 94 °C for 3 min, and then PCR was performed with 30 cycles
consisting of a denaturing step of 94 °C 30 s, an annealing step
of 58 °C for 30 s and an elongation step of 72 °C for 30 s, the
final step at 72 °C was extended to 8 min. The expected size of
each PCR product is 308bp in type A, 300bp in type B, 386bp
in type C. Products after gel electrophoresis by 1.3 % gelose
consisting of a denaturing step of 94 °C 30 s, an annealing step
of 58 °C for 30 s and an elongation step of 72 °C for 30 s, the
final step at 72 °C was extended to 8 min. The expected size of
each PCR product is 308bp in type A, 300bp in type B, 386bp
in type C. Products after gel electrophoresis by 1.3 % gelose
were observed under ultraviolet radiation comparing with
stander DNA.

Statistical analysis
The statistical software SPSS was used for statistical analysis,
the level of significance was P<0.05.

RESULTS
GGTmRNA in tissues
In normal liver tissues (12 cases), type A was the main type, it
was found in all livers (100 %), type B was not found, type C
was found in 3 cases, the GGTmRNA expression was monogenic
(type A) in 9 livers and polygenic (type A + type C) in 3 cases (Table 1).

In liver tissues with acute hepatitis, chronic hepatitis,
cirrhosis or alcoholic hepatopathy, type A was the main type
(81.82-93.33 %), type C was found occasionally (13.33-
23.08 %), the distribution of types of GGTmRNA was similar
as in normal liver tissues (P>0.05), type B was found in 3
cases with chronic hepatitis, 3 cases with cirrhosis, the
prevalence of type B was higher than normal liver tissues, but it was
not significantly different (P>0.05).

In 32 cancerous tissues with HCC, type B was the main
type, it was found in 28 cases, the prevalence of type B in
cancerous tissues (87.5 %) was significantly higher than in
normal livers (P<0.05); type A was found in 15 cases, its
prevalence in cancerous tissues (46.88 %) was lower than in
normal livers(P<0.05); the type C was not significantly
different between cancerous tissues and normal liver tissues
(P>0.05). In 21 adjacent paracancerous tissues, 21 distal
cancerous tissues and 11 noncancerous tissues, the main type
was type A and B, the prevalence of type A (87.51 %, 90.48 %,
81.82 %) was similar as in normal livers (P>0.05); however the
prevalence of type B (80.95 %, 76.19 %, 72.73 %) was
significantly higher than in normal liver tissues (P<0.05); the
prevalence of type C was similar as in normal livers (P>0.05).

In nontumor tissues of livers with hepatic benign tumor and
secondary carcinoma of liver, the distribution of GGTmRNA was similar as in normal livers (P>0.05).

The relation between types of GGTmRNA and size of HCC:
The prevalence of type A in cancerous tissues of larger sized HCC
is lower than in that of smaller sized HCC, the monogenic pattern
of type B tended to be found more frequently in larger sized HCC,
but the difference was not significant (P>0.05 ). Table 2.

| Group | No. of Samples | Type of GGTmRNA |
|-------|---------------|-----------------|
|       | A (%)          | B (%)           | C (%)           |
| 1.Normal | 12             | 12 (100%)       | 0               | 3 (25.00%)      |
| 2.Acute hepatitis | 15             | 14 (93.33%)     | 1 (6.67%)       | 2 (13.33%)      |
| 3.Chronic hepatitis | 18             | 16 (88.89%)     | 3 (16.67%)      | 4 (22.22%)      |
| 4.Cirrhosis | 11             | 9 (81.82%)      | 3 (27.27%)      | 2 (18.18%)      |
| 5.Alcoholic hepatopathy | 13            | 12 (92.31%)     | 1 (7.66%)       | 3 (23.08%)      |
| 6.Hepatic benign tumor | 10       | 10 (100%)       | 0               | 1 (10.00%)      |
| 7.Secondary carcinoma of liver | 13 | 11 (84.62%) | 0 | 3 (23.08%) |
| 8.HCC | 32             |                 |                 |                |
| Cancerous tissues | 32         | 15 (46.88%)*    | 28 (87.5%)*     | 2 (6.25%)      |
| Adjacent Paracancerous tissues | 29             | 18 (65.71%)     | 17 (60.95%)*    | 3 (14.29%)      |
| Distal Cancerous tissues | 21          | 19 (90.49%)     | 16 (76.19%)*    | 2 (9.52%)       |
| Noncancerous tissues | 11          | 9 (81.82%)      | 8 (72.73%)*     | 1 (9.09%)       |

*P<0.05 vs. normal liver.

