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• LIVER CANCER •

Is the expression of γ-glutamyl transpeptidase messenger RNA an indicator of biological behavior in recurrent hepatocellular carcinoma?

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Abstract

AIM: To investigate the correlation between gamma-glutamyl transpeptidase (γ-GTP) expression in the primary HCC and post-resection recurrence and its biological behaviors.

METHODS: Forty consecutive patients having curative resection for HCC were included in this study. The primers for reverse-transcription polymerase chain reaction (RT-PCR) were corresponding to the 5'-noncoding human γ-GTP mRNA of fetal liver (type A), HepG2 cells (type B), and placenta (type C). Both the cancer and non-cancerous tissues of the resected liver were analyzed. The correlations between the expression of γ-GTP and the clinicopathological variables and outcomes (recurrence and survival) were studied.

RESULTS: Those with type B γ-GTP mRNA in cancer had significant higher recurrence rate than those without it (63.6 % *vs* 14.3 %). Both those with type B in cancer and in non-cancer died significantly more than those without it (45.5 % *vs* 0 % and 53.6 % *vs* 0 %, respectively). By multivariate analysis, the significant predictors of recurrence included high serum AFP (P=0.0108), vascular permeation (P=0.0084), and type B γ-GTP mRNA in non-cancerous liver (P=0.0107). The significant predictors of post-recurrence death included high serum AFP (P=0.0141), vascular permeation (P=0.0130), and daughter nodules (P=0.0053). As to the manifestations (recurrent number ™ℝ2, recurrent extent ™ℝ2 segments, extra-hepatic metastasis, and death) in recurrent patients, there were no statistical significant differences between those with type B in the primary tumor and those without it. The difference between those with type B in non-cancerous liver and those without it also was not significant.

CONCLUSION: Patients of HCC with type B γ-GTP mRNA both in cancer and in non-cancerous tissue had a worse outcome, earlier recurrence, and more post-recurrence death.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the leading malignant tumors with a poor prognosis in areas of high hepatitis B and C prevalence. During the last 10 years, efforts have been made worldwide toward earlier detection and safer surgical resection of HCC. However, despite these recent diagnostic and therapeutic advances, postoperative recurrence is still common[1-4]. How to predict recurrence before resection is a challenging problem for surgeons. Certain characteristics related to HCC recurrence have been reported widely and variably in the literatures[1-14]. γ-glutarmyl transpeptidase (γ-GTP) is an important enzyme catalyzing hydrolysis of glutathione and transfer of the γ-glutamyl residue and is widely distributed in mammalian tissues[15-23]. The enzyme has been used as an important marker enzyme for some neoplasms, HCC and preneoplastic lesions of the liver[24-31]. According to Tsutumi's study, changes in the expression of hepatic γ-GTP mRNA may be related to the development of HCC[24]. The aim of this study is to elucidate the role of the expression of γ-GTP in both cancerous and non-cancerous liver tissues of primary lesion in the prediction of HCC recurrence.

MATERIALS AND METHODS

Patients and tumor samples

Forty patients with HCC who underwent curative hepatectomy at the Department of Surgery, Mackay Memorial Hospital from January 1997 to June 1998, whose tissue specimens histopathologically found to have no degeneration or necrosis, were included in this study. Freshly resected specimens were used. Clinical details were available from medical records on all patients (Table 1). The mean age of patients was 52.4± 16.6 years (range 16-82) with a male to female ratio of 26:14. The operations included major resections (12 partial lobectomies, 18 lobectomies and 5 extended lobectmies) and minor resections (4 segmentectomies and 1 subsegmentectomy). After the resection, all patients were followed up at out-patient-clinic receiving regular clinical assessment, periodic abdominal ultrasonography (every 2 to 3 months during the first 5 years, then every 4 to 6 months thereafter) to detect tumor recurrence, serum alpha-fetoprotein (AFP) and liver biochemistry (every 2 months during the first 2 years, then every 4 months during the following 3 years, and every 6 months thereafter). Abdominal computed tomography was also done (every 6 months during the first year, then every year). Liver specimens were stored at -80 ¡æuntil RNA extraction.