Table 2 GGTmRNA and the size of HCC

| Group | Small sized HCC | Large sized HCC | Enormous sized HCC |
|-------|----------------|----------------|-------------------|
| Cancerous tissues | 8            | 16             | 8                 |
| type A       | 5(62.50%)     | 7(43.75%)      | 3(37.50%)         |
| Cancerous tissues | 8            | 16             | 8                 |
| type B       | 6(75.00%)     | 15 (93.75%)    | 7(87.50%)         |
| Cancerous tissues | 8            | 16             | 8                 |
| type B       | 5(62.50%)     | 14 (87.50%)    | 7(87.50%)         |
| Peri-B |                |                |                   |
| Peripheral type B | 8            | 16             | 8                 |
| type B       | 5(62.50%)     | 14 (87.50%)    | 7(87.50%)         |

GGTmRNA in peripheral blood
In peripheral blood of 32 patients with HCC, type A was found
in 2 cases (6.25 %), type B was found in 12 cases, the
prevalence of type B (37.5 %) was significantly higher than
normal (P<0.05), type C was found in 1 case (3.13 %). In
peripheral blood of patients with acute hepatitis, type A was
found in 2 cases. In chronic hepatitis group, type A was found
in 1 case. Type B and C were not found in acute and chronic
hepatitis group. In the other groups, GGTmRNA was not found.
In 8 cases of small sized HCC, type B was found in 5
noncancerous tissues (62.5 %) and in peripheral blood of 2
cases (25 %). In the 8 cases, there were 3 patients with serum

HAN GQ et al. The analysis of γ-glutamyl transpeptidase gene in different type liver tissues 277
In 21 cases of HCC with AFP positive, type B was found in peripheral blood of 8 cases (38.1 %). In 11 cases of HCC with AFP negative, type B was found in peripheral blood of 4 cases (36.36 %). Type B was not significantly different between them ($P>0.05$). Therefore the prevalence of type B in peripheral blood was not related to the prevalence of AFP.

DISCUSSION

Hepatocellular carcinoma is one of the common malignant tumor[17-29], because of its severe malignance, quick development, early intrahepatic metastasis, mostly being combined with cirrhosis, frequent recurrence, the prognosis of HCC still remains dismal[30-41]. By now, surgery is still the most efficient treatment for HCC, but about 70 percent of patients with HCC lost the opportunity of surgery, since they did not go to see a doctor until the tumor reached an advanced stage, and HCC recurred more frequently after surgery, on the other hand, HCC is not susceptible to radiotherapy, chemotherapy and other synthetic treatments[42-44], so it is imperative to clarify the pathogenic mechanism of HCC and to find efficient methods for early diagnosis of HCC. The epidemiological studies suggested that the prevalence of HCC in patients with hepatopathy had been obviously increasing in China. At present, the pathogenic mechanisms of HCC are not well known, it is reported that the occurrence and evolvement HCC may be a process of polygenic and multiple steps, which related to polygenic expression, such as repair of DNA, signal transduction, cell cycle regulation etc[45-52].

It is still difficult to monitor the canceration of liver cells in preneoplastic stage and early stage of HCC[53]. If we could monitor the changes of the structure and function of some genes, we would find the patients with high risk of HCC, forecast the possibility of occurrence of HCC before cytological changes, and then we could prevent, make a diagnosis and give treatment on molecular level.

It has been reported that HCC synthesizes and secretes many proteins, polypeptides or isoenzymes such as AFP, GGT etc. they may be used as important marks for the diagnosis of HCC. GGT is closely relate to biotransformation, metabolism of nucleic acid, and the occurrence of carcinoma, it may be used as a mark for detection of bibulosity and the canceration of nucleic acid, and the occurrence of carcinoma, it may be used as important marks for the diagnosis of HCC. GGT is closely related to the shift of GGT mRNA. The serum level of GGT is mostly higher in patients with alcoholic hepatopathy, but the distribution of types of GGT mRNA in these liver tissues was similar as in normal liver tissues. This result suggested that alcohol did not induce the shift of GGT mRNA.