Patients in the control group included 5 healthy volunteers, 5 individuals with chronic active hepatitis without HCC and 5

individuals with liver cirrhosis without HCC. All received liver biopsies for γ-GTP mRNA study during exploratory laparotomy.

Table 1 Demography

Characteristics	Total (*n*=40)
Age (years)	52.4±16.6 [16-78]
Male26 (65%)	
Liver cirrhosis	33 (82.5%)
Child-Pugh class A:B	32:8 (80%:20%)
Tumor size	
Small (<3 cm)	6 (15.0%)
Median (3-10 cm)	23 (57.5%)
Large (>10 cm)	11 (27.5%)
HBsAg (+)	22 (55.0%)
Anti-HCV (+)	15 (37.5%)
AFP	
Below 20 μg /L	17 (42.5%)
Over 1000 μg/L	14 (35.0%)
Capsule	
Complete	18 (45.0%)
Incomplete or absent	22 (55.0%)
Edmondson-Steiner grade	
I & II	19 (47.5%)
III & IV	21 (52.5%)
Vascular permeation	17 (42.5%)
Satellite formation	19 (47.5%)
Multiple HCC	13 (32.5%)
Major resection	32 (80.0%)
Minor resection	8 (20.0%)

Reverse transcription-polymerase chain reaction

For HCC liver specimens, both nucleotide probes to each type of γ-GTP mRNA were shown in Table 2. To rule out any false positive, cancerous and non-cancerous tissue were studied for the expression of γ-GTP mRNA, respectively. For the resected specimens, the non-cancerous tissue was taken at least 1.0 cm far from HCC. For control group, only non-cancerous liver tissue was studied.

Table 2 Nucleotide sequences of the primer sets and specific oligonucleotide probes to each type of γ-GTP 5'-noncoding mRNA

Type of γ-GTP mRNA or	Primers probes	Nucleotide sequences
Primer sets		
A type	Sense	5'-CAC AGG GGA CAT ACA GTG AG-3'
	Antisense	5'-GAA ATA GCT GAA GCA CGC GC-3'
B type	Sense	5'-GGA TTC TCC CAG AGA TTG CC-3'
	Antisense	5'-GAA GGT CAA GGG AGG TTA CC-3'
C type	Sense	5'-GCC CAG AAG TGA GAG CAG TT-3'
	Antisense	5'-TCC AGA AAG CAG CTA GAG GG-3'
Oligonucleotide probes		
A type		5'-GGG CAG GGC TTG GTG AAT GGT AGC TGT GAT TAT CAT CAT G-3'
B type		5'-CGC AGG ATG GTG TGC GAG GAC CCC GAG CTG GTG TTC CAG GC-3'
C type		5'-ATA GAG ACA CCG ATT CCT GGA GGT CCA AAG AGC CTC AGG A-3'

Total RNA was extracted from the homogenized liver specimens by the method of Chomocynsky *et al*[32]. cDNA of γ-GTP mRNAs were amplified by reverse-transcription polymerase chain reaction (RT-PCR) using three different primer sets, which were specific for the three types. Six oligonucleotides, designed from cDNA sequences of γ-GTP at the 5'-noncoding region of human fetal liver, human placenta, and HepG2 cells were synthesized for use as the primers in PCR. The expected size of each PCR product was 308 bp in fetal liver (type A), 300 bp in Hep G2 (type B), and 386 bp in placenta (type C). The existence of intron sequences between each PCR primer set was confirmed by human γ-GTP genomic cloning and partial sequencing. The primer sets for type A, type B, and type C were corresponded to the sequences on γ-GTP cDNA from fetal liver, Hep G2 cells and placenta, respectively. An aliquot of the amplified γ-GTP cDNA fragments was analyzed on a 1 % agarose gel and transferred onto a Hybond-N membrane. The amplified γ-GTP cDNA were hybridized on Southern blots with oligonucleotide probes which were specific for each type of γ-GTP mRNA.

As the probes for type A, type B, and type C, 3 oligonucleotides were synthesized and were labeled at their 3' end using fluorescein d-UTR. The hybridized bands were reacted with HRP-labeled anti-fluorescein antibody and visualized on film using the ECL 3'-oligolabelling and detection systems of Amersham Life Science (Buckinghamshire, England), according to the instruction manual.

Parameters

The difference of γ-GTP expression in diverse clinicopathologic parameters were evaluated. Parameters included the presence of liver cirrhosis (confirmed by the operative findings and also by the pathology examination of the specimen), hepatitis B surface antigen (HBsAg), hepatitis C virus infection (anti-HCV), Child-Pugh classification of liver reverse (class A *vs* B), serum alpha-fetoprotein (AFP, <20 ng/ml *vs* 20-1 000 ng/ml *vs* >1 000 ng/ml) titer, tumor size (<3 cm, 3-10c m, >10 cm), cell differentiation grade (Edmondson-Steiner grade l-ll *vs* III-IV), encapsulation (complete, infiltration by HCC or absent) and vascular permeation (including vascular invasion and/or tumor thrombi within the portal vein or hepatic vein), daughter nodules or satellite nodules, multi-centric HCC and clinical evidence of recurrence, recurrence free interval, survival and death. The expression of γ-GTP mRNA of both cancerous tissue and non-cancerous tissue of the liver from resected specimens were compared with the above parameters by both univariate (UV) and multivariate (MV) analysis, respectively.

Statistical analysis

Statistical program (BMDP), Student's *t*-test or Mann-Whitney U test for continuous variables, χ^2 test of Fisher's exact test for categorical variables, and logistic regression and COX proportional hazards model for multivariate analysis were used. *P* value <0.05 was considered as a significant difference.

RESULTS

The types of γ-GTP mRNAs in livers obtained from 15 control patients were shown in Table 3. The γ-GTP mRNA expression was monogenic in 13 patients and polygenic in 2 patients. Type A was found in all patients (100 %), type B was found in one patient (6.7 %) and type C was found in one patient (6.7 %). In those with HCC, from the cancerous portion of the livers, the distribution of the type of γ-GTP mRNA was one (2.5 %) case of type A (monogenic), 24 (60.0 %) cases of type B (monogenic), 15 (37.5 %) cases of both type A and B (polygenic), and none of type C. From their non-cancerous portion of the livers, the distribution of the type of γ-GTP

mRNA was 12 (30.0 %) cases of type A (monogenic), 16 (40 %) cases of type B (monogenic), 12 (30 %) cases of type A and type B (polygenic) and none of type C (Table 3). Among those with HCC, the frequency of type A with type B (polygenic) or type B (monogenic) was 97.5 % in cancerous tissues and 70.0 % in non-cancerous tissues.

Table 3 Frequency of γ-GTP mRNA in liver

Tissues	mRNA types (%)				
	A	A+B	B	A+B or B	C
HCC					
Cancerous	1 (2.5)	15 (37.5)	24 (60.0)	39 (97.5)	0 (0)
Non-Cancerous	12 (30.0)	12 (30.0)	16 (40.0)	28 (70.0)	0 (0)
	A	B		C	
Control	15 (100.0)	1 (6.7)			1 (6.7)

The correlation between those with type B and type B with type A in cancerous tissue and non-cancerous tissue and patients' characteristics were shown in Table 4. Gender, positivity of HBsAg, and Chlid-Pugh class A or B showed no statistically significant differences between the presence and the absence of type B and type B with type A γ-GTP mRNA.

Table 4 Correlation between type B γ-GTP mRNA and clinical parameters

Parameters	Cases	Type B / A+B(%)	
		HCC liver	Non HCC liver
Male	26	23 (88.5)	20 (76.9)
Female	14	10 (71.4)	8 (43.9)
HBsAg (+)	22	19 (86.4)	17 (60.7)
(-)	18	14 (77.8)	11 (39.3)
HCV (+)	15	14 (93.3)	14 (93.3)[b]
(-)	25	19 (76.0)	14 (56.0)[b]
Child A	32	25 (75.8)	21 (65.6)
Child B	8	8 (100.0)	7 (87.5)
AFP (ng / ml) <20	17	11 (64.7)[a]	9 (32.1)[c]
=20	23	22 (95.6)[a]	19 (82.6)[c]

Denotes: [a]*P*=0.0295; [b]*P*=0.0151; [c]*P*= 0.0430.