Studies about small sized HCC have been the important incident in the history of HCC in the past 20 years. Early diagnosis and treatment are the keys to increase survival rate and decrease recurrence rate. The detection of serum alpha-fetoprotein (AFP) is an important method for early diagnosis of HCC, especially in patients with high risk of HCC[55,56]. However, the negative rate of AFP is higher in patients with small sized HCC. In present study, among the 8 patients with small sized HCC, type B was detected in noncancerous liver tissues of 5 patients, and in peripheral blood of 2 cases, however, AFP was positive in serum of only 2 patients. Moreover, in peripheral blood, type B of GGT mRNA was found in 4 of 11 HCC patients with AFP negative (36.36 %). These results suggested that the detection of unique type B of GGT mRNA may provide a useful tool for the diagnosis of the small sized HCC and HCC with AFP negative.

Since there are lots of RNA enzymes in blood plasma, RNA will be degraded by RNA enzymes as soon as it appears in plasma, so there are not dissociative GGT mRNAs in blood plasma in normal blood plasma. In this study, GGT mRNAs were not found in normal peripheral blood, however, among 32 cases of HCC, type B of GGT mRNA was found in peripheral blood of 12 cases (37.5 %). GGT mRNAs were not found in peripheral blood in other groups. So it may be inferred that cancerous cells exist in peripheral blood of this 12 patients. These results suggest that the shift of type of GGT mRNA are closely related to the development of HCC, and that analysis of GGT mRNA expression may provide a useful tool for early diagnosis of HCC.

REFERENCES

1. Ma XD, Sui YF, Wang WL. Expression of gap junction genes connexin32 and connexin43 and their proteins in hepatocellular
carcinoma and normal liver tissues. World J Gastroenterol 2000; 6: 66-69
2 Wang Y, Liu H, Zhao Q, Li X. Analysis of point mutation in site 1896 of HBV procore and its detection in the tissues and serum of HCC patients. World J Gastroenterol 2000; 6: 395-397
3 Mei MH, Xu J, Shi QF, Yang JH, Chen Q, Qin LL. Clinical significance of serum intercellular adhesion molecule detection on patients with hepatocellular carcinoma. World J Gastroenterol 2000; 6: 406-410
4 Qin Y, Li B, Tan YS, Sun ZL, Zuo FQ, Sun ZF. Polymorphism of p16INK4a gene and rare mutation of p15INK4b gene exon2 in primary hepatocellular carcinoma. World J Gastroenterol 2000; 6: 411-414
5 Lin NF, Tang J, Mohamed-Ismail HS. Study on environmental etiology of high incidence areas of liver cancer in China. World J Gastroenterol 2000; 6: 572-576
6 Tian DY, Yang DF, Xia N, Zhang ZG, Lei HB, Huang YC. The serological prevalence and risk factor analysis of hepatitis G virus infection in Hubei Province of China. World J Gastroenterol 2000; 6: 585-587
7 Zhong DR, Ji XL. Hepatic angiomyolipoma misdiagnosis as hepatocellular carcinoma: a report of 14 cases. World J Gastroenterol 2000; 6: 608-612
8 Riordan SM, Williams R. Transplantation of primary and reversibly immortalized human liver cells and other gene therapies in acute liver failure and decompensated chronic liver disease. World J Gastroenterol 2000; 6: 636-642
9 Li Y, Su JJ, Qin LL, Yang C, Luo D, Ban KC, Kessler TW, Roeck-Buck BD. Chemopreventive effect of oltipraz on AFB1 induced hepatocarcinogenesis in tree shrew model. World J Gastroenterol 2000; 6: 647-650
10 Xu HY, Yang YL, Gao YY, Wu QL, Gao GQ. Effect of arsenic trioxide on human hepatoma cell line BEL 7402 cultured in vitro. World J Gastroenterol 2000; 6: 688-692
11 Huang Xf, Wang CM, Dai XW, Li ZJ, Pan BR, Yu LB, Qian B, Fang L. Expression of chromogranin A and cathepsin D in human primary hepatocellular carcinoma. World J Gastroenterol 2000; 6: 693-696
12 Xu HY, Yang YL, Guan XL, Song G, Jiang AM, Shi LJ. Expression of regulating apoptosis gene and apoptosis index in primary liver cancer. World J Gastroenterol 2000; 6: 721-724
13 Chen YP, Liang W, Zhang L, He HT, Luo XQ. Transfusion transmitted virus infection in general populations and patients with various liver diseases in south China. World J Gastroenterol 2000; 6: 738-741
14 Ma XD, Sui YF, Wang WL. The expression of gap junction protein connexin32 in human hepatocellular carcinoma, cirrhotic and normal human liver tissues. J Zheng 1999; 18: 133-135
15 The prevention and cure project for virus hepatitis. Zhonghua Ganzangbing Zazhi 2000; 8: 324-329
16 Worman HJ, Lin F, Mamiya N, Mustacchia PJ. Molecular biology and the diagnosis and treatment of liver diseases. World J Gastroenterol 1998; 4: 185-191
17 Lee JH, Ku JI, Park YJ, Lee KU, Kim WH, Park JG. Establishment and characterization of four human hepatocellular carcinoma cell lines containing hepatitis B virus DNA. World J Gastroenterol 1999; 5: 289-296
18 He XW, Wang JL. The current status and prospect in the gene therapy of liver cancer. Hua ren Xiu hua Zazhi 1998; 6: 158-159
19 Fu JM, Yu XF, Shao YF, Telomerase and primary liver cancer. Shijie Hua ren Xiu hua Zazhi 2000; 8: 461-463
20 Zhai SH, Liu JB, Liu YM, Zhang LL, Du ZP. Expression of HBSAg, HCV-Ag and AFP in liver cirrhosis and hepatocarcinoma. Shijie Hua ren Xiu hua Zazhi 2000; 8: 524-527
21 Su YM, Zhu SN, Lu SL, Gu YH. HCV genotypes expression in hepatocellular carcinoma by reverse transcription in situ polymerase chain reaction. Shijie Hua ren Xiu hua Zazhi 2000; 8: 874-878
22 Tang YW, Yao XQ. Regulating effect of HCC cells on the activation of stellate cells. Shijie Hua ren Xiu hua Zazhi 2001; 9: 202-204
23 Cui J, Yang DH, Qin HR. Mutation and clinical significance of cfms oncogene in hepatocellular carcinoma. Shijie Hua ren Xiu hua Zazhi 2001; 9: 392-395
24 Cheng H, Liu YF, Zhang HZ, Shen WA, Zhang SZ. Construction and expression of anti-HCC immunotoxin of sFv-TNF-α and GFP fusion proteins. Shijie Hua ren Xiu hua Zazhi 2001; 9: 640-644
25 Jiang YG, Wang YM, Li QF. Expression significance of HLA-DR antigen and heat shockprotein 70 in hepatocellular carcinoma. Shijie Hua ren Xiu hua Zazhi 2001; 9: 1139-1142
26 Wu MC. The review of hepatology study in China. Shijie Hua ren Xiu hua Zazhi 2000; 8: 1201-1204
27 Meng ZH, He ZP. Current situation of gene therapy studies in inhibition of liver cancer. Shijie Hua ren Xiu hua Zazhi 1999; 7: 350-352
28 Tang QY, Yao DF, Liu YH, Meng XY, Yang SH, Wu XH, Wu W, Lu JX: Abnormal expression and methylation of γ-glutamyl transferase genes in human hepatoma tissue. Zong honghua Xiu hua Zazhi 1999; 18: 168-171
29 Ning XY, Yang DH. Study and progress on in vivo gene treatment of primary hepatocarcinoma. Shijie Hua ren Xiu hua Zazhi 2000; 8: 89-90
30 Tang ZY, Sun FX, Tian J, Ye SL, Liu YK, Liu KD, Xue QJ, Chen, Xia Jl, Qin LX, Sun SL, Wang L, Zhou J, Li Y, Ma ZC, Zhou XD, Wu ZQ, Lin ZY, Yang BH. Metastatic human hepatocellular carcinoma models in nude mice and cell line with metastatic potential. World J Gastroenterol 2001; 7: 597-603
31 Wang WX, Dong JY, Zhou SY, Li WL, Zhou Y. Modification of ricin and its hepatotoxicity and activity against hepatocellular cancer in mice. World J Gastroenterol 1998; 4: 307-310
32 Wang ZX, Hu GF, Wang HY, Wu MC. Expression of liver cancer associated gene HCC3A. World J Gastroenterol 2001; 7: 821-825
33 Tang ZY. Hepatocellular carcinoma-Cause, treatment and metastasis. World J Gastroenterol 2001; 7: 445-454
34 Cui J, Zhou XD, Liu YK, Tang ZY, Zile MH: Abnormal betacatenin gene expression with invasiveness of primary hepatocellular carcinoma in China. World J Gastroenterol 2001; 7: 542-546
35 Huang XF, Wang CM, Dai XW, Li ZJ, Pan BR, Yu LB, Qian B, Fang L. Expressions of chromogranin A and cathepsin D in human primary hepatocellular carcinoma. World J Gastroenterol 2000; 6: 693-696
36 Liang Y, Lu B, Cui ZF, Li XD, Guo YJ, Lu YJ. The expression of Fas/Fasl in hepatocellular carcinomas. Shijie Hua ren Xiu hua Zazhi 2001; 9: 1364-1368
37 Lee JH, Ku JI, Park YJ, Lee KU, Kim WH, Park JG. Establishment and characterization of four human hepatocellular carcinoma cell lines containing hepatitis B virus DNA. World J Gastroenterol 1999; 5: 289-295
38 Parks R, Garden O. Liver resection for cancer. World J Gastroenterol 2001; 7: 766-771
39 Xu HY, Yang YL, Guan XL, Song G, Jiang AM, Shi LJ. Expression of regulating apoptosis gene and apoptosis index in primary liver cancer. World J Gastroenterol 2000; 6: 721-724
40 Lin NF, Tang J, Ismail HS. Study on environmental etiology of high incidence areas of liver cancer in China. World J Gastroenterol 2000; 6: 572-576
41 Gao F, Chen YJ, Yu XL. Drug induce the apoptosis of hepatocellular cells. Shijie Hua ren Xiu hua Zazhi 2001; 9: 688-688
42 Zhang JK, Chen HB, Sun JL, Zhou YQ. Effect of dendritic cells on LPK cells induced at different times in killing hepatoma cells. Shijie Hua ren Xiu hua Zazhi 1999; 7: 673-675
43 Cao W, Wang ZM, Liang ZH, Zhang HX, Wang YQ, Guan YL, Xia WN, Pan BR. Effects of antiogenesis inhibitor TNP-470 with lipiodol in arterial embolization of liver cancer in rabbits. Shijie Hua ren Xiu hua Zazhi 2000; 8: 629-632
44 Lu B, Dai YM. Abnormal cycle regulation of cells in the HCC. Shijie Hua ren Xiu hua Zazhi 2001; 9: 205-208
45 Wang Q, Lin ZY, Feng XL. Alterations in metastatic properties of hepatocellular carcinoma cell following H ras oncogene transfection. World J Gastroenterol 2001; 7: 355-359
46 Xu DX, Chen WS, Ye ZJ. The antisense gene of growth factor receptor reversing the malignant phenotype of human hepatoma cells. Shijie Hua ren Xiu hua Zazhi 2001; 9: 175-179
47 Yang JQ, Yang LY, Zhu HC. Mitomycin C. Induced apoptosis of
human hepatoma cell. Shijie Huaren Xiaohua Zazhi 2001; 9: 268-272