From the univariate analysis, in cancerous portion of HCC livers, a significant correlation was found between type B and type B with type A expression of γ-GTP mRNA and high serum AFP level (*P*=0.0295). In non-cancerous portion of HCC livers, a significant correlation was found between type B and type B with A expression of γ-GTP mRNA and positivity of HCV antibody (*P*=0.0151) and high serum AFP level (*P*=0.0430).

The correlations between the types of γ-GTP mRNA expression and pathological parameters were shown in Table 5. From the univariate analysis, in cancerous portion of HCC livers, a significant correlation was found between type B and type B with type A γ-GTP mRNA and the presence of daughter nodules (*P*=0.0089).

In non-cancerous portion of HCC livers, a significant correlation was found between type B and type B with type A and the Edmondson-Steiner grade I and II *vs* III and IV (*P*=0.0226), the absence of tumor capsule (*P*=0.0014), the presence of vascular permeation (*P*=0.0042) and the presence of daughter nodules (*P*=0.0012). In both cancerous and non-cancerous livers, no statistically significant difference was shown between type B and type B with type A and the presence of liver cirrhosis, tumor size and multi-centric HCC.

From outcome point of view, among those 39 patients whose cancerous liver with type B or type B with A γ-GTP mRNA, 21 patients (53.8 %) had recurrence (Table 6). As for the other patients without type B and B with A did not have had recurrence. The difference was statistically significant (*P*=0.0328). Among the former, 15 patients (38.5) died. Among the latter, no patient died. The difference was also statistically significant (*P*=0.0328). The death was related to tumor recurrence.

Table 5 Correlation between types of γ-GTP mRNA and pathological parameters

Parameters	Cases	Type B / A+B(%)	
		HCC liver	Non HCC liver
Cirrhosis (+)	33	27 (81.8)	22 (66.7)
(-)	7	6 (85.7)	6 (85.7)
Size< 3 cm	6	6 (100.0)	4 (66.7)
3-10 cm	11	7 (63.6)	5 (45.4)
=10 cm	23	20 (86.9)	19 (82.6)
Differentiated grade I / II	19	14 (73.7)	10 (52.6)[b]
III / IV	21	19 (90.5)	18 (85.7)[b]
Complete capsule (+)	18	13 (72.2)	8 (44.4)[c]
(-)	22	20 (90.9)	20 (90.9)[c]
Vascular permeation (+)	17	16 (94.1)	16 (94.1)[d]
(-)	23	17 (73.9)	12 (52.2)[d]
Daughter nodules (+)	19	19 (100.0)[a]	18 (94.7)[e]
(-)	21	14 (66.7)[a]	10 (47.6)[e]
Multi-centric (+)	13	12 (92.3)	12 (92.3)
(-)	27	21 (77.8)	16 (59.2)

Denotes: [a]*P*=0.0089; [b]*P*=0.0226; [c]*P*=0.0014; [d]*P*= 0.0042; [e]*P*=0.0012.

Table 6 Correlation between γ-GTP mRNA and their outcome

γ-GTP mRNA		Cases	Recurrence (%)	Death (%)
Type B in HCC liver	(+)	33	21 (63.6)[a]	15 (45.5)[b]
	(-)	7	1 (14.3)[a]	0 (0)[b]
Type B in non-HCC liver	(+)	28	22 (78.6)[c]	15 (53.6)[d]
	(-)	12	0 (0)[c]	0 (0)[d]

Denotes: [a]*P*= 0.0328; [b]*P*=0.0328; [c]*P*:<0.0001; [d]*P*: 0.0011

Among 28 patients whose non-cancerous liver tissue was type B or type B with type A, 22 patients (78.6 %) had recurrence. Among the other 12 patients without type B or type B with type A, no patient had recurrence. The difference had statistical significance (*P*<0.0001). Among the former, 15 patients (53.6 %) died, while among the latter, no patient died. The difference had statistical significance (*P*=0.0011).

The correlations between the parameters and recurrence were shown in Table 7. In UV analysis, statistically significant difference was found in high serum AFP (*P*=0.0001), large size (*P*=0.0213), differentiation grade (III and IV) (*P*=0.0049), absence of complete capsule (*P*=0.001), vascular permeation (*P*<0.0001), presence of daughter nodules (*P*=0.0003), type B (including type B and type B with type A) in cancerous liver (*P*=0.0094) and type B in non-cancerous (including type B and type B with type A) liver (*P*=0.0003). In MV analysis, only AFP (*P*=0.0108), vascular permeation (*P*=0.0048), and type B γ-GTP mRNA in non-cancerous liver had significant difference (*P*=0.0107).

Table 7 Significant factors in recurrence and death

Parameters	Recurrence		Death	
	UV	MV	UV	MV
Sex	n.s.	n.s.	n.s.	n.s.
Age	n.s.	n.s.	n.s.	n.s.
Child-Pugh's classification: A *vs.* B	n.s.	n.s.	n.s.	n.s.
HBsAg (+)	n.s.	n.s.	0.0166	n.s.
HCV (+)	n.s.	n.s.	n.s.	n.s.
AFP<20 *vs.* 20-1000	0.0001	0.0108	0.0006	0.0141
γ-GTP mRNA (+)	n.s.	n.s.	n.s.	n.s.
Pathology of HCC:				
Size: <3 cm *vs.* 3-10 cm	0.0213	n.s.	0.0080	n.s.
Cirrhosis	n.s.	n.s.	n.s.	n.s.
Edmondson-Steiner grade				
Complete capsule (+)	0.0001	n.s.	0.0007	n.s.
Vascular permeation (+)	<0.0001	0.0084	<0.0001	0.0130
Daughter nodules (+)	0.0003	n.s.	0.0001	0.0053
Multicentric (+)	0.0549	n.s.	0.0550	n.s.
Type B γ-GTP mRNA in HCC liver	0.0094	n.s.	0.0119	n.s.
Type B γ-GTP mRNA in non-HCC liver	0.0003	0.0107	0.0046	n.s.

Denotes: n.s.=not significant.

Significant factors relevant to post-recurrence survival in univariate analysis included negative HBsAg (P=0.0166), low serum AFP level (P=0.0006), small tumor size (P=0.0080), complete capsule (P=0.0007), absence of vascular permeation (P<0.0001), absence of daughter nodules (P=0.0001), absence of multi-centric HCC (P=0.550), absence of type B γ-GTP mRNA in cancerous tissue (P=0.0119) or non-cancerous tissue of HCC liver (P=0.0046). However, in MV analysis, only serum AFP (P=0.0141), vascular permeation (P=0.0130) and daughter nodules (P=0.0053) had significant difference.

DISCUSSION

γ-glutarnyl transpeptidase (γ-GTP) is a plasma membrane-bound enzyme which has major importance in the metabolism of glutathione[33,34]. Stark *et al* mentioned that metabolism of glutathione by γ-GTP in pre-neoplastic liver foci may initiate an oxidative process leading to a radical-rich environment and result in oxidative damage[25]. Such damage may contribute to foci progress to malignancy. It has been reported that HCC of rat and human both expresses γ-GTP enzymes with unique carbohydrate moieties compared with normal liver enzymes[20,24,28,35]. The presence of the unique γ-GTP isoform for HCC in patient serum had been used as a marker for the diagnosis of HCC[28]. γ-GTP was used as an important marker enzyme for chemically induced HCC, because it is present in both primary HCC and pre-neoplastic lesions of the liver or some other liver diseases[24-27,30,31,36-40]. It has been recently used as a response indicator in the treatment of hepatitis[41].

Experimentally, Mallory bodies develop in mice treated chronically with griseofulvin, and HCC is also found in these animals[42]. In hepatocytes developing Mallory bodies, histologically detectable γ-GTP activity was observed from the early stage of development. These results strongly suggested that changes in γ-GTP in livers may be closely related to the phenotypical expression of carcinogenesis of hepatocytes.