49 Li BA, Wang HY, Chen ZJ, Wu MC. The association between signal-regulatory protein-alpha and hepatocellular carcinoma. Zhonghua Yixue Zazhi 1999; 79: 268-270

50 Zheng SX, Li XG, Zhou LJ, Zhu XZ. Construction of pcDNA3/p16 plasmid and its inhibitory role in the proliferation of hepatoma cell line. Shijie Huaren Xiaohua Zazhi 2000; 8: 49-51

51 Lu DD. The research progress of the relationship between DNA methyl and hepatocellular carcinoma. Zhonghua Ganzangbing Zazhi 1999; 7: 251-252

52 Yang DH, Zhang MQ. Cell apoptosis and hepatocellular carcinoma. Zhonghua Ganzangbing Zazhi 1999; 7: 123-124

53 Yao DF, Jiang DR, Wu XH, Zhu YS, Huang JF, Mong XY. Experimental study on value of carcino-embryonic gamma-glutamyl transferase isoenzymes for monitoring carcinogenesis of hepatocytes. Zhonghua Ganzangbing Zazhi 2000; 8: 30-32

54 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

55 Zhou XP, Chen WX. The image diagnosis of hepatocellular carcinoma. Shijie Huaren Xiaohua Zazhi 2000; 8: 439-440

56 Tian FZ. Tumor markers of hepatocellular carcinoma. Shijie Huaren Xiaohua Zazhi 2000; 8: 440-441