Furthermore, many previous studies concerning γ-GTP in HCC strongly suggest that changes in hepatic γ-GTP expression may closely related to the development of HCC. However, its mechanisms are not well known, and the reports of genomic analysis on the specific γ-GTP to HCC is not common. Recently, human γ-GTP complementary DNA (cDNA) sequences from fetal liver, placenta, and HepG2 cells have been published. These cDNA sequences showed identical GGT protein structure. The most significant difference among these cDNAs exists in the 5'-noncoding region, suggesting that (1) human γ-GTP mRNA might be regulated by alternative-splicing mechanisms in this region, or (2) they are derived from different γ-GTP genes. Pawlak *et al* reported that at least four different γ-GTP genes or pseudogenes are present in human genome[16]. However, pathophysiological roles of the genetic polymorphisms of γ-GTP genes are not well known. In Tsutsumi's study, polymorphisms of γ-GTP mRNAs at the 5'-noncoding region were analyzed.

The results obtained in placenta, fetal liver and Hep G2 cells indicate that the system used in the study is specific to define three types of γ-GTP mRNA[24]. Differences of γ-GTP in tissues have been attributed to sialic acid contents[15]. In normal liver, the main type of γ-GTP mRNA was type A[24]. The expression was monogenic in most cases and polygenic in some cases. In the polygenic cases, type C was found commonly and type B was found occasionally. According to Tsutsumi's report, in cases with liver diseases but not HCC, the distribution of the of γ-GTP mRNA was nearly the same as in normal livers[24]. Our results are similar with the above. On the other hand, the main type of γ-GTP mRNA in cancerous tissue was type B. In Tsutsumi's series, type B was found in all cases, and in more than half of the cases, only type B was detected[24]. In our series, type B was found in 60 % of patients and the combination of type A with type B and only type B are detected in 97.5 %. Tsutsumi reported that in non-cancerous tissues from livers with HCC, the main types of γ-GTP mRNA were type A and B[24]. Both types were found in all cases, except for one case in which type B was not detected. In this study, type B was found in 40 % of patients and both type B with Type A and type B were detected in 70 % of patients. The prevalence of type B was significantly higher in both cancerous and non-cancerous tissues of liver with HCC than that in livers without HCC. The prevalence of type A in cancerous tissues, but not in non-cancerous tissues, was significantly lower than that in livers without HCC. These results strongly suggested that the γ-GTP mRNA expression in the human liver may shift from type A to type B during the development of HCC. The high prevalence of type B in non-cancerous tissues of livers with HCC suggested that the shift of the γ-GTP mRNA may occur from the preneoplastic stage of hepatocytes. The shift of mRNA expression may occur early in the development of recurrence or even in pre-neoplastic stage. Based on that, we used the shift of the γ-GTP mRNA as a tool to predict the recurrence of HCC during the follow-up after resection of primary lesion of HCC.

The high recurrence rate after resection is one of the main factors in the poor outcome for HCC patients[1-6]. Tumor recurrence limits the long-term survival. However, tumor recurrence is well correlated with tumor invasiveness. From the literatures, tumor invasiveness may be determined from high serum AFP, hepatitis vascular permeation, the grade of cell differentiation, infiltration or absence of capsule, size, coexisting cirrhosis, presence of daughter nodules, and multiple lesions[1-14]. Our study suggested that the presence of type B (Hepa G cells) in both HCC tissue and non-cancerous liver tissue of resected HCC specimens was closely related to some invasiveness parameters of HCC. The presence of type B γ-GTP mRNA in cancerous tissue was correlated statistically with high serum level of AFP, daughter nodules, post-resection recurrence and post-recurrence survival. Whereas, the presence of type B γ-GTP mRNA in non-cancerous liver tissue was correlated significantly with hepatitis C infection, high serum level of AFP, Edmondson-Steiner grade III and IV of cellular

differentiation, absence of infiltration of capsule, vascular permeation, daughter nodules, post-resection recurrence and postrecurrence survival.

The presence of type B γ-GTP mRNA which is detected from non-cancerous portion of liver tissue of the resected HCC specimens may be considered as its presence in the remnant liver of the patients who had receiver resection. High level of serum AFP had been considered as a poor prognostic index[5]. According to our study, it was correlated well with the presence of type B γ-GTP mRNA in both cancerous tissue and non-cancerous tissue. It was also correlated statistically significantly with tumor recurrence and survival.

More hepatitis C infection, but not hepatitis B infection was found in the presence of type γ-GTP mRNA in the remnant live. It was also corresponded with a higher recurrence and less survival. Some literature has also mentioned that the prognosis of hepatitis C infection is worse[1,11]. Vascular permeation, indicating tumor invasiveness, consists of either tumor invasion of the hepatic vein, portal vein and/or hepatic artery, or tumor thrombi within the vessels. It may be detected preoperatively by ultrasonography, arteriography or portography, intraoperatively by ultrasonography or direct observation, or postoperatively by pathological examination of surgical specimens. Vascular permeation is the most consistent significant prognostic factor of postoperative tumor recurrence[2, 3, 8-10, 11-13]. In univariate analysis, the presence of type B the prognosis in the remnant liver is significantly related to vascular permeation and in the COX model, patients with vascular permeation had significantly more recurrence and less survival.

Whether the grade of differentiation of HCC is a determinant of recurrence after resection has been debated for a long time[1, 2, 9, 11]. The histological differentiation of the HCCs in this study correlated significantly with γ-GTP, and the presence of type B γ-GTP mRNA increased with increasing dedifferentiation. Our findings are consistent with that shift to type B γ-GTP mRNA in the remnant liver may be associated with the progression of HCC as an event in hepato-carcinogenesis.

The exact mechanism of capsular formation is not known. A tumor capsule may act as a barricade preventing the spread of cancer cells and has a positive role in the prognosis of HCC[2, 5, 7, 8, 10, 13]. The invaded capsule was regarded as incomplete in our series. We found that the expression of type B γ-GTP mRNA in the remnant liver was higer in patients with no capsule and incomplete capsule. Multifocal HCCs are also a controversial issue. Some consider them an early metastasis via the portal vein but some consider them multicentric. The former is a poor prognostic factor but the latter might not be. Without the aid of molecular biology, it is difficult to differentiate daughter nodules, intrahepatic metastatic nodules and multicentric HCC[14]. In the present study, we selected daughter or satellite nodules according to the criteria of the Liver Cancer Study Group of Japan. As for the evaluation of prognosis after recurrence, some authors reported that the most significant factor affecting the survival time of patients with intrahepatic recurrence was the number of tumor nodules at the time of recurrence[1, 2, 4, 8, 9, 12]. In our study, those with the presence of daughter nodules showed a higher presence of type B γ-GTP mRNA in both remnant liver and the original resected HCC tissues.

Tumor size has been emphasized as one of the significant prognostic factors because vascular invasion and daughter lesions may increasingly develop as the tumor grows[2-5, 8, 11-13]. In our study, no correlation between the presence of type B γ-GTP mRNA and tumor size was found. In addition, tumor size also had no correlation with recurrence or survival in our patients. From our experience, some large HCCs were the result of expansive growth and had slow intraportal or distant spread. Our studies showed tumor invasiveness and prognosis was correlated with the presence of HCV infection, high serum level of AFP, vascular permeation, the grade of cell differentiation, infiltration or absence of capsule and daughter nodules. They were also all associated with the expression of type level mRNA in the remnant liver. Among them, serum AFP level and daughter nodules were associated with the presence of type B in HCC tissues. It suggested that the shift of type A to type B of γ-GTP mRNA in the liver tissues were strongly related to the development of HCC, including the progression of preneoplastic tissue and the potential of post-resection recurrence, the invasiveness of HCC and less survival of the patients. It was recommended that the expression of γ-GTP mRNA in both cancerous tissue and non-cancerous tissue of the resected HCC specimens may play a significant role in predicting the prognosis of HCC in patients after resection.

